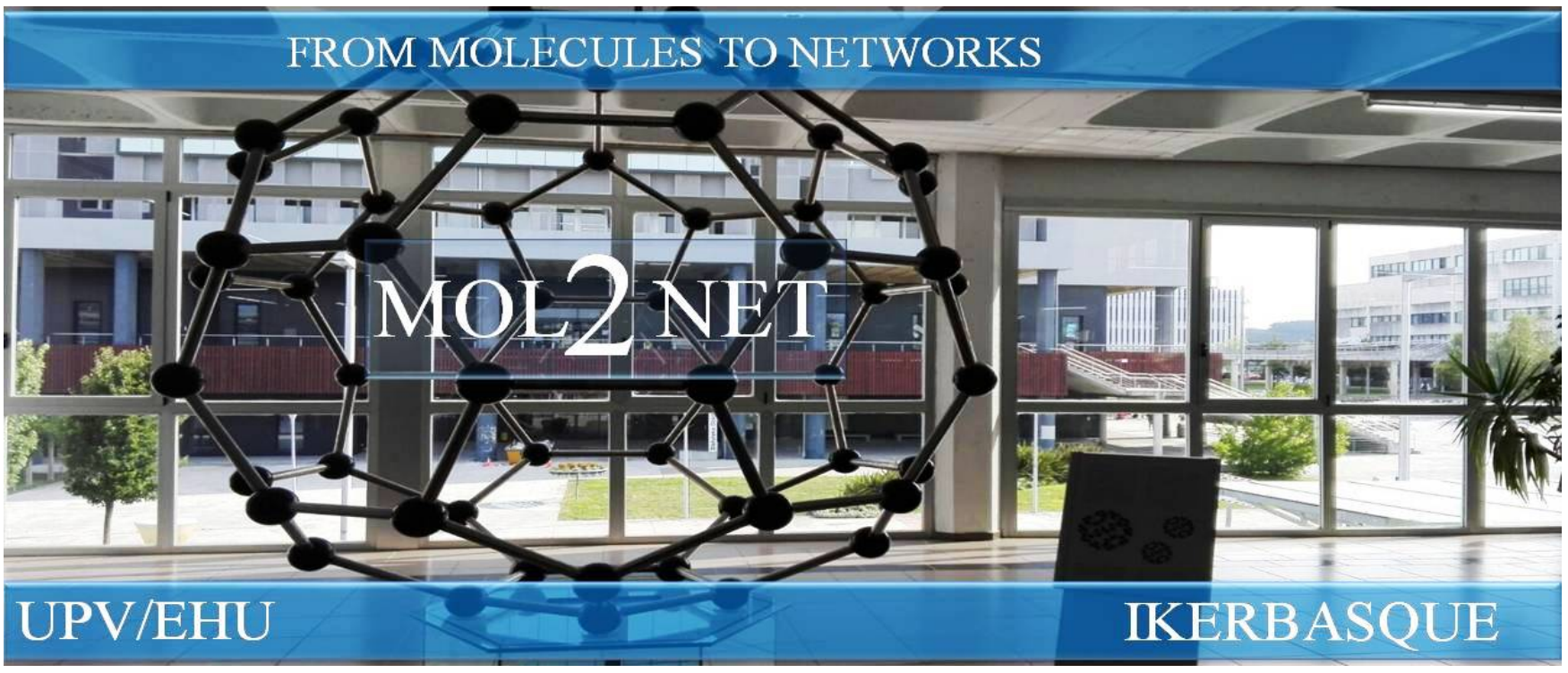
MOL2NET-03, doi: 10.3390/mol2net-03-05179, http://sciforum.net/conference/mol2net-03



MOL2NET International Conference on Multidisciplinary Sciences

(3rd edition)

MOL2NET: FROM MOLECULES TO NETWORKS
(PROCEEDINGS BOOK) Vol. 3.

YEAR-ROUND CONFERENCE
15 January–15 December 2017

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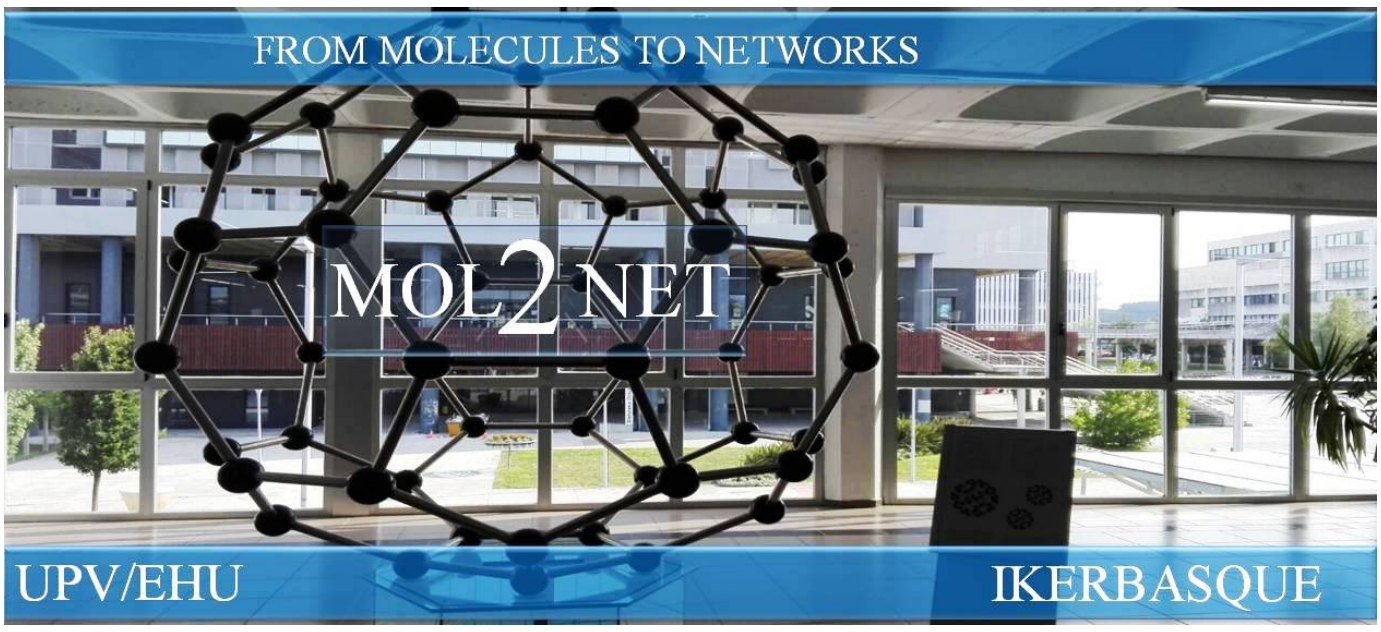
MOL2NET 2017, International Conference on Multidisciplinary Sciences, 3rd edition



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MOL2NET YEAR-ROUND CONFERENCE SERIES & WORLDWIDE ASSOC WORKSHOPS

NOTES: MOL2NET is a year-round conference series with multiple associate workshops worldwide running and open to submissions almost all the year, please read [Workshops/Sections Schedule]. We suggest you to **download** the [Template File] to write your communication and to **read carefully** the [Instructions to authors] about publication model, copyright, authors responsibilities, *etc.*
[Home] [Facebook] [2015] [2016] [Welcome Videos] [Mandarin, 官话] [Hindi, हिन्दी] [Euskera] [Castellano] [Português] [Français]

Welcome from the Conference Chair

We are glad to invite all colleagues worldwide to participate on a new edition of this International Conference Series. The official title of this conference series is MOL2NET International Conference Series on Multidisciplinary Sciences. This is a scientific conference series running on a **year-round conference** scheme (opened for submissions and publications almost all the year) in order to fit on the same platform 3 general sections and more than 10 associate workshops worldwide (see schedule bellow).

MOL2NET (the conference running title) is the acronym of the lemma of the conference: FROM MOLECULES TO NETWORKS. This running title is inspired by the possibility of multidisciplinary collaborations in science. The **topics of interest** include, but are not limited to, Chemistry (All areas), Mathematics (Applied), Physics (Applied), Materials Science, Nanotechnology, Biology and Life Sciences (All areas), Medicine, Biomedical Engineering, Education, along with Computer Sciences, Data Analysis, Statistics, Artificial Intelligence, Deep Learning, Bioinformatics, Systems Biology, and Complex Networks Sciences. See the following note to authors on **topics outside the scope** of the conference and associated workshops [Note on topics].

Headquarters and Supporters

MOL2NET runs both in person (host universities of associated workshops) and online at platform SciForum maintained by the editorial MDPI, Basel, Switzerland. The Scientific Headquarters (HQs) of this conference series are in the Faculty of Science and Technology, University of Basque Country (UPV/EHU), Biscay. However, the idea of this multidisciplinary conference emerged from the melting pot formed as the result of multiple collaborations of professors from many centers worldwide. Locally, the founders and strongest supporters of the conference are professors endowed by IKERBASQUE, Basque Foundation for Sciences, professors from the two departments Department of Organic Chemistry I and Department of Organic Chemistry II of the University of Basque Country (UPV/EHU), and professors from the Department of Computer Sciences of the University of Coruña (UDC).

Internationally, professors from Dept of Biological Chemistry & Molecular Pharmacology (BCMP), Harvard Medical School, USA; the Center for the Study of Biological Complexity of the Virginia Commonwealth University (VCU), USA; the Natural Resources Research Institute, of the University of Minnesota, USA; CNRS-Université Paris-Sud, Paris, France, Universität Rostock, Germany, and other institutions are also founders and/or supporters of this conference, please see full committees lists [Honor Committee] [Co-Chairs Committee] [Scientific Committee (Abroad)] [Scientific Committee (Local)] and [MOL2NET-IKERBASQUE Staff].

Steps for Participation

(0) Register, Sign in/Login, to Sciforum platform [Sciforum login]

(1) Submit the title and abstract, select a section or workshop (do not upload paper here) [Submit New Abstract]

(2) Wait for Sciforum abstract acceptance email, follow the link, and/or login to upload paper, doc and pdf format

(3) Download template doc and prepare your communication [Template File]

(4) Wait for paper acceptance and publication emails (follow link to proofread your paper) (Asap after upload)

(5) Communicate with chairpersons if corrections are necessary, including past editions (All the year)

(6) Log in to post comments, questions, or answers in a section or one of the workshops

(7) Contact chairpersons if you need author (publication) or attendance (posting) certificate for conference and/or workshops (All the year)

Other Editions in This Series

Workshops/Sections Schedule

General Schedule: MOL2NET International Conference Series on Multidisciplinary Sciences, MDPI Sciforum, Basel, Switzerland has online sections (3) and workshops (>10) associated. The conference usually **runs online** at the online platform SciForum maintained by the editorial MDPI, Basel, Switzerland. However, the platform is open o a year-round basis almost all the days of the year in course, MOL2NET-03 in 2017, for practical reasons; e.g., accommodation of many workshops in different dates along the year, reception of communications, etc.

Submission and Publication of communications: As we mentioned before, despite the official dates the online sections (1-3) open at the beginning of the year in course and are open all the year for submissions of communications in order to enable the accommodation of all workshops in different dates along the year. The associated workshops open in different dates along the year (see specific workshop pages). Submission of papers to all sections/workshops is open until the last days of the year in course (2017 for present edition) or the first days of the following. The **publication of communications** is continuous all the year upon acceptance.

Schedule for Online participation: After publication of papers is closed we open the online platform for online participation. The authors will be able to post online comments and/or answers to comments in this workshop/section and also in the other general sections and/or >10 international workshops of the MOL2NET conference (many of them also run both online and in person). The participants are entitled to receive participation certificates for MOL2NET conference and all the workshops they participate upon request to the respective chairpersons. See committees of MOL2NET and each workshop.

Schedule for in person participation: Many of the workshops associated to MOL2NET are going to be held also ***in person*** **(face-to-face)** in different universities of USA, Spain, Italy, Mexico, Chile, etc. This in person workshops have specific schedules in parallel to their online versions. Please, go to the homepages of these workshops and/or contact their chairpersons for further details.

Notes for participants

MOL2NET conference runs both online (general sections) and/or in person (associated workshops). No physical presence is needed for online participation saving traveling costs. We accept experimental works, theoretical works, or experimental-theoretic works in the areas mentioned. Proceedings will be Published Online, Open Access, and **Totally Free of Charges** (no cost). For details about in person (face-to-face) participation on associated workshops contact the respective members of the local committees.

Before to submit your communication recommend to download and use the [Template File] to write your communication; we strongly recommend you to read carefully the following **notes to participants** about publication model, copyright, authors responsibilities, *etc* Firstly, be aware that the works published here belongs to two main modalities preliminary communications or comments on previous works.

Preliminary communications. These are short communications of unpublished results but they are not post-print journal papers. In this sense, committee and/or external reviewers check only scope and apparent scientific soundness. They have the same editorial process than for an online Preprint service. Therefore, all works receive doi number and are indexed in databases (GoogleScholar, Publons, *etc*.).The works may receive also comments from registered participants (public post-publication review). The authors are encouraged to submit their works to a peer-reviewed scientific journals of MDPI or other editorials during or after finalization of the conference, as per SciForum copyright rules.

Research Highlights. These short communications are comments on already published papers. They are short notes to comment about the more interesting points, highlights, etc. of works previously published by the authors or other groups. In this case, committee and/or external reviewers check also only scope and apparent scientific soundness. The works may receive also comments from registered participants (public post-publication review).

In any case, **it is the authors responsibility,** to ensure the veracity of the contents, checking similarity to other works, and carry out a proper citation of previous works. The committee is not responsible of this previous aspects in this publishing modality. In this sense, we strongly recommend the authors to use online text-similarity checking services to avoid any form of plagiarism or copyright violation. The authors may be requested to modify the communication (re-write their texts) in the case that high similarity is detected and reported to the committee. In these cases, the manuscript could be put on standby or withdrawn temporarily until the authors re-submit the proper version. The authors are also allowed to submit short reviews, comments, letters, or discussions of papers already published if they guarantee sufficient difference to previous public contents.

MDPI Journals Issues (Organized by MOL2NET Committee Members)

In parallel, the members of committees and/or authors are encouraged to edit special issues for different journals of the editorial MDPI (http://www.mdpi.com/). The special issue is now in call for papers, submissions are welcome in *a posteriori*, in parallel, or totally independently from the conference. Manuscripts should be submitted online at www.mdpi.com by registering and logging in to this website. Once you are registered, click here to go to the submission form. In order to send a proposal of associated workshop and/or special issue contact the chairperson of the conference Prof. González-Díaz H.

Entropy (Call for Papers): Special issue entitled Graph and Network Entropies. Journal Entropy (ISSN 1099-4300), **JCR IF = 1.821**. Topics: network sciences; walk entropies; algegraic graph theory; spectral methods; matrix functions. Editor: Prof. Dr. Ernesto Estrada, Department of Mathematics & Statistics, University of Strathclyde, Glasgow G11XQ, UK.

Nanomaterials (Call for Papers): Special issue entitled: Experimental Nanosciences, Computational Chemistry, and Data Analysis. Journal Nanomaterials (ISSN 2079-4991), **JCR IF = 3.55**. Editors: Prof. Dr. González-Díaz H, UPV/EHU, Spain, Prof. Dr. B. Rasulev , NDSU, USA, Dr. Shameer Khader, Philips, Cambridge, MA, USA, Prof. Dr. H. Kušić, UZ, Croatia**.**

IJMS (Published): see also, as example, the previous special issue on Data Analysis in Molecular Sciences. This issue was published in 2016 on the International Journal of Molecular Sciences (IJMS), **IF = 3.257**. The issue included 18 papers in total (some of them from the conference).

Associated Workshops

In addition, you can participate both online or in person (face-to-face) in some of the workshops we organize in different universities worldwide (see workshops section). Publication of all communications of the workshops will be also online via the platform SciForum. We welcome proposals for organization of workshops in different universities. Please, do not hesitate to contact conference chairperson and/or scientific committee presidents. Some of the workshops open for 2017 edition are: SRI-09,

STU, Miami, FL, USA (Multidisciplinary Sciences); IWMEDIC-05, UDC, Coruña, Spain (Biomedical Engineering); SIUSCI-01, SIU, Miami, FL, USA (Multidisciplinary Sciences); WMCUP-01, UP, Padova, Italy (Medicinal Chemistry); LAWSCI-01, UPV/EHU, Bilbao, Spain (Bioethics and Legal Sciences); WRSAMC-02, UFPB, Paraiba, Brazil (Medicinal Chemistry).

Past Editions

MOL2NET-01, the first edition of this conference series, was held in Dec 2015. This first conference attracted more than 100 papers and 300+ authors and/or committee members representing 30+ universities of 20+ countries. Some of the world top universities and centers represented in the lists of committee members and/or authors were: **Harvard** Medical School, Boston, USA; **Stanford** School of Medicine, USA; **Virginia** Commonwealth University (VCU), USA; University of **Minnesota Duluth**, MN, USA; Conservatoire National des Arts et Métiers, **CNAM París**, France; University of **Pennsylvania**, USA; Miller School of Medicine, University of **Miami**, USA; **EMBL-EBI** European Bioinformatics Institute, Cambridge, UK; **CAS** Chinese Academy of Science, China; **ZJU** Zhejiang University, China.

MOL2NET-02, the 2nd edition of this conference series, was held in Dec 2016. This 2nd conference published 150 communications of 400+ authors 30+ universities of 20+ countries. Some of the world top universities and centers represented in the lists of committee members and/or authors were: **Harvard** Medical School, Boston, USA; **Stanford** School of Medicine, USA; **Virginia** Commonwealth University (VCU), USA; University of **Minnesota Duluth**, MN, USA; Conservatoire National des Arts et Métiers, **CNAM París**, France; University of **Pennsylvania**, USA; **Miami** Dade Collegue (MDC), USA; **EMBL-EBI** European Bioinformatics Institute, Cambridge, UK; **CAS** Chinese Academy of Science, China; **ZJU** Zhejiang University, China.

Past Workshops

SRI-08 The 8th Annual Undergraduate Summer Research Symposium of Saint Thomas University, Miami, USA, Sept, 2016. Symposium of the Summer Research Institute (SRI), HQ Saint Thomas University (STU), Miami, FL, USA. Workshop supported by STE-TRAC Miami Dade College (MDC) grant. **Topics:** Multidisciplinary sciences, Applied physics, Environmental Sciences, Biomolecular Sciences, etc. **Chairpersons:** Prof. David Quesada and Prof. Humberto Gonzalez-Diaz (Online Publication). (**38 communications**)

IWMEDIC-04, IV International Workshop on Medical Imaging, Medical Coding, and Clinical Data Analysis of University of Coruña (UDC). Was held presentially at the University Hospital Complex of A Coruña (June, 20, 2016), Hospital Médico Quirúrgico San Rafael (June, 21,2016), and Faculty of Computer Sciences, UDC (June, 20-22, 2016). **Topics:** Medical Imaging, Medical Informatics, Bioinformatics, etc. **Chairperson:** Prof. Alejandro Pazos. (**30 communications**)

UFIQOSYC-01, Workshop was held at the Department of Organic Chemistry II, University of Basque Country UPV/EHU. **Topics:** Organic Chemistry, Chemical Catalysis, Organic Synthesis. **Chairpersons:** Prof. Esther Lete, Prof. Esther Domínguez Pérez, and Prof. Jose Luis Vicario. (**17 communications**)

BMEICB-02 Second Bioinformatics Meeting of The School of Bioinformatics Engineering, University of Talca, Talca, Chile, (Oct, 13-14, 2016). **Topics:** Bioinformatics, Informatics Engineering, Medical Informatics, Chemoinformatics, etc. **Chairpersons:** Prof. Gabriel Nuñez and Prof. Julio Caballero. (MOL2NET Contact). (**12 communications**)

SUIWCS-01, Soochow University International Workshop Series on Computer Sciences. The SUIWCS-01 workshop was held in person at the the School of Computer Science and Technology of Soochow University (SUDA), PCR, China (Nov, 2016). **Topics:** Machine Learning, Reinforcement Learning, Bioinformatics, Medical Informatics, Chemoinformatics, etc. **Chairpersons:** Prof. Quan Liu, Assist. Prof. Xiaoke Zhou (MOL2NET Contact). (**12 communications**).

People, Media Channels, and Social Networks

We are uploading flyers and promotional videos (in different languages) to the MOL2NET accounts in different social networks such as: FACEBOOK group with +10000 followers; and TWITTER account @mol2net. In addition, we have uploaded topic-specific pages with lists of contacts of people related to the conference. In this page you can find people with research interests focused on one specific area such as Organic Chemistry, Computational Chemistry, Materials or Nanoscience, etc. In this sense, to contact people related to all areas of Chemistry you may visit [Section 01], but to contact people related to Organic Chemistry & Medicinal Chemistry specifically (organic synthesis, catalysis, drug discovery, *etc*.) you can visit also the page Organic Chemistry People, as well as [Section 08].

THANK YOU FOR YOUR SUPPORT!!!

Sincerely yours

MOL2NET Chairman

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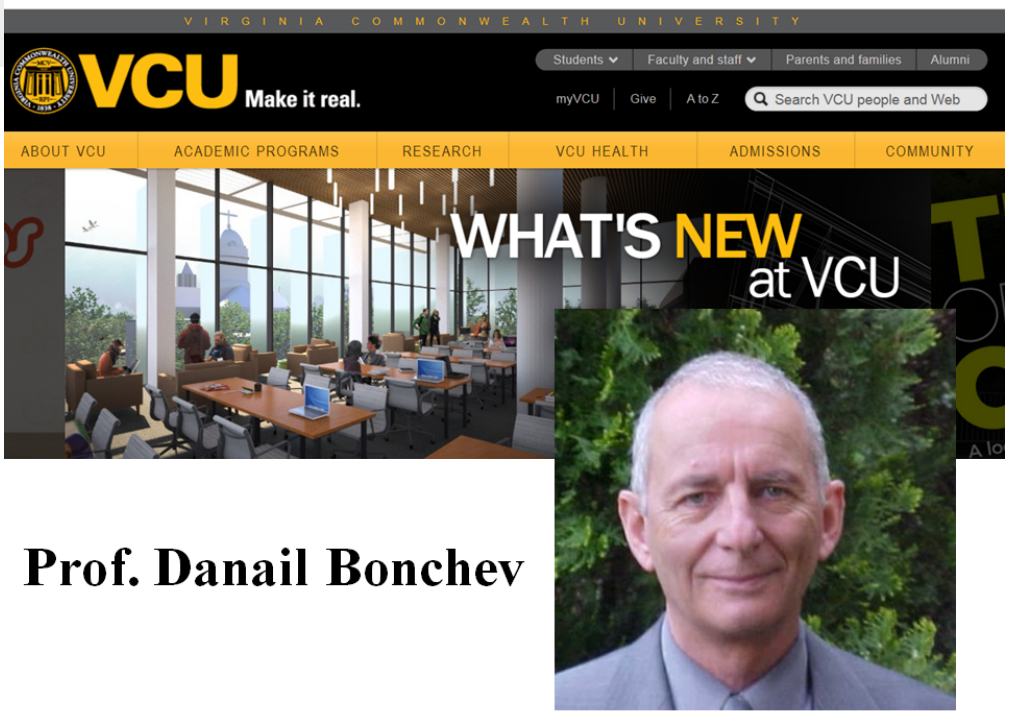

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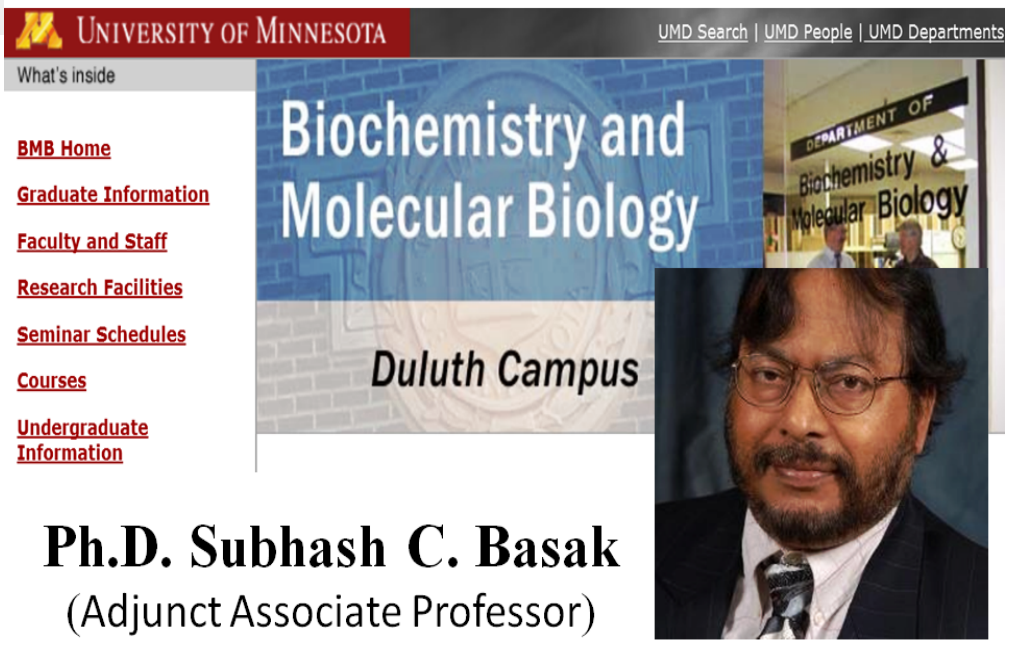

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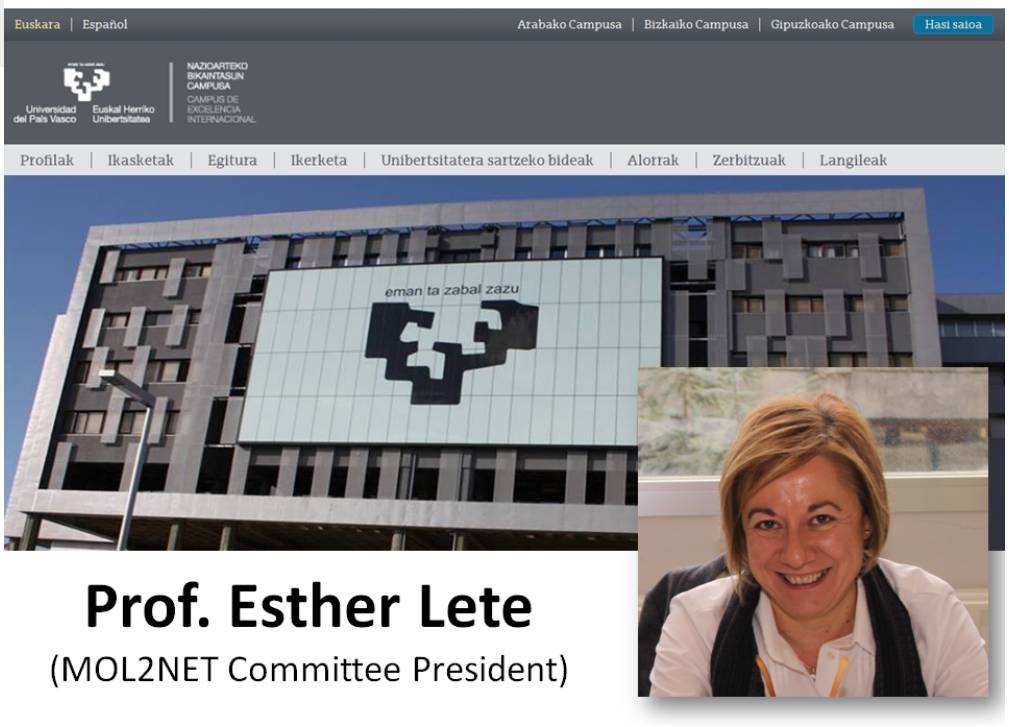

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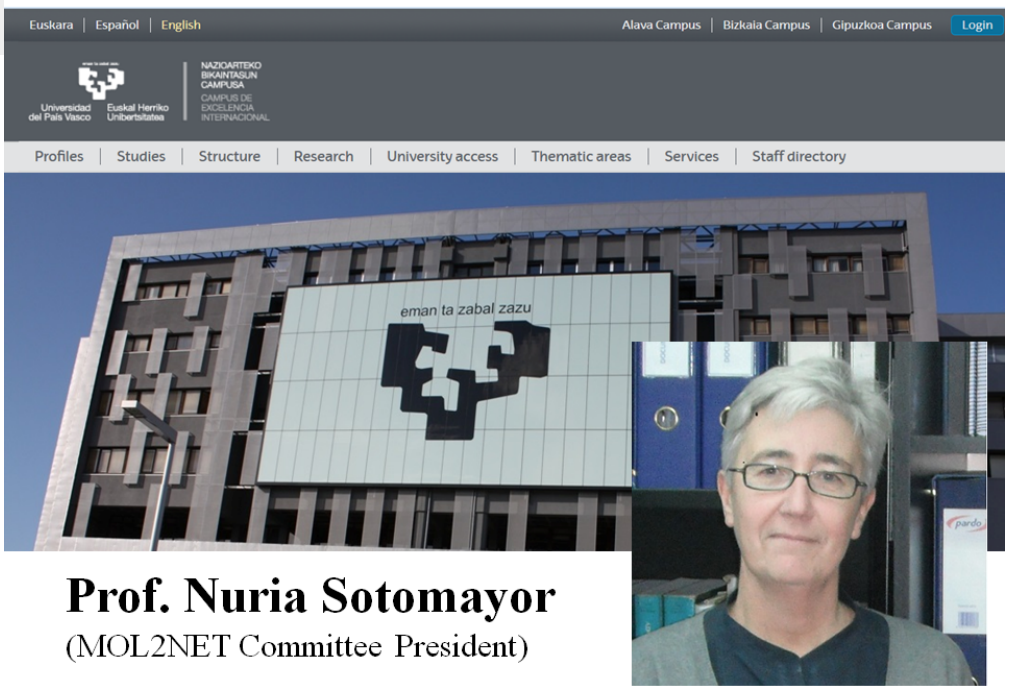

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Thank you for your support!!!

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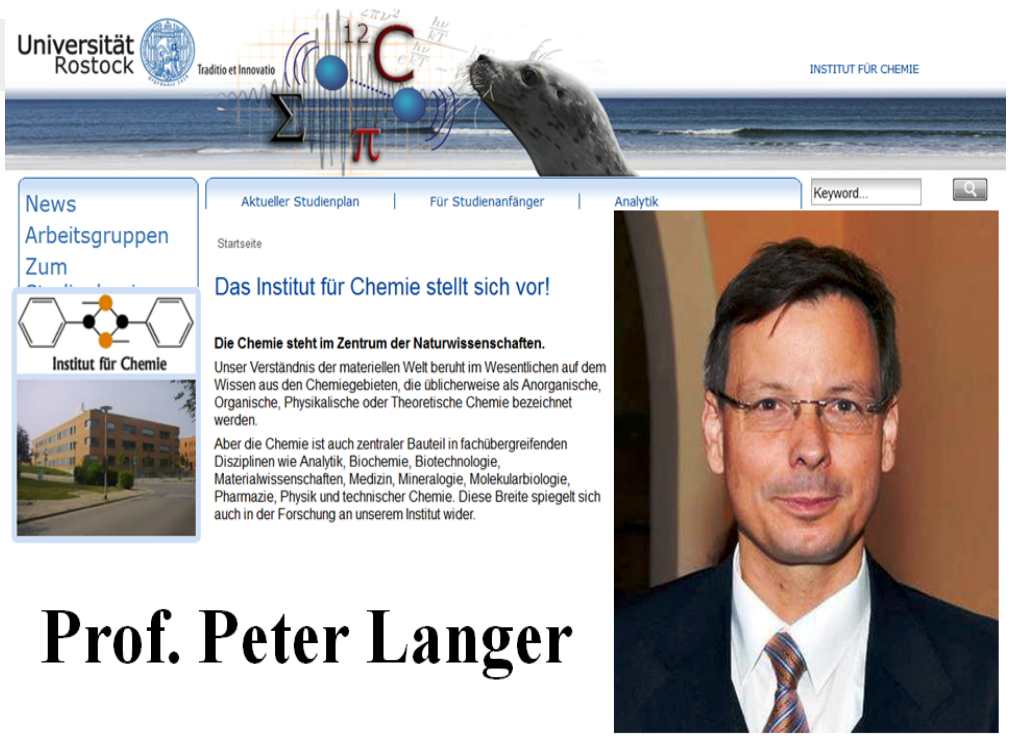

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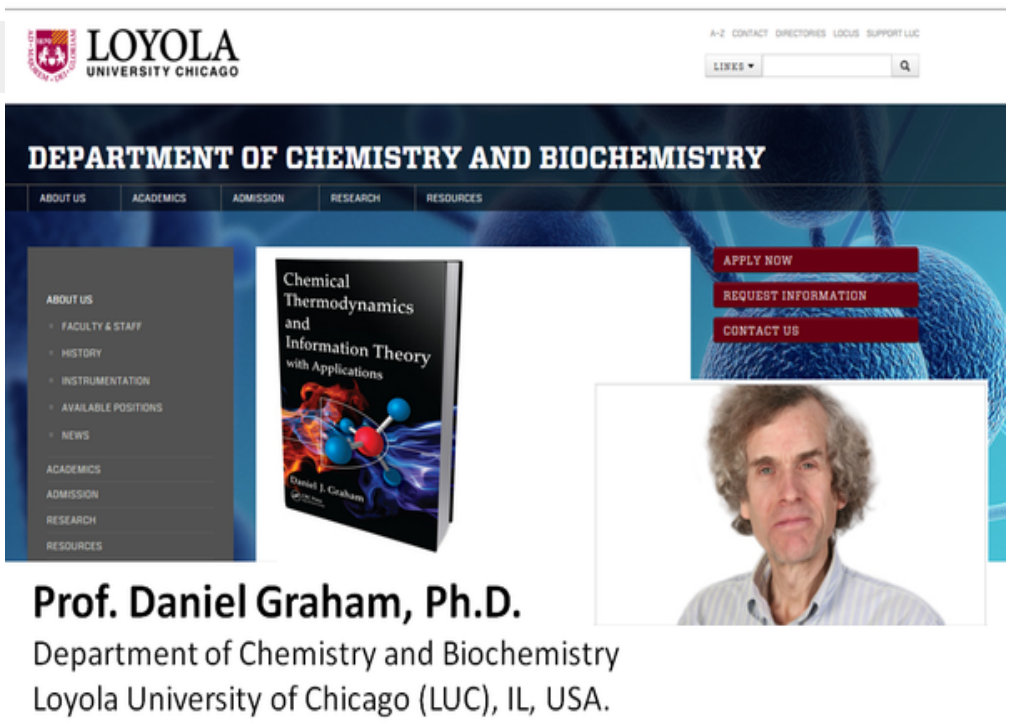

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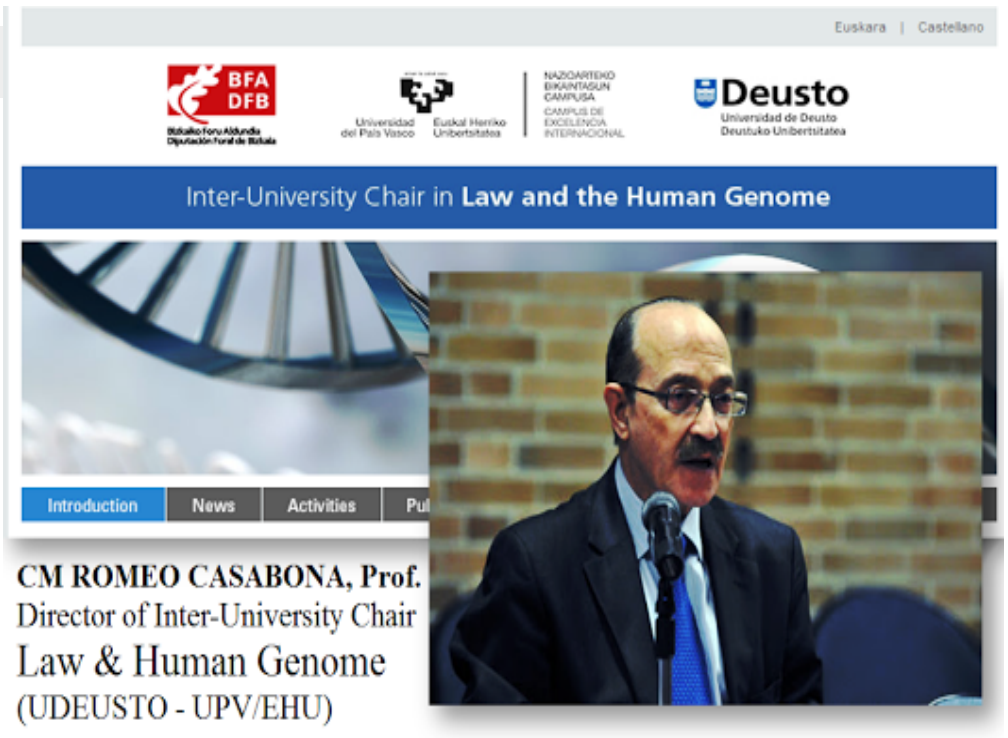

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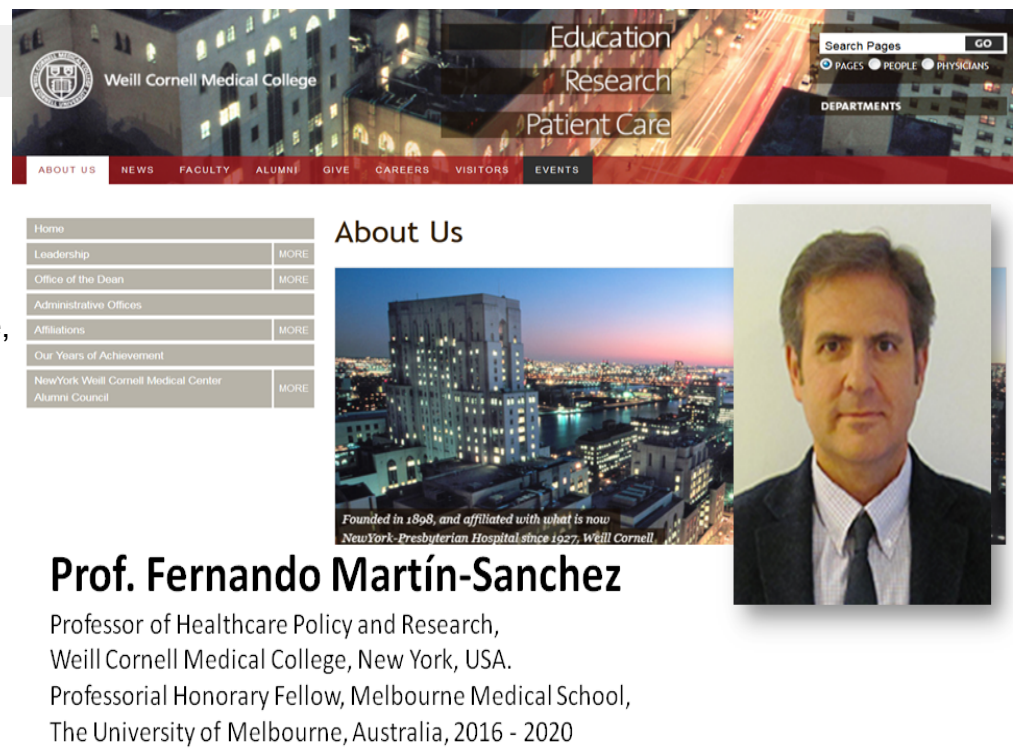

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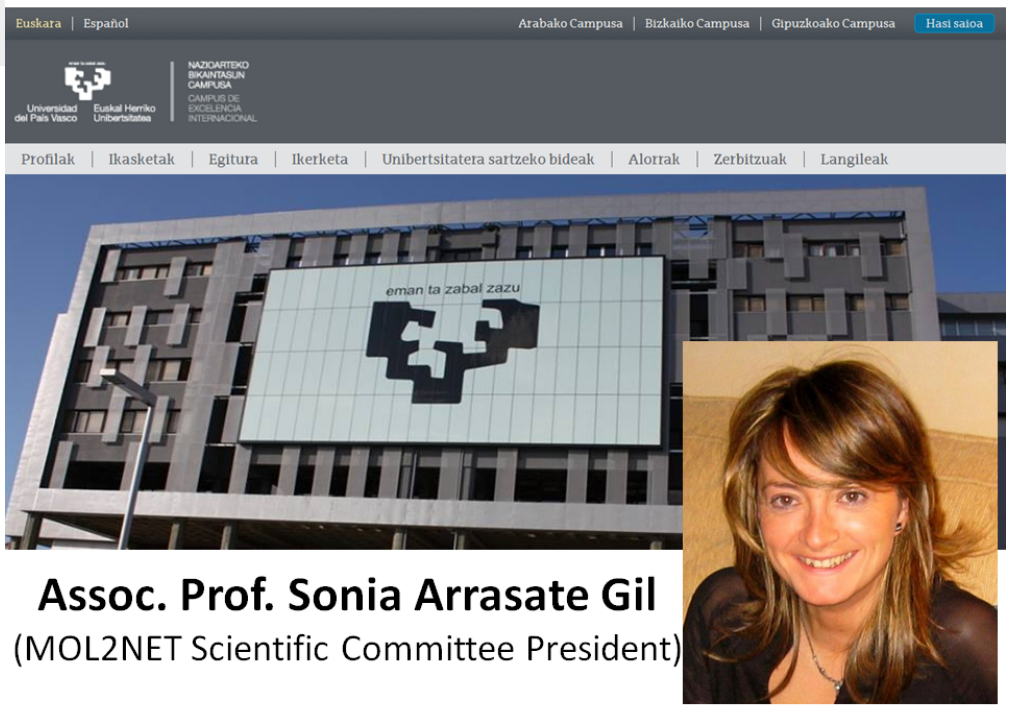

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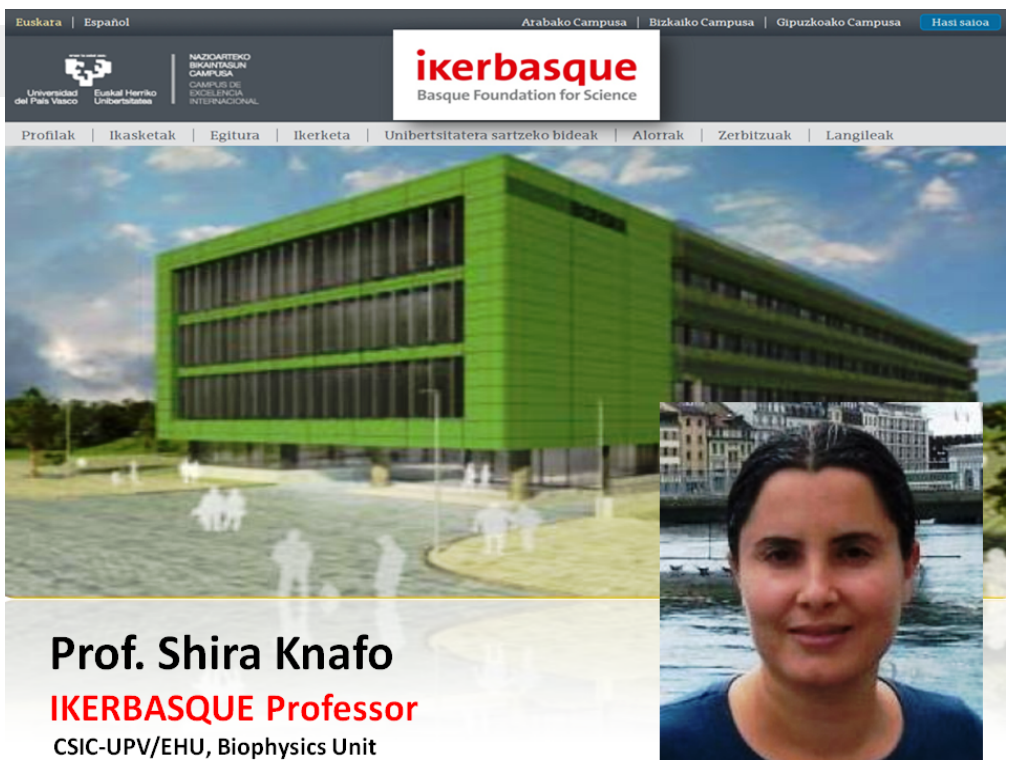

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MDPI JCR Journals Special Issues

The members of committees and/or authors are encouraged to edit special issues for different journals of the editorial MDPI (http://www.mdpi.com/). In this edition we opened/continued with two special issues entitled:

Nanomaterials (Published): Special issue entitled: Experimental Nanosciences, Computational Chemistry, and Data Analysis. Journal Nanomaterials (ISSN 2079-4991), **JCR IF = 3.55**. Editors: Prof. González-Díaz H, UPV/EHU, Spain, Prof. B. Rasulev , NDSU, USA, Dr. Shameer Khader, Philips, Cambridge, MA, USA, Prof. H. Kušić, UZ, Croatia**.**

IJMS (Published): Special issue entitled: Data Analysis in Molecular Sciences. Journal: International Journal of Molecular Sciences IJMS (ISSN 1422-0067), **IF = 3.257**. Topics: Cheminformatics, Computational Chemistry, Bioinformatics, Data Analysis, Proteomics, Biological Networks, Editors: Prof. González-Díaz H, University of the Basque Country UPV/EHU, Biscay, Spain. Prof. R. Todeschini, University of Milano-Bicocca, Italy, Prof. A. Pazos Sierra, University of Coruña UDC, Spain, Prof. S. Arrasate, University of the Basque Country (UPV/EHU), Biscay, Spain.

Conference series: MOL2NET, International Conference on Multidisciplinary Sciences

» For hosting your own conference's website on Sciforum, please get in touch with us

- MOL2NET 2018, International Conference on Multidisciplinary Sciences, 4th edition (15 January–15 December 2018)
- MOL2NET 2017, International Conference on Multidisciplinary Sciences, 3rd edition (2017)
- MOL2NET 2016, International Conference on Multidisciplinary Sciences, 2nd edition (2016)
- MOL2NET, International Conference on Multidisciplinary Sciences (2015)

01: Chemistry (All Areas), Soft Matter Physics, Pharmaceutical Sciences, Polymer, and Nanosciences

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This section covers: All areas of Chemistry, including, Inorganic, Analytical, Physical, Organic, Medicinal, and Theoretical Chemistry as well as Soft Matter Physics, Biophysics, Polymers, Materials, Pharmaceutical Sciences, and Nanosciences.

The topics of interest include both experimental and theoretical research in these areas. Including, but not limited to, Organic synthesis, Chemical reactivity, Catalysis, Solid State Chemistry, Inorganic Crystals, Crystal Symmetry, and Complexes. Physicochemistry and Analytical chemistry techniques; Spectroscopy (X-Ray, NMR, IR, EPR, Mass Spectroscopy), Chromatography and sample preparation techniques, TEM and SEM Microscopy.

The section also covers experimental and/or theoretical analysis of artificial polymers, biopolymers, materials, nanomaterials, etc. Experimental and theoretical study of carbon nanomaterials (Graphene, Fullerenes, Nanotubes, Diamonoids), Ceramic materials, Alloys. Biopolymeric Nanomaterials for biosciences (drug carriers, diagnosis tools, medical imaging) including Dendrimer, Protein nanoparticle. Shape Memory Polymers, Nanopatterning, and Surface Imprinting.

Last but not least, the section includes studies in theoretical and computational methods: Chemoinformatics and Computer Aidded Drug Design (CADD) methods, Computational Chemistry, Quantum Mechanics (*Ab inition*, DFT, MP3, AM1 methods), Monte Carlo (MC) algorithm, Quantitative Structure-Reactivity, Structure-Property, or Structure-Retention Relationships (QSPR/QSRR) models in organic, inorganic, physical and analytical chemistry. Experimental Design, Chemometrics, Formulation Design, and Data Analysis in Analytical chemistry and Pharmaceutical sciences.

See as example, the communications of the same section in previous editions:

MOL2NET 2016, Section 01 (Chemistry All Areas, Nanosciences, Polymers)

MOL2NET 2015, Section 01: Section A (Chemistry) , Section C (Soft Matter Physics, Polymers, Materials)

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List of papers (31)

Antibacterial Potential Of The Alpha-pinene Positive Enantiomer Against The Strain *Proteus mirabilis* show abstract

by Letícia de Sousa Eduardo , Ticiane Costa Farias , Gildoberg Nunes da Silva , Francisca Patrícia da Silva Lopes , Sávio Benvindo Ferreira *

Potent antioxidant activity of Kigelia africana flower fractions on cell-free systems. show abstract

by Enoel Hernández-Barreto *

4,6,6-trimethylbicyclo [3.1.1] hept-3-ene: Analysis of the Inhibitory Effect of Monoterpere on *Pseudomonas aeruginosa* Strain show abstract

by Ticiane Costa Farias , Letícia de Sousa Eduardo , Siluana Benvindo Ferreira , Zilka Nanes Lima , Sávio Benvindo Ferreira *

Exploring different strategies to improve the magnetic response of cobalt doped ferrite nanoparticles show abstract

by Itziar Galarreta , Maite Insausti *, Izaskun Gil de Muro , Idoia Ruiza de Larramendi , Luis Lezama

Influence of Carbon Nanotubes in Nano-carboapatite show abstract

by Wanderson Geraldo Knupp *, Ihosvany Camps Rodriguez , Mirta Mir Caraballo *

Lattice dynamics of carbon nanotube interacting with hydroxyapatite show abstract

by Wanderson Geraldo Knupp *, Ihosvany Camps Rodriguez *, Mirta Mir Caraballo

Enhancement of quantum efficiency of hybrid photoelectrochemical cell: Effect of functionalized carbon nanotube with Cu doped ZnO nanocomplex show abstract

by Poonam Bandyopadhyay *, Papiya Nandy , Ruma Basu , Sukhen Das , Debbethi Bera

Use of novel metallic nanoparticles for improvement of electrical properties of PVDF-HFP polymer film show abstract

by Biplab Kumar Paul , Sukhen Das , Ananda Lal Gayen , Poonam Bandyopadhyay , Smarajit Manna , Ruma Basu , Durga Shankar Bhar , Papiya Nandy *

Standardization of the Safety Level of the Use of DMSO in Viability Assays in Bacterial Cells show abstract

by Raquel Carlos de Brito , Gildoberg Nunes da Silva , Ticiane Costa Farias , Paula Benvindo Ferreira , Sávio Benvindo Ferreira *

New Microbiological and Pharmacokinetic models show abstract

by Jose I. Bueso-Bordils *, Pedro A. Alemán-López , Sara Costa-Piles , Maria J. Duart , Luis Lahuerta-Zamora , Rafael Martín-Algarra , Gerardo M. Antón-Fos

Synthesis and Anti-mycobacterial evaluation of coumarin derivatives show abstract

by Rodrigo Santos Aquino de Araújo , Karla Patrícia de Oliveira Luna , Lilian Maria Lapa Montenegro , Andrea Santos Lima , Ricardo Olímpio de Moura , José Maria Barbosa-Filho , Francisco Jaime Bezerra Mendonça-Júnior *

Nanodesk project: development of a web platform for the selection of the nanoparticles of interest for the plastic industry show abstract

by Riccardo Concu *, Maria Natalia Dias Soeiro Cordeiro

Synthesis and antibacterial activity of novel bisacodyl derivatives show abstract

by Fatma ABDMOULEH , Charlotte RICCOBONO , Maxi ZHU , Ciaran ROGERS , Ameni HADJ MOHAMED , Christophe RICCO , Mehdi EL ARBI ,Mamdouh BEN ALI , Clotilde FERROUD , Maité SYLLA-IYARRETA VEITIA *

Diyne formation from alkynes in the presence of palladium pincer complexes show abstract

by Esther Domínguez , Garazi Urgoitia , María Teresa Herrero , Raul Sanmartin *

Efficient preparation of carboxylic acids from alkynes show abstract

by Esther Domínguez , Garazi Urgoitia , María Teresa Herrero , Raul Sanmartin *

A more efficient catalyst for the cycloisomerization of alkynoic acids show abstract

by Galder Llorente , Aimar García , Esther Domínguez *, Garazi Urgoitia , Iratxe Astarloa , María Teresa Herrero , Nerea Conde , Raul Sanmartin *

Enaminoketone derivatives as key intermediates for the synthesis of 4-quinolones show abstract

by Ander Alvaro , Aimar García , Esther Domínguez *, Galder Llorente , Garazi Urgoitia , Iratxe Astarloa , María Teresa Herrero , Raul Sanmartin *

In Silico Repurposing, Design, Synthesis and Biological Evaluation of Bisacodyl Analogues show abstract

by Maité SYLLA-IYARRETA VEITIA *, Dany SIVERIO MOTA , Vanessa LERARI , Marta MARIN , Rosa M. Giner , Liliana Liliana VICET MURO , Yankier Rivero Guerra , Françoise Dumas , Clotilde FERROUD , Peter A. M. DE WITTE , Alexander D. CRAWFORD , Vicente J. ARAN , Yovani MARRERO PONCE

Employment of hyphenated approach for metabolomic fingerprinting of phenolics from *Torilis leptophylla* roots show abstract

by Noshin Nasreen , Nabil Semmar , MUHAMMAD FARMAN *, Saud Nasim Ahmad

02: OMICs, Biotechnology, Bioinformatics, Neurosciences, and Biomedical Engineering

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This section covers experimental and computational methods in Life Sciences in general: OMICS, Molecular Biology, Biotechnology, Bioinformatics, Biomedical Engineering, and related topics. Experimental and theoretical methods applied to drug discovery, biomarkers and target validation, vaccine design, in biosciences. In experimental studies: Pharmacological assays, Toxicity and Cytotoxicity studies, Molecular Biology and Biotechnology. Proteomics, Genomics, and Metabolomics (OMICS methods) like Sequencing, Cloning, DNA microarrays, and Mass Spectroscopy in Clinical Proteomics.

The section also include the use of computational techniques in the previous fields. Some examples are: Molecular Mechanics and Molecular Dynamics (MM/MD) for Drug-protein Docking studies, Quantitative Structure-Activity / Toxicity Relationships (QSAR / QSTR) models. Bioinformatics analysis of Disease Biomarkers and Computational vaccine design (Alignment and Alignment-free techniques). Determination of the 3D proteins structure using NMR and X-ray techniques. Experimental and computational study of RNA (Rnomics), secondary RNA structure prediction, miRNA biomarkers. As well as, Computational Systems Biology, Complex Networks Analysis for OMICS, Networks Analysis in Ecology, Mathematical Biology, etc.

The section includes in addition: Biomedical Engineering, and Medical Informatics. Biomedical research, experimental and/or computational medical diagnostic tools in cancer research, neurosciences, clinic and biomedical engineering. Including, but not limited to, EEG and structural NMR in clinical diagnosis in neurology and brain research. EEG, fNMRI, microscopy, tomography, study for tissue connectivity analysis, including the use of experimental techniques and complex networks computational analysis in neurosciences, bone tissue connectivity, vascular system connectivity, etc.

See, as example, the presentations of the same section on last editions:

MOL2NET 2016, Section 02 (OMICS, Biotech, Bioinfo, Biomed Eng)

MOL2NET 2015, Section B (Med Chem, Omics, Biosciences)

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List of papers (14)

by Irati Rodilla , Aitziber Mendiguren , Joseba Pineda *

Electrophysiological study of the effect of cannabidiol on the dorsal raphe nucleus serotonergic neurons show abstract

by Erik Aostri *, Joseba Pineda *, Aitziber Mendiguren *

List of Accepted Abstracts (2)

Recent updates on Computer-aided drug discovery: Time for a Paradigm Shift show abstract

by Talambedu Usha , Shanmugarajan Dhivya , Chinaga SureshKumar , Arvind Kumar Goyal , Sushil Middha *

Acidic and Alkaline Enzymes Discrimination using Split Amino Acid Composition inheriting Chou's Pseudo Amino acid composition incorporating Probabilistic Neural Network Model show abstract

by Zaheer Ullah Khan *, Dr. Farhad Ali

03: Computational Sciences, Statistics, Artificial Intelligence, Complex Networks, Machine Learning, and Big Data

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This section covers the different applications of computer sciences, data analysis, statistics, modelling techniques, etc. in multidisciplinary sciences. The topics include, but are not limited to, connectivity analysis in biology, environment, epidemiological, and social networks; including the computational analysis of metabolic pathways in Metabolomics, Protein interaction networks in proteomics, food webs, and other biological-ecological networks like host-parasite, prey-hunter, etc. Geographical Information Systems (GIS), land covering networks, atmospheric reactions networks. Study of social collaboration, electronic social networks (Facebook, Twitter, etc.), disease spreading networks and epidemiology, vaccination models in epidemic networks, legal and law citing networks, networks in sociology and criminology, etc.

This section covers also: technological, industrial, and economic connectivity, including the analysis of computer connectivity, Internet, wireless networks, satellite networks, electrical networks, airport and other transport networks, financial networks, trade networks, etc. In addition, we cover pure theoretical aspects in network science and data analysis theory, including but not limited to theoretical studies in network sciences, topological indices, node centrality, network robustness, multiplex networks, network attack, and new spatial statistical analysis, time series analysis, biostatistics, machine learning and big data analysis methods.

This section is also aimed at presenting the most commonly used software tools in Multidisciplinary Science. Include, but is not limited to, new scientific software, web servers, databases, etc. with applications in Chemistry (all branches), Bioinformatics, Proteomics, Biotechnology, Medical Informatics and Biomedical Engineering, Computer Science, etc.

The short communications should present computational tools that may be desktop/web/mobile applications/scripts, open code or private software. The tool may be original or a pipe of other tools. It should contain a software description, case uses in order to understand how to employ it, links to the open repositories (GitHub, GitLab, Personal Webs, etc.) or official Webs of the private products, and references of the publications where the tools have been applied. The authors may include in the communication a link to their personal webs, web servers, repositories, databases, etc.

Special attention will be paid to the links to tutorials (blogs, videos, etc.), print screens with the tools in action, pseudocodes, examples of input and outputs, script examples while using the tools, and links to the social network posts for the tools. The emphasis of this section is on the software per se. Communications that make use of a software to solve a practical problem but do not put emphasis on describing it could be suitable for other sections.

We also welcome submissions related to: Client–server model — *Client–server computing*, Grid computing with a cluster of networked, loosely coupled computers to perform very large tasks, Distributed Fog computing paradigm, Dew computing, Mainframe computer for big data processing in large research organizations, Utility computing, and Peer-to-peer computing alternatives in science and medical informatics.

Enjoy programming for science!

See examples in similar sections of the last editions:

MOL2NET 2016, Section 03 (Computational Sciences)

MOL2NET 2015, Section E (Statistics, Artificial Intelligence)

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List of papers (10)

New insights to understand the CoMFA and CoMSIA analysis within the framework of Density Functional Theory. Toward a generalized methodology. show abstract

by Alejandro Morales-Bayuelo *

Prediction of mRNA expression in cow's milk using mRNA secondary structures and Machine Learning classifiers show abstract

by Rodrigo Martín *, Yong Liu , Omar Landaeta , Luis Felipe Llamas , Chuanshe Zhou , Zhiliang Tan , Haibo Zhang , Cristian Robert Munteanu *

Computational study of aromatic compounds inhibiting *Trypanosoma cruzi* glyceraldehyde 3-phosphate dehydrogenase show abstract

by Pablo Henrique Delmondes *, Fabricio Tarso de Moraes , Ricardo Stefani

SMANN: Screening Model of Artificial Neural Networks for Brain Connectom show abstract

by Enrique Barreiro , Maykel Cruz-Monteagudo , Cristian Robert Munteanu *, Alejandro Pazos

FRAMA 1.0: Framework for Moving Average Operators Calculation in Data Analysis show abstract

by Bernabe Ortega-Tenezaca *, Viviana F. Quevedo-Tumailli , Humbert Gonzalez-Diaz *

Optimizing queries via search server ElasticSearch: a study applied to large volumes of genomic data show abstract

by Alex Camargo *, Diego Rodrigues Mengarda *, Vinicius Rosa Seus *

Data compression with Python: application of different algorithms with the use of threads in genome files show abstract

by Vinicius Rosa Seus , Alex Camargo *, Diego Rodrigues Mengarda

The KP algorithm for the analysis of the optimal flow of information show abstract

by Bernabe Ortega-Tenezaca *, Carlos Israel Núñez Miranda *

Cálculo de pK_a de ácidos aspárticos en tiorredoxina a través de dinámica molecular show abstract

by Jans Alzate Morales *, Aharon Gómez , Esteban Vöhringer-Martinez

Prediction of RIFIN proteins with gene orientation network indices show abstract

by Viviana Quevedo *, Bernabé Ortega-Tenezaca , Julio César Vargas-Burgos , Alejandro Pazos-Sierra , Humbert González-Díaz

List of Accepted Abstracts (1)

by [not defined] show abstract

04: SRI-09: 9th Summer Research Institute Symposium, STU-MDC, Miami, USA, 2017

NOTES: Before to submit your communication we strongly recommend to read to read carefully the **notes to participants** at the end of the welcome message and/or the notes in the following page [Notes to authors] about publication model, copyright, authors responsibilities, *etc*.

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Welcome Message

Nine years have gone since the First STU-MDC Summer Research Institute (SRI) Symposium. Over the years, faculty members from both institutions, School of Science, Technology, and Engineering Management of Saint Thomas University (STU) and Miami Dade College (MDC), have partnered to provide undergraduate students hands-on experiences and computational projects that will prepare them for the new challenges of the modern world and the job market. The commitment of the faculty and staff of the School of Science, Technology and Engineering Management has permitted to introduce students into modern techniques of molecular biology, statistical analysis, mathematical modeling, computational physics, bioinformatics, nano sciences and a large variety of characterization techniques. During this last edition, students exchanged experiences with researchers from University of Miami, Florida International University (FIU) and also from NASA,

For the second time, this year, the memories of the SRI will be hosted online by the MOL2NET conference series. It means, that all communications will be published online at Sciforum platform. All presentations will be peer reviewed and a DOI number will be assigned. MOL2NET conference of Sciforum is one of the platforms with international recognition for scientific exchange. The variety of topics included within this new edition acknowledges the inter-disciplinarity, teamwork, and networking of modern science. Feel free to contact researchers and participants to foster more collaboration efforts. St. Thomas University School of STEM is open to new ventures and joint ventures. Interested to see previous edition can follow the link SRI-08 and witness the quality of 38 submissions with a total of 21 publications. The new edition SRI-09 received a total of 27 submissions.

Special thanks to our partner Miami Dade College (MDC) and the grant **STEM-SPACE P03C1160161**. The received contributions provided funding for equipment, logistics, and minor activities associated with the SRI. Ten speakers from and local Technological companies came to St. Thomas to share their wisdom as an in kind contribution.

Thank you for your support!

SRI Chairman

Prof. Dr. David Quesada, Associate Professor of Physics, (MOL2NET Committee)
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Dr. Aliuska Duardo-Sanchez (Law.Lic., Ph.D. Legal Informatics)
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Department of Public Law, University of Basque Country (UPV/EHU),
Campus Biscay, Leioa, 48940, Spain.

Advisory Chair

Prof. Dr. González-Díaz H., IKERBASQUE Professor

(1) Department of Organic Chemistry II, University of the Basque Country UPV/EHU , 48940, Leioa, Biscay, Spain.
(2) IKERBASQUE, Basque Foundation for Science , 48011, Bilbao, Biscay, Spain.
ORCID: https://orcid.org/0000-0002-9392-2797

[SRI09 PHOTO GALLERY]

List of papers (14)

Analysis of microbial communities reflect diel vertical migration in the Gulf of Mexico show abstract
by Dora P Maul *, Claudia Gorbea , Amanda Lobato , Reinaldo Sanchez-Arias , Kevin Boswell , Cole Easson , Jose Lopez

Evaluating medicinal plants for anticancer properties: testing plant extracts for cytotoxicity show abstract
by Alexis Tapanes-Castillo *, Dora P Maul , Luis C Fernandez-Torres , Maria D Pina , Vadym Trokhymchuk , Andrea Peterson , Carlos C Planchart

Differential expression of native potatoes genes in response to drought conditions show abstract
by Diana Martinez , Dora P Maul *, Laynet Cornelio , Olga P Ponce , Yanexis Zarut

Twitter Data Mining and Predictive Modeling in R show abstract
by Reinaldo Sanchez-Arias *, Eliana Espinosa

Creating a Model to Predict Student Success using WeBWorK data show abstract
by Reinaldo Sanchez-Arias *, Jose Muguira

Analysis of the Gentrification Process in the City of Miami show abstract

by Reinaldo Sanchez-Arias *, Sabrina Romero

Biomedical modeling of Magnetic Nanoparticles Fluid Hyperthermia for Cancer treatment show abstract

by David Quesada *, Maria V Barreat

Assessment of Microclimatic conditions of St. Thomas University forest show abstract

by David Quesada *, Kaden Loring

Time series analysis of the EEG signals for Epilepsy seizure forecast show abstract

by David Quesada *, Mang S Cing

Time series analysis and forecast of respiratory conditions in Florida show abstract

by David Quesada *, Paula De la Plaza

Preliminary antioxidant activity analysis of Brazilian pepper tree (*Schinus terebinthifolius*) extracts via TLC, FRAP, and DPPH show abstract

by Alexis Tapanes-Castillo *, Cristina Balistreri , Daniel A Russo , Maria D Pina , Jonathan Brown

Antioxidant properties of oyster plant (*Tradescantia spathacea*) extracts using different methods show abstract

by Alexis Tapanes-Castillo *, Cristina Balistreri , Daniel A Russo , Maria D Pina

Preliminary study of the antioxidant activity of *Vitis rotundifolia* (muscadine grape) extracts using different methods show abstract

by Alexis Tapanes-Castillo , Cristina Balistreri , Daniel A Russo , Maria D Pina *, Trevaun Williams

Assessment of the temperature comfort of a model house show abstract

by David Quesada *, Armando Rios

List of Accepted Abstracts (13)

Antioxidant capacity of *Moringa oleifera* and *Casuarina equisetifolia* show abstract

by Chelsea E Trost , Christine Curiac , Jason Alvarodiaz , Jennifer J Cerda , Luis C Fernandez-Torres *

Antioxidant capacity of *Vitis rotundifolia* and *Petiveria alliacea* show abstract

by Chelsea E Trost , James D Hankemeyer , Luis C Fernandez-Torres *

Antioxidant Capacity of Okinawa Spinach (*Gynura bicolor*) show abstract

by Chelsea E Trost , Christine Curiac , Jason Alvarodiaz , Jennifer J Cerda , Luis C Fernandez-Torres *, Dalyn Valentin

Antioxidant properties of *Hamelia patens* and *Annona muricata* show abstract

by Jason Alvarodiaz , Jennifer J Cerda , Luis C Fernandez-Torres *

Calcium-functionalized biochar for removal of contaminants in water show abstract

by Luis C Fernandez-Torres *, Fransesca Sarmiento , Victoria Slavina

Doped Sn:S thin film absorbers for tin sulfide solar cells show abstract

by Luis C Fernandez-Torres *, Osvy Rodriguez

Evaluating the role of DLGAP1 transcripts in autism biology show abstract

by Alexis Tapanes-Castillo *, Andrea Peterson , Derek Dykxhoorn , Leana M. Ramos , Milagros D Mulero

Gene Expression of Chloroplastic Antioxidant Ascorbate Peroxidases during Dehydration Conditions in Spinach Leaves show abstract

by Dora P Maul *, Cristina Pappaterra

Investigation of the antioxidant properties of the invasive plant Brazilian pepper tree show abstract

by Luis C Fernandez-Torres *, Casey Panella

Iron oxide-functionalized biochar for removal of discarded prescription drugs show abstract

by James D Hankemeyer , Luis C Fernandez-Torres *, Victoria Slavina

Primer design for detection of antioxidant genes Ascorbate Peroxidase and Catalase in *Spinacea oleracea, Camellia sinensis,* and *Daucus carota* show abstract

by Dora P Maul *, Grecia Cotto-Gonzalez

Reforestation of Dade County Slash Pine Urban Forest show abstract

by Richard M Aponte , Alfred Cioffi *, Venansa Denis , Steven Osorio

Synthesis and characterization of cobalt phosphate nanomaterials show abstract

by Kasey L Rivera *, Jaya Borgatta , Robert Hamers

05: WCUCW-01 West Coast University Capstone Workshop, WCU, Miami, USA, 2017

WCUCW-01

NOTES: We suggest you to **download and use** the [Template File] to write your communication. We also strongly recommend you to **read carefully**the [Instructions to authors] about publication model, copyright, authors responsibilities, *etc*.

[REGISTER/LOG IN TO SUBMIT]

Dear colleagues worldwide, we invite you to join us at WCUCW-01 West Coast University Capstone Workshop, 2017, Miami, FL, USA. This is the Capstone workshop for West Coast University (WCU), Miami, FL, USA. This workshop series held each academic term is devoted to fostering both education and research in multidisciplinary sciences. Nursing students completing their General Education courses at WCU form groups of three and take on the roles of scientist, medical researcher, or public health professional, all of them approaching an assigned topic from their field of expertise. They are each required to devise a study on their topic, pretend to conduct it, generate logical data to support a hypothesis, write an academic paper sharing their findings, and defend their data in a presentation.The workshop education goals focus on professors and students in the WCU Miami campus Capstone course. However, we also welcome researchers and students from other nationwide WCU campuses and researchers from other institutions around the world.

The Capstone workshop is associated with MOL2NET-03 International Conference on Multidisciplinary Sciences, MDPI Sciforum, Switzerland, HQ UPV/EHU, Bilbao, Spain. It will therefore **run both online and in person.** The workshop runs online at the platform SciForum maintained by the editorial MDPI, Basel, Switzerland.It runs in person at WCU, Miami campus, during the Capstone course. The **topics of interest** include, but are not limited to, the following: Multidisciplinary Science (all areas), Health Science (all areas), Chemistry (all areas), Biology and Life Sciences (all areas), Environmental Science (all areas), Social Sciences (all areas), Biomedical Engineering, Mathematics (Applied), Data Analysis, Education. See the following note to authors on **topics outside the scope** of the conference and associated workshops [Note on topics].

Steps for Participation

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(1) Submit the title and abstract, select a section or workshop (do not upload paper here) [Submit New Abstract]

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(3) Download template doc and prepare your communication [Template File]

(4) Wait for paper acceptance and publication emails (follow link to proofread your paper, ASAP after upload)

(5) Communicate with chairpersons if corrections are necessary, including past editions (all year)

(6) Log in to post comments, questions, or answers in a section or one of the workshops

(7) Contact chairpersons if you need author (publication) or attendance (posting) certificate for conference and/or workshops (all year)

Notes to Participants

To see a detailed schedule of this workshop/section and of the entire conference follow this link:[MOL2NET workshops schedule]

Preliminary communications are not post-print journal papers. In this sense, committee and/or external reviewers check only scope and apparent scientific soundness. They have the same editorial process as for an online Preprint service. Therefore, all works receive DOI numbers and indexed in GoogleScholar. The works may also receive comments from registered participants (public post-publication review). The authors are encouraged to submit their works to peer-reviewed scientific journals of MDPI or other editorials during or after finalization of the conference, as per SciForum copyright rules.

Comments on previous works are short notes to comment about the more interesting points, highlights, etc. of works previously published by the authors or other groups. In this case, committee and/or external reviewers also only check the scope and apparent scientific soundness. The works may also receive comments from registered participants (public post-publication review).

After publication of papers is closed, we shall open the online platform, and the authors (professors, researchers, and students) will be able to post online comments, questions, and/or answers to comments in this workshop/section and also in the other general sections and/or >10 international workshops of the MOL2NET conference. (Many of them also run both online and in person.)

We strongly recommend that you carefully read the following **notes to participants** about publication model, copyright, authors' responsibilities, *etc.* Be aware, **it is the author's responsibility** to ensure the veracity of the contents(checking similarity to other works) and to carry out a proper citation of previous works. The committee is not responsible of these aspects in this publishing modality. We therefore strongly recommend the authors to use online text-similarity checking services to avoid any form of plagiarism or copyright violation. The authors may be requested to modify the communication (re-write their texts) in the case that high similarity is detected and reported to the committee. In these cases, the manuscript could be put on standby or withdrawn temporarily until the authors re-submit the proper version. The authors are also allowed to submit short reviews, comments, letters, or discussions of papers already published if they guarantee sufficient difference to previous public contents.

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List of papers (6)

Literature and Empathy in Nursing Students show abstract

by Brigitte Fernandez , Melissa Cueto *

Genetics of Hypertension show abstract

by Valerie Puig , Melissa Cueto *

06: INDOWSCI-01; US-EU India Worldwide Workshop on Multidisciplinary Sciences, NSIT, Delhi, India, 2017

INDOWSCI-01; US-EU India Worldwide Workshop Series on Multidisciplinary Sciences, Duluth, USA, 2017

NOTES: We suggest you to **download and use** the [Template File] to write your communication. We also strongly recommend you to **read carefully**the [Instructions to authors] about publication model, copyright, authors responsibilities, *etc*.

[REGISTER/LOG IN TO SUBMIT]

INDOWSCI-01; US-EU India Worldwide Workshop Series on Multidisciplinary Sciences is aimed to become a reference international science workshop series. This workshop is associated to MOL2NET-03 International Conference Series on Multidisciplinary. In fact, the first edition will **run online only** at the online platform SciForum maintained by the editorial MDPI, Basel, Switzerland**.** After publication of papers is closed we shall open the online platform and the author will be able to post online comments and/or answers to comments in this workshop/section and also in the other general sections and/or >10 international workshops of the MOL2NET conference (many of them also run both online and in person). To see a detailed schedule of this workshop/section and all the conference follow this link [MOL2NET workshops schedule].

In any case, you should consider university of University of Minnesota, MN, USA and Netaji Subhas Institute of Technology, New Delhi, India (see chairpersons below) as the two main host institutions.We expect to organize in person workshops in parallel to the online version for the next editions.

INDOWSCI series focused on the interchanges of ideas among India researchers working in India or abroad (U.S., Europe, etc.) with colleagues from other countries across the world. The foundations of this workshop are in the multiple collaborations of Professors / Researchers of Indian researchers like Dr. Suhbash C. Basak, University of Minnesota, USA, Prof. Kunal Roy, Jadavpur University, Dr. Shameer Khader, Philips Healthcare, Cambridge, MA, USA, with their international colleagues at Virginia Commonwealth University (VCU), University of Coruña (UDC), University of Basque Country (UPV/EHU), IKERBASQUE, Basque Foundation for Sciences, and many other institutions in US-EU, India, and other countries.

The workshop is inspired on the Indo-U.S. Workshops series organized by Dr. Suhbash C. Basak during many years, as example of multicultural melting pot devoted to promote interdisciplinary collaborations in science. The works on this initiative started in 1990; the first event was organized in 1998 at Visva Bharati University, West Bengal, India. The Lecture Series was mainly to train young scholars. The third line of events was geared to benefit the South American scientists and young scholars. Two events have been held in Colombia. Some reports of these workshops are: *[J. Chem. Inf. Comput. Sci.*, 1999, 39 (2), pp 179–179], [*J. Chem. Inf. Comput. Sci.*, 2001, 41 (3), pp 479–479], *[J. Chem. Inf. Model.*, 2006, 46 (1), pp 1-1], These workshops included:

(1) Indo-US Workshop on Mathematical Chemistry Series, beginning in 1998 in India and having six more events both in USA and India

(2) Indo-US Lecture Series on Discrete Mathematical Chemistry, four events mainly focusing on the training of young scholars.

(3) Mathematical Chemistry Workshops of the Americas (involving countries of North and South Americas)

This INDOWSCI workshop is associated to MOL2NET-03 International Conference on Multidisciplinary Sciences, MDPI Sciforum, Switzerland, HQ UPV/EHU, Bilbao, Spain. Consequently, it aims to promote results on Multidisciplinary Science (All Areas), including but not limited to, the following **topics of interest:** Chemistry (All areas), Mathematics (Applied), Physics (Applied), Materials Science, Nanotechnology, Biology and Life

Sciences (All areas), Medicine, Biomedical Engineering, Education, along with Computer Sciences, Data Analysis, Statistics, Artificial Intelligence, Deep Learning, Bioinformatics, Systems Biology, and Complex Networks Sciences. See the following note to authors on **topics outside the scope** of the conference and associated workshops [Note on topics].

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(6) Login to post comments, questions, or answers in a section or in one of the MOL2NET workshops (**2017**-Dec-15 to Dec-20)

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Notes to Participants

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Preliminary communications are not post-print journal papers. In this sense, committee and/or external reviewers check only scope and apparent scientific soundness. They have the same editorial process than for an online Preprint service. Therefore, all works receive doi number and are indexed in databases (GoogleScholar, Publons, *etc*.).The works may receive also comments from registered participants (public post-publication review). The authors are encouraged to submit their works to a peer-reviewed scientific journals of MDPI or other editorials during or after finalization of the conference, as per SciForum copyright rules.

Comments on previous works are short notes to comment about the more interesting points, highlights, etc. of works previously published by the authors or other groups. In this case, committee and/or external reviewers check also only scope and apparent scientific soundness. The works may receive also comments from registered participants (public post-publication review).

In any case, **it is the authors responsibility,** to ensure the veracity of the contents, checking similarity to other works, and carry out a proper citation of previous works. The committee is not responsible of this previous aspects in this publishing modality. In this sense, we strongly recommend the authors to use online text-similarity checking services to avoid any form of plagiarism or copyright violation. The authors may be requested to modify the communication (re-write their texts) in the case that high similarity is detected and reported to the committee. In these cases, the manuscript could be put on standby or withdrawn temporarily until the authors re-submit the proper version. The authors are also allowed to submit short reviews, comments, letters, or discussions of papers already published if they guarantee sufficient difference to previous public contents.

Scientific Committee

INDOWSCI Honor Chairpersons

Dr. Suhbash C. Basak, Natural Resources Research Institute, Duluth, MN, USA. **(North America)**

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Prof. Kunal Roy, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata, India. **(Asia)**

Dr. Shameer Khader, Dept. of Medical Informatics and Research Informatics, Northwell Health, NY, USA **(North America)**

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Dr. Anuraj Nayarisseri, Principal Scientist, Eminent Biosciences, Vijaynagar, Indore, Madhya Pradesh, India.

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ORCID: https://orcid.org/0000-0002-9392-2797

List of papers (7)

Intelligent consensus predictor: Towards more precise predictions for external set compounds show abstract
by Kunal Roy *, Pravin Ambure , Supratik Kar , Probir K Ojha

QSAR with ETA indices: Insecticidal activity of plant derived compounds against zika virus vector Aedes aegypti show abstract
by Priyanka De , Rahul B Aher , Kunal Roy *

Inhibition of Secretory Aspartyl Protease of *Candida albicans* by metabolites of *Streptomyces chrestomyceticus* strain ADP4 show abstract
by Vartika Srivastava , Rajeev K Singla , Ashok K Dubey *

***In silico* molecular GRIP docking studies of antioxidant potentials of black tea compounds** show abstract
by Koushik Bhandari , Baishakhi De *

Applications of Structure Based Drug Design Approaches towards Design and Development of Calcium Channel Blockers show abstract
by Vaishali M Patil *, Neeraj Masand

Bioenergy: A Sustainable Energy option show abstract
by S Krishna Sundari *, Saloni Sachdeva , Prakhar Agarwal , Sakshi Awasthi

Ecotoxicological assessment of pharmaceuticals using computational toxicology approaches: QSTR and interspecies QTTR modelling show abstract
by Kabir Khan , Supratik Kar , Hans Sanderson , Kunal Roy *, Jerzy Leszczynski

07: IWIMSM-01: Iberoamerican Workshop on Modelization and Simulation, UCV, Valencia, Spain, 2017

IWIMSM-01: Iberoamerican Workshop on Modelization and Simulation Methods, Valencia, Spain, 2017

[Submit New Abstract]

Welcome Message

We are glad to invite all colleagues worldwide to participate on the workshop IWIMSM-01: Iberoamerican Workshop on Modelization and Simulation Methods, Valencia, Spain, 2017. This is a joint workshop promoted by professors of the Institute of Molecular Science (ICMol) and the Faculty of Pharmacy of the University of Valencia (UV), and the Catholic University of Valencia (UCV), Valencia, Spain. MOL2NET (the conference running title) is the acronym of the lemma of the conference: From Molecules to Networks. IWIMSM-01: Iberoamerican Workshop on Interdisciplinary Modelization and Simulation Methods, Valencia, Spain, 2017. This workshop is associated to the MOL2NET International Conference Series on Multidisciplinary Sciences.

Scope and Target Audience

This conference is in large measure targeted at under-graduated, MSc, and PhD students, as well as Professors, Engineers, and Researchers focused on the application of Computational Modelling and Simulation techniques. The fields of application include, but are not limited to, Medicinal Chemistry, Computational Chemistry, Materials and Nanosciences, Bioinformatics, Biotechnology, Systems Biology, Biomedical Engineering, Environmental Sciences, Social Sciences, Scientometrics, etc. The algorithms used may also come from different areas including, but not limited to, Discrete Mathematics, Mathematical Analysis, Complex Networks Sciences, Statistics, Data Analysis, Artificial Intelligence, Machine Learning, Deep Learning, etc.

Abstract preparation and submission

Before to submit your communication we recommend to download and use the [Template File] to write your communication. We also strongly recommend you to read carefully the **notes to participants** at the following page [Instructions to authors] about publication model, copyright, authors responsibilities, *etc*. Submit your communications in two steps, first submit your title, authors, and abstract here: [Submit New Abstract]. Next, upload your communication after abstract approval.

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(3) Download template doc and prepare your communication [Template File] (until **Dec-15**)

(4) Wait for paper acceptance and publication emails (follow link to proofread your paper) (Asap after upload)

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(6) Log in to post comments, questions, or answers in a section or one of the workshops (**Dec-15** to **Dec-20**)

(7) Contact chairpersons if you need author (publication) or attendance (posting) certificate for conference and/or workshops (All the year)

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Prof. Facundo Pérez Giménez, Department of Physical Chemistry, Faculty of Pharmacy, University of Valencia, Spain.

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Dr. Riccardo Zanni, Department of Physical Chemistry, Faculty of Pharmacy, University of Valencia, Spain.

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Prof. Natalia D.S. Cordeiro, Department of Chemistry and Biochemistry, University of Porto, Porto, Portugal.

Prof. Carolina Horta Andrade, Universidade Federal de Goias, Setor Leste Universitario, Goiania, Brazil.

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(2) IKERBASQUE, Basque Foundation for Science , 48011, Bilbao, Biscay, Spain.
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List of papers (9)

Direct spectrophotometric method to determine cell density of *Isochrysis galbana* in serial batch cultures from a larger scale fed-batch culture in exponential phase show abstract
by Jerónimo Chirivella-Martorell , Ángel Serrano-Aroca *

Enhancement of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)'s properties for advanced industrial applications show abstract
by Ariagna Laritza-Rivera-Briso , Belén Frígols-Garrido , Miguel Martí-Jiménez , Ángel Serrano-Aroca *

Standardization of commercial cinnamon essential oils by gas chromatography-mass spectrometry analysis show abstract
by Antolín Cantó , Maria Amparo Blázquez *

Estudio de la pradera de *Posidonia oceanica* (L.) Delile del área litoral del municipio de Teulada (Alicante). show abstract
by Valero Mario , Jose Tena *, Torres Javier , Royo Miguel

Application of molecular topology to the prediction and optimization of mosquito repellent activity of N-acyl-piperidine derivatives show abstract
by Ramón García-Domenech *, Maria Angeles Martinez Rodriguez , Raimundo Segui Lopez-Peñalver , Gemma Alcacer Tomas , Jorge Galvez ,Maria Galvez-Llompart

Patterns of scuba diver behaviour to assess environmental impact on marine benthic communities: a suitable tool for management of recreational diving on Benidorm island (Western Mediterranean sea). show abstract
by Jiménez-Gutierrez Santiago , Martín Germán , Jose Tena *

Comunidad de poliquetos de los fondos blandos de la playa de Canet d'en Berenguer (Valencia-Spain). show abstract
by Royo Miguel , Torres Javier , Jose Tena *, Valero Mario

An analysis of the multiple choice test evaluation show abstract
by Juan Luis González-Santander *, Germán Martín

Antioxidant activity of 5-FU and new fluorinated uracil derivates show abstract

by Carlos Casanova , Mari Luz Moreno Sancho , María Miranda , Inmaculada Almansa , Antonio Falcó , Mari Ángeles Navarro , Santos Fustero ,Salvador Mérida , Vincent M. Villar *

List of Accepted Abstracts (1)

Retos del empleo de la secuenciación masiva de nueva generación (NGS) de la macrofauna bentónica para la evaluación de la calidad ambiental marina. show abstract

by Javier Torres *

08: LAWSCI-01: Challenges in Law, Technology, Life, and Social Sciences, UPV/EHU, Bilbao, Spain, 2017

MOL2NET2017, LAWSCI01

[SUBMIT HERE] (Select MOL2NET Conference, Section LAWSci)

Dear colleagues worldwide,

We are glad to invite you to participate in the workshop LAWSci, Challenges in Law, Technology, Life, and Social Sciences. This workshop will be held from 25-30 Oct, **2017**, online at Sciforum platform. This is a workshop associated to the MOL2NET International Conference Series on Multidisciplinary Sciences, MDPI Sciforum, Switzerland, HQ UPV/EHU, Bilbao, Biscay, Spain.

LAWSci workshop series promotes multidisciplinary collaborations and debate in the frontiers of Law, Technology, Life, and Social Sciences. The interaction between bio-science and ICTs has forged great developments in many fields. However, the appreciation of these discoveries is sadly, all too often, accompanied by a lack of understanding of the legal implications. This conference series aims to provide a reference to the various legal avenues that are available for the protection of scientific advances, but also the legal instruments to protect society from unwanted effects. It constitutes a study of some of the legal implications of bioscience and ICT advances, weighing their impact on society and the law's role in shaping that effect.

In this sense, the presentations will be focused on legal trends in different fields covering, but not limited to: patentability in plants and human genomics, clinical procedures' standards, patients' personal data protection, informed consent, regulatory issues in drug discovery, biomedical research legislation, toxicology, medico-legal problems such as healthcare malpractice, medical insurance or ethics in medical practice, software protection in chemo-informatics, bioinformatics, medical informatics, and social sciences, taxes in the biotechnology industry and causality/liability in environmental pollution, criminology, *etc*. The conference will run on-line and free, saving traveling and participation costs (subscriptions, open publication, participation in forum, certificates, etc., are **free of cost**).

Publication Model and Authors Responsibility:

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In any case, **it is the responsibility of the authors,** to ensure the veracity of the contents, checking similarity to other works, and carry out a proper citation of previous works. The committee is not responsible of this previous aspects in this publishing modality. In this sense, we strongly recommend the authors to use online text-similarity checking services to avoid any form of plagiarism or copyright violation. Some workshops in this conference series use specialized services to checked for possible text similarity. For instance, MODEC is using the official account of their organizing university for URKUND web server. Please, be aware that the authors may be requested to modify (re-write their texts) the communication

in the case that high similarity is detected and reported to the committee. In these cases, the manuscript could temporarily withdrawn until the authors re-submit the proper version. The authors are also allowed to submit short reviews, comments, letters, or discussions of papers already published if they guarantee sufficient difference to previous public contents.

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by Irina Pérez-Trujillo *

Genetic Manipulation on Plants: Intellectual Property Issues. show abstract

by Anisley Negrín Ruiz *, Lázaro Pino Rivero

Worldwide cyber-attacks: their impact on the international health services and their answer in the Spanish criminal code show abstract

by Iker Conal *

Biotechnology in plants genomics: a legal and bioethics overview. show abstract

by Anisley Negrín Ruiz *, Lázaro Pino Rivero

Challenges in Law, Technology, Life, and Social Sciences show abstract

by Aliuska Duardo-Sanchez *

Criminalistics and its role in prevention show abstract

by Osvaldo Brito Febles *

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by Josefina Chinea Guevara *

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by Graciela García *

Some historic considerations on democracy show abstract

by Mayda Gallardo Villavicencio *

Considerations on contraventions and environmental education show abstract

by Irina Pérez Trujillo *

Analysis of the *ne bis in idem* principle in a court ruling show abstract

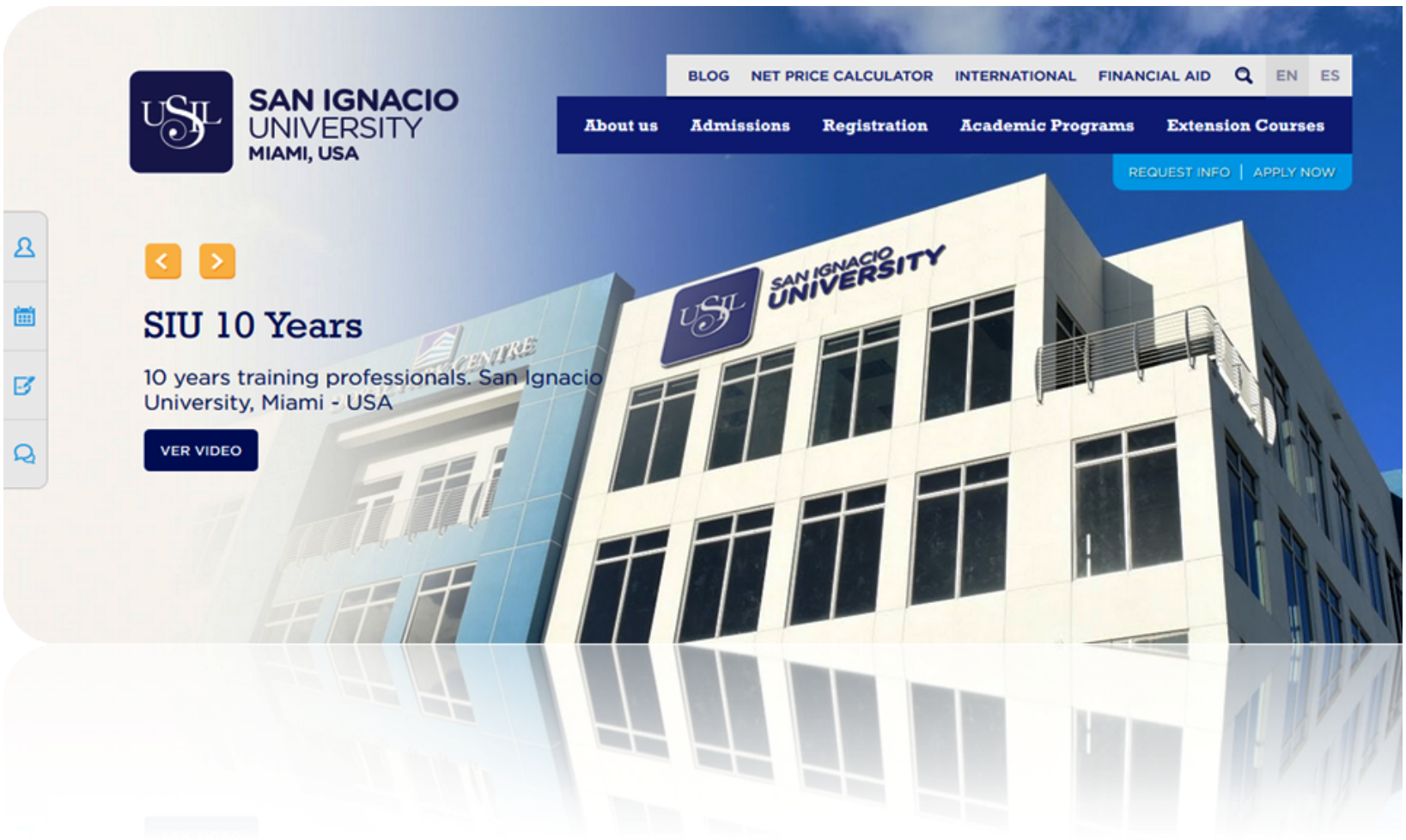
by Sirats Díez-Fernández *

Importance of coaching and mentoring tools for training business leaders show abstract

by Pablo Henrique Delmondes *, Wemerson Borges Afonso

09: SIUSCI-01: San Ignacio University Sciences Workshop, SIU, Miami,USA, 2017

SIUSCI-01



[SUBMIT HERE] (Select MOL2NET Conference, Section SIUSCI)

Dear colleagues worldwide,

We are glad to invite you to participate in the workshop SIUSCI-01. SIUSCI-01 workshop series will be held at San Ignacio University (SIU), FL, Miami, USA. San Ignacio University is an innovative educational institution dedicated to the creation of the leaders of tomorrow accredited by the Accrediting Council for Independent Colleges and Schools (ACICS). The present workshop focus on topics of multidisciplinary sciences relevant to the interests of SIU students and professors. SIUSCI-01 is also devoted to strength the collaborations and networking between SIU professors and students with other students and researchers in FL education system and worldwide.

In this sense, SIUSci-01 is a workshop associated to and hosted online by the MOL2NET Conference Series on Multidisciplinary Sciences, MDPI Sciforum, Basel Switzerland. This means that all communications are going to be published online at Sciforum platform. All presentations will be peer reviewed and a DOI number will be assigned. MOL2NET conference of Sciforum is one of the platforms internationally recognized for scientific exchange. This annual edition is full of diversity in topics, approaches, and integration of disciplines, representing one of the common paradigms of modern science, interdisciplinary teamwork, and networking. I hope you will enjoy the program and the presentations.

Publication Model and Authors Responsibility:

Before to submit your work be aware that the editorial process is the same than for a **PREPRINT** service. Therefore, all works receive doi number and are indexed in databases (GoogleScholar, Publons, *etc*.). However, the works published here are **preliminary communications and not post-print journal papers**. In this sense, committee and/or external reviewers check only scope and apparent scientific soundness. The works may receive also comments from registered participants (public post-publication review). The authors are encouraged to submit their works to a peer-reviewed scientific journals of MDPI or other editorials during or after finalization of the conference, as per **SciForum copyright rules**.

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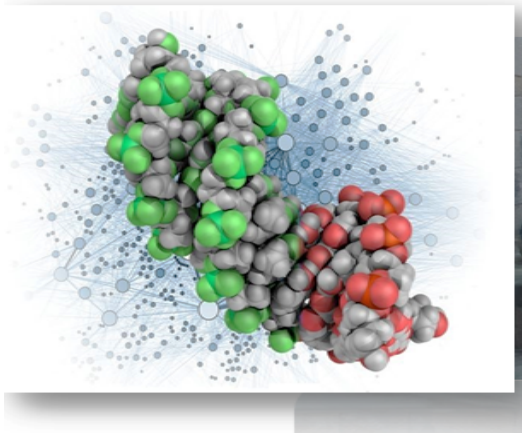
10: EJIBCE-01; Meeting of Young Researchers in Structural Computational Biology, UC, Coimbra, Portugal, 2017

EIJCBE-01



EJIBCE 2017

[REGISTER/LOG IN TO SUBMIT NEW ABSTRACT OR COMMUNICATION HERE]

About

The Meeting of Young Researchers in Structural Computational Biology (EJIBCE 2017) aims at bringing together the Portuguese scientific community working in the field in a free-of-charge meeting, to provide a forum for discussion and sharing with no strings attached. This third edition builds on the success of previous editions in Porto, Lisbon, Coimbra and Oeiras, which gathered ca. 60 participants and had over 10 oral communications in each edition.

Mission & Objectives

Given the current economic situation in Europe, and particulaly in Portugal, it is increasingly harder to obtain funding and have an optimistic approach to science and collaborations. Besides, the recent austerity measures put in place by the Portuguese government led many researchers to join groups and institutes abroad, and eventually lose touch with the Portuguese scientific community and developments.

After obtaining a doctoral degree or finishing a postdoctoral appointment, many Portuguese scientists abroad look back to their country looking for an opportunity to continue their careers and contribute to the development of Portuguese science. Others wish to remain abroad, but at the same time reach out to the Portuguese community and cultivate stronger ties to its researchers. However, what groups are there on Computational Structural Biology? What do they actually focus on? Are they open to collaborations?

This meeting was created in order to answer these and other similar questions. Its main goal is to provide a simple no-frills environment where Portuguese researchers in Computational Structural Biology, active both in Portugal and abroad, can meet and get to know each other's research and ambitions. Ultimately, we hope that in doing so, we will be able to stimulate new collaborations and broaden the horizons of the Portuguese community of Computational Structural Biologists.

EJIBCE 2017

EJIBCE 2017 builds up on the format of previous editions, focusing on a small number of oral communications interspersed with several breaks to encourage discussion and exchange of ideas. Throughout the day, 11 researchers will present their results. Seven will be selected from the submitted abstracts by the Scientific Committee, based on the quality and originality of the work, and will be given 15 minutes to speak, followed by a short discussion. The remaining 4 oral communications will be delivered by invited keynotes, who will be given 30 minutes to speak plus 10 for discussion. The program is available as a Google Calendar [Here].

This workshop is associated to the MOL2NET-03 International Conference Series on Multidisciplinary Sciences. This is a year-round conference series with multiple associated workshops worldwide (online and in person). In fact, This year's edition will run both, in person at University of Coimbra Physic's Department and online at the web platform SciForum maintained by the editorial MDPI, Basel, Switzerland**.** It means that, after publication of all papers of all the workshops associated to the conference we shall open the online platform and the authors of general sections and all workshops will be able to post online comments and/or answers to comments in this workshop/section and also in the other general sections and/or workshops. To see a detailed schedule of the conference follow this link [MOL2NET workshops schedule].

Guest Speakers

João Ribeiro received his diploma degree in Bioinformatics in 2009 from the Faculty of Biotechnology, Portuguese Catholic University, and his Ph.D. in Sustainable Chemistry in 2014 from the Faculty of Science, the Faculty of Pharmacy and the Institute of Biomedical Sciences Abel Salazar of University of Porto in association with the Nova Lisboa University, advised by Prof. Maria J. Ramos. During his Bioinformatics degree, he had the opportunity to combine Biology and Biochemistry with software development to produce user-friendly Bioinformatics tools. The pursuit for the integration of Biochemistry and software development followed João to his Ph.D studies, where he developed third-party tools (plugins) for the widely used molecular visualization program VMD, developed by Klaus Schulten's Theoretical and Computational Biophysics (TCB) group. These plugins aimed to assist non-expert users of Computational Chemistry software at different stages of the computer-aided drug design process, such as molecular docking, protein mutagenesis and molecular dynamics simulation analysis. João joined the TCB group in 2014 to employ molecular dynamics simulation to study the cellulose degradation process for biofuel production while keeping the software development always present in his work. He is currently the main developer of the newest VMD plugin to assist MD novice users in the preparation, execution and analysis of MD simulations, QwikMD. This plugin smooths the initial learning curve imposed by the MD programs and allows more advanced users to speed-up tedious structure preparation procedures.

João M. Damas is a biochemist from background (Universidade de Lisboa, 2007) that decided to follow computational molecular biophysics. During more than eight years he was a researcher in the Protein Modelling laboratory (www.itqb.unl.pt/pm), under the supervision of Prof. Cláudio M. Soares, where he specialized in molecular dynamics and related techniques and applying them to understanding biomolecules at the molecular level. One year and a half ago, João joined Acellera Ltd. (www.acellera.com), a company focused on software and hardware solutions for computerized drug discovery, where he is a Researcher and Software Developer. He is one of the main developers and maintainers of HTMD, a Python package to prepare, handle, simulate, visualize and analyze molecular systems, as well as of other Acellera software, such as ACEMD, AceCloud, and AceFlow.

António Ribeiro is a postdoctoral researcher in bioinformatics and computational chemistry at the European Bioinformatics Institute (EBI), in Cambridge. António finished his biochemistry master's degree in 2009 at the Faculty of Sciences of the University of Porto. His master thesis was developed in the computational biochemistry group under the supervision of Professors Maria João Ramos and Pedro Fernandes, and focused on the application of computational methods (QM and QM/MM) to study chemical and enzymatic reactions. His PhD thesis, finished in 2013, was done in part at the same group, but also at the University of Calabria, with Professor Nino Russo. The thesis described the computational study of the catalytic mechanism of several enzymes, namely HIV-1 Integrase and Protein Phosphatase 5. After the PhD, António used other computational methods like virtual screening and free energy calculations to identify potential hit compounds for HIV-1 Integrase. Since June, 2016, he is part of Professor Janet Thornton's group at the EBI, where he is developing a database of enzyme mechanisms and catalytic sites. This database (https://www.ebi.ac.uk/thornton-srv/m-csa/) will be used to understand enzymatic function and enzyme evolution.

João M. Martins graduated with a Bachelor's degree in Chemistry with the University of Porto in 2011, having started his work in computational structural biology during his undergraduate studies. He later obtained the degree of master in Chemistry from the same university, researching quantum chemistry and theoretical structural biology during his Master's thesis work. Working under Prof. Kresten Lindorff-Larsen, he obtained the PhD degree in Biochemistry in 2017 with the Structural Biology and NMR lab (SBiNLab) of the University of Copenhagen. His work has been focused on protein-protein interfaces, hotspot theory and small molecule docking under the supervision of Irina Moreira while at the University of Porto and mutation studies through free energy perturbation and integration of experimental data in protein simulation. Currently, he works as a postdoctoral research scientist at the The Image Section of the University of Copenhagen computer science department. His research focuses on protein structure and function studies through machine learning and neural networks' methods.

Statement of Responsibility

questions should be addressed to the organization by email (ejibce@gmail.com). Registration to this event implies acceptance of these conditions.

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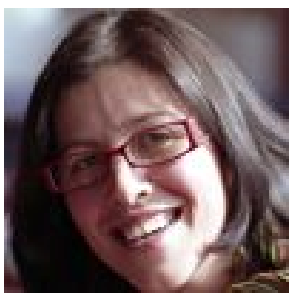

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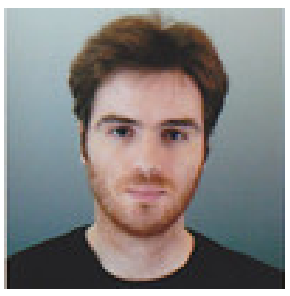

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by António Ribeiro *, Gemma L. Holliday , Nicholas Furnham , Janet M Thornton

***In silico* studies on the pH induced membrane insertion of pHLIP peptides** show abstract

by Tomás Silva *, Diogo Vila-Viçosa , Yana K. Reshetnyak , Oleg A. Andreev , Miguel Machuqueiro

"*In silico*" estimation of encapsulation-induced pK_a shifts in drugs. show abstract

by Diogo Reis *, Miguel Machuqueiro , Diogo Vila-Viçosa

Enhancing protonation sampling via a pHRE replica exchange scheme show abstract

by Diogo Vila-Viçosa *, Pedro B. P. S. Reis , Chris Oostenbrink , Miguel Machuqueiro

Structural mechanism of HER2-antibodies complexes by molecular dynamics studies show abstract

by Rita Melo *, Jose G. Almeida , Sandra Cabo Verde , Zeynep H. Gumus , I. S. Moreira , J. D.G. Correia

Improving pKa calculations of membrane inserting amino acids using replica exchange CpHMD simulations show abstract

by Pedro b. p. s. Reis , Diogo Vila-Viçosa , Miguel Machuqueiro *

Effect of protonation state on the interaction of Hoechst 33342 with lipid membranes – An experimental and computational study. show abstract

by Patrícia Santos , Hugo Filipe *, João P. Prates Ramalho , Luís M. S. Loura , Maria João Moreno *

Using big-data to understand the protein interface landscape show abstract

by José G. Almeida *, Alexandre M. J. J. Bonvin , Irina S. Moreira

Creating a valid *in silico* Dopamine D2-receptor model for small molecular docking studies show abstract

by Beatriz Bueschbell *, António J. Preto , Carlos A. V. Barreto , Anke C. Schiedel , Irina S. Moreira

Structural and dynamic understanding of the ghrelin receptor high constitutive activity show abstract

by Carlos A.V. Barreto *, Miguel Machuqueiro , Alexandre M.J.J. Bonvin , Irina S. Moreira

Mapping the conformational and structural regulators involved in the inhibition of the human 20S proteasome inhibitors show abstract

by Pedro M. P. Fernandes *, Bruno L. Victor , Rita C. Guedes

Alpha-helical and beta-sheet membrane- membrane protein dimers: centralizing information show abstract

by Pedro Matos Filipe *, António José Preto , Panos Koukos , Alexandre Bonvin , Irina Moreira

Characterization of the membrane permeability of different proteasome inhibitors using molecular dynamics methods show abstract

by Bruno Victor *, Pedro M. P. Fernandes , Romina A. Guedes , Rita C. Guedes

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by Pedro Renault *, Jacky Marie , Jean-Louis Banères , Nicolas Floquet

Quantum Chemical Modeling of Citrate Synthase reaction mechanism show abstract

by Eduardo Oliveira *

11: EHUDW-01: First EHU-DELFIN Programme Workshop, UPV/EHU, Bilbao, Spain, 2017

EHUDW-01: First EHU-DELFIN Programme Workshop, Bilbao, Jul, 2017

The UPVEHUDW01: First UPV-EHU DELFIN Program Workshop, Bilbao, Jul, 2017 is a scientific and educational workshop organized by professors of the University of the Basque Country (UPV/EHU) and IKERBASQUE, Basque Foundation for Sciences. The workshops is associated to the MOL2NET International Conference Series on Multidisciplinary Sciences, MDPI Sciforum, Basel, Switzerland.

The workshop aim is to promote the scientific, educational, and cultural interchange of professors and students of UPV/EHU with students of the international education network DELFIN, Mexico. The DELFIN program was created in 1995 with the aim of strengthening the collaborative culture between the Higher Education Institutions and Research Centers that are members of the Program, through the mobility of professors-researchers, students and the dissemination of scientific and technological products. In particular to strengthen the development of research and national graduate.

Specifically, student mobility is promoted through academic research stays, within the framework of the Summer of Scientific and Technological Research of the Pacific. This mobility program strengthens the vocation of young people for science and technology and influences their decision to join postgraduate programs at home and abroad.

EHUDW-01 Steering Committee

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Department of Odontology II

José Manuel Aguirre Urizar
Eduardo Ginestal Gómez

Department of Pharmacology

Nerea Jauregizar Albonigamayor

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by Cindy Neyel Rojas González *

Comparative Study of Invasive Candidiasis in Mexico and Spain show abstract
by Diana Franco *, Guillermo Quindós Andrés *, Iñigo De La Fuente , Laureano Ribacoba , Manuel Fernández Rodríguez , Andrea Guridi , Elena Sevillano , Elena Eraso

Overview of PT-QSRR models for predicting yield of reaction show abstract
by Ramiro Corona-Jiménez *

Editorial: EHUDW01, First EHU-DELFIN Program Workshop, Bilbao, Jul, 201 show abstract
by Susana Serrano-Gazteluurrutia *, Guillermo Quindós *, Elena Eraso *, Aliuska Duardo-Sanchez

Study of microbiological laboratory techniques for the etiological diagnosis and guidance in the treatment of invasive candidiasis show abstract
by Amanda Patricia Solís Gutiérrez *, Katherine Miranda Cadena , Iñigo De la fuente *, Cristina Marcos Arias *, Estibaliz Mateo *, Andrea Guridi *, Elena Sevillano *, Lucila Madariaga *, Elena Eraso *, Guillermo Quindós *

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by Diana Franco *

Oral diseases and microorganisms associated in people with intellectual disabilities: A comparison between Spain and Mexico show abstract
by Paola De Anda *, Eduardo Ginestal *, Iñigo De La Fuente *, Iratxe Aspiazu *, José Manuel Aguirre *, María Ángeles Echebarria *, Elena Eraso *, Guillermo Quindós *

12: MODEC-02: Workshop on Natural Products and Agro-Industrial Procesess in Amazon, UEA, Puyo, Ecuador, 2017

MODEC, 2nd Edition

[Workshop Editorial & Full Committee]

MODEC 2017 (Submissions Open)

SUBMIT TO MODEC HERE!!!

NOTE ON PUBLICATION MODEL: *Before to submit your work be aware that the editorial publication model of this workshop is similar to a PREPRINTservice. It means that works presented here have to be considered only as preliminary communications and not as final post-print versions of journal papers. In this sense, even when all the works published are revised by at least one member of the committee and/or external reviewer this level of revision checks only apparent scientific soundness and general scientific interest. In a second level, collective post-publication review, the works published may receive comments (published in the form of posts) from all the participants registered in the conference. The authors are prompted to use all these opinions to write the full versions of their works and submit them to publication in a peer-reviewed scientific journals according to SciForum copyright rules. In the specific case of MODEC workshop all papers will be checked for possible similarity using the official count of the organizing university for URKUND web server. In any case, the authors are the only one responsible of the veracity of the contents, checking similarity to other works, citing properly previous works, etc.*

MODEC2017, International Workshop on the Natural Products and Agro-Industrial Processes in Ecuadorian Amazon region

Welcome to the MODEC2017 workshop. This is Amazon State University's (UEA) first workshop, devoted to the promotion and application of the Multidisciplinary Sciences to the development of natural products and agro-industrial processes in Ecuadorian Amazon regions.

This workshop runs both online and on location at the university. The online portion of the workshop is powered by the SciForum platform of MDPI, hosted by the MOL2NET International Conference Series on Interdisciplinary Sciences.

Additionally, the physical component of the workshop is also scheduled to run through the Department of Earth Sciences (Facultad de Ciencias de la Tierra) at Universidad Estatal Amazónica (UEA), Puyo, Ecuador. Please, contact the workshop chairmen for further details. Paper submission is already open and the publication of papers for conference purposes in SciForum platform is free of cost.

Publications are expected to be short papers consisting of 1-3 pages. Be aware that the submission is a two step process. First you must register and submit a tentative title, authors list and abstract. Next, you need to submit your full publication upon acceptance of the abstract by the committee. Full publications will be published online, free of charge, with doi number as soon as possible after acceptance. If you are planning to submit a publication, please use the following template, MODEC Template.doc. Click on the following link to register and/or submit your communication Sumit to MODEC here.

All submitted papers should fall under one of the following categories:

- Computational chemistry, Cheminformatics, and Bioinformatics
- Mathematical modeling
- Organic and Functional Foods
- BioTrade: Natural Products of the Amazon
- Production systems with agro-business and forestry purposes or biomass for energy purposes
- Environmental impacts
- Agro-industrial development processes

Schedule:

- Abstracts submission until 2017-Oct-01, Envio de los abstract hasta el 01 de octubre de 2017.
- Abstract acceptance until 2017-Oct-03.

- Communications submission until 2017-Nov-01.
- Communications acceptance until 2017-Nov-15.
- Communications publication until 2017-Nov-30.

Please, click the following link to read the editorial paper with welcome message and call for papers as well as the full description of the workshop with details of topic, short biographies of the members of the committee, contact details, personal pictures, etc., [Workshop Editorial & Full Committee]

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Prof. Amaury Pérez Martínez received his Bs. in Chemical Engineering in 2002, his MSc degree in Chemical Engineering Processes and Analysis in 2007, and his PhD degree in Chemical Engineering in 2013. He has worked in the development of new technology in the Chemical Industry. Currently, he is an Assistant Professor of Agro industrial Engineering at Amazon State University (UEA) in Puyo, Ecuador. His research interests include philosophy of process design, simulation, and sustainable development

Institution: Universidad Estatal Amazónica, Ecuador

Email: amperez@uea.edu.ec

Honoray Chairman

Prof. Dr. C. Julius Caesar Vargas Burgos, Full Professor and Rector of Amazon State University (UEA), Puyo, Ecuador

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Technological proposal for a garlic-derived inulin extraction process show abstract
by Marco Vinicio Lara Fiallos *, Maria Caridad Julian Ricardo , Mario Romeo Calle Miñaca , Pedro Miguel Barba Estrella , Reynerio Álvarez Borroto, Isnel Benitez Cortés , Pascual Alfredo Lara Gordillo , Amaury Pérez Martínez *

Quality indicators in *Ochroma pyramidale* seeds from three sites in the Ecuadorian Amazon for reforestation in degraded areas show abstract
by Yudel García *, Yasiel Arteaga , Reinier Abreu , María de Decker , Yamila Lazo

The water relations of *Inga multinervis* for efficient water use in forest systems show abstract
by Yasiel Arteaga *, Yudel García , Reinier Abreu , María de Decker , Dunia Chávez , Yamila Lazo

Ethnopharmacology, biological activity and chemical characterization of *Mansoa alliacea*. A review about a promising plant from Amazonian region show abstract
by Angélica Tasambay Salazar *, Laura Scalvenzi , Andrea Stefany Piedra Lascano , Matteo Radice

Amazonian fruits availability as fruits agro-alimentary chain primary elements of Pastaza (Puyo), Ecuador. show abstract
by Neyfe SAblòn Cossìo *, Ruth Irene Arias Gutiérrez , Manuel Lázaro Pérez Quintana , Fabiola Sánchez , Horacio Bautista

Polyphenols extracts from (*Theobroma cacao*) and Chuchuhuaso (*Maytenus macrocarpa*) as possible natural Amazonian antioxidant show abstract
by Manuel Lázaro Pérez Quintana *, Luis Silva , Matteo Radice , Luis Bravo , Janeth Sánchez , Andrea Riofrio

Antiproliferative activity of *Psidium guajava* essential oil: a preliminary study show abstract
by Matteo Radice *, Matteo Chiurato , Alessandra Guerrini , Francesco Lozupone

Stochastic model on the mobility of petroleum in the soils of the Ecuadorian Amazon show abstract
by Marco Heredia *, Maria de Decker , Valeria Villalta , Mayra Vargas

Rural community's territorial system dynamics at the Anzu river valley in the Kichwa Amazon territory to propose change scenarios show abstract
by Ruth Irene Arias Gutiérrez *, Angelina Herrera Sorzano , Manuel Lázaro Pérez Quintana , Roberto González Sousa , Elisa López

The market of *Ilex guayusa*. Products, stakeholders, prototypes and trends in the Ecuadorian Amazon Region. show abstract
by Nancy Elizabeth Lema Paguay , Maite Ximena Reinosa Galora , Yuri Danny Abad Cordero , Tania Marobel Heras Calle , Matteo Radice , Neyfe SAblòn Cossìo *

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by Sávio Benvindo Ferreira *, Tassiana Barbosa Dantas , Daniele de Figuerêdo Silva , Edeltrudes de Oliveira Lima

Design of innovative products from elements of the Ecuadorian Amazon show abstract
by KARLA LILIANA SALAGATA TIRADO , Leidy Paola Pico Poma , Lessly Estefania Ramírez Herrera , Johnny Osvaldo Guevara Ocampo , Anthony Ordoñez Barros , David Sancho Aguilera , Neyfe SAblòn Cossìo *

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by Carlos Alfredo Bravo Medina *, Bolier Torres , Reinaldo Alemán , Daysi Changoluisa , Haidee Marín , Hector Reyes , Henry Navarrete

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by Reinaldo Aleman *, Carlos Bravo , Jorge Antonio Freile , Javier Dominguez , Derwin Viafara , Jorge Luis Alba , Edgar Ruben Iza

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by Edwin M. Collahuaso González , Amaury Pérez-Martínez , Julio Abel Loureiro Salabarría , Karel Diéguez-Santana *

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by Karel Diéguez-Santana *, Sheyla F Pintado , Ronny G Tomalá , Gabriela Moreno Samaniego , Katherin Gaibor , Mayra Zambrano , Robinson J. Herrera Feijoo , Henry Navarrete Pico

Affectation of *thrips palmi* in the quality and production of the naranjilla in the Ecuadorian Amazon. show abstract
by Edgar Ruben Chicaiza Reisancho *, Luis Antonia Diaz-Suntaxi , Santiago Aguiar-Novillo , Pablo Ernesto Arias , Patricio Ruíz Marmol , Jose Escobar-Machado , Christian Guillen

Use of cocoa polyphenols (Theobroma cacao) as natural Amazonian antioxidant in the elaboration of sausage fresh show abstract
by Luis Antonio Silva *, Manuel Pérez , Matteo Radice , Luis Bravo , Janeth Sánchez

Cocoa polyphenols (*Theobroma cacao*) as natural Amazonian antioxidant in sausage fresh. show abstract
by Luis Antonio Silva *, Manuel Pérez , Luis Bravo , Matteo Radice , Janeth Sánchez , Marco Andino

Chemical Characterization of The Agroindustrial By-Products Destined for Pig Feeding show abstract
by Santiago Aguiar-Novillo *, Edgar Ruben Chicaiza Reisancho *, Willam Caicedo-Quinche , Luis Antonia Diaz-Suntaxi , Pablo Ernesto Arias ,Patricio Ruíz Marmol , Jose Escobar-Machado , Christian Guillen

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by Luis Antonia Diaz-Suntaxi *, Edgar Ruben Chicaiza Reisancho *, Segundo Valle , Pablo Ernesto Arias , Santiago Aguiar-Novillo , Patricio Ruíz Marmol , Jose Escobar-Machado , Pedro Lopéz- Trabanco

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by CHRISTIAN CORDOVA *

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by Danae Cañadas *

Formulation and processing of gluten and lactose-free mulberry ice cream based on soybeans and an average sugar content show abstract
by fernando Sánchez *

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by vinicio silva *

Development of a nutraceutical drink based on lacto serum and pitahaya pulp show abstract
by JESUS RAMON LOPEZ *

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by Irma Lucia Cañadas Salazar *

Application of a mathematical model for estimating the useful life of the tomato tree (*Solanum betaceum*) using a combination coating with sande latex (*Brosimun utile*). show abstract
by Vicky Solórzano *

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Commercialization and direct sale of yogurt frozen in the amazonia, with aesthetic presentation in the shape of ice cream and with accompaniment of tropical fruits. show abstract
by EDISON ERNESTO ZUÑIGA *

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Mathematic modeling to optimize the removal process of straction of the poliophenolic compound of Guayusa (Ilex guayusa) show abstract
by Darío Rafael Hidalgo Nuñez *

Mathematical model for the preparation of a slimming drink with apio tomato flavor (Apium graveolens), ginger and apple vinegar, endless with stevia (stevia rebaudiana bertoni) and bee honey. show abstract
by Paola Alexandra Velastegui *

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by ADRIANA ISABEL RODRÍGUEZ *

Mathematical modeling for the extraction of oil from the Amazonian fruit PITON Grias neuberthii L show abstract
by Derwin Viáfara Banguera *

Mathematical Modeling for the Study of Meat Preservation of *Colossoma macropomum* show abstract
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Mathematical modeling in the fermentation process to obtain the biol from the liquid residues (blood, ruminal content, etc.) coming from the municipal camal of the city of Macas
by OLIVA SUAREZ *, Amaury Pérez Martínez , Karel Dieguez Santana
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Mathematical modeling of production citric acid from sugar cane bagasse using native *Aspergillus niger* from Ecuadorian Amazon
by Henrry Tuquerres *
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Mathematical modeling of the calorific value by means of bromatological analysis and calorimeter bomb of the sawdust of Pigüe (*Pollalesta discolor*).
by Janeth Miryam Cárdenas *
show abstract

Modeling a diagnosis to the Kallari biocontrolling plant as a quality assurance system based on the application of bpm (good manufacturing practices) in the production of cured vanilla and powder.
by Johnatan Cardenas *
show abstract

Modeling and simulation process of extraction essential oil of Cinnamon by steam witch vapor
by Wilson Noe Aldaz *
show abstract

Modeling of the dairy Agroindustrial chain of the province of Chimborazo case canton Guano year 2017
by Ramiro German Jaramillo *
show abstract

Modeling of the drying process of Garlic Sacha leaves for Agroindustrial use
by GENOVA CABRERA *
show abstract

Modeling of ultrasonic assisted extraction to obtain a polyphenolic extract with high antioxidant activity from the paratima cecropiifolia Martius (monte grapes).
by Jorge Julio Reyes Mera *
show abstract

Use of garlic of the mountain (Mansoa Aliácea), in the elaboration of Spanish chorizo
by Mauricio Brito *
show abstract

13: WRSAMC-02: Workshop in Medicinal Chemistry, UFPB, Paraiba, Brasil, 2017

WRSAMC2017, 2nd Edition

NOTES: Before to submit your communication we strongly recommend to read carefully the [Notes to Authors] about, participation modalities, publication model, copyright, authors responsibilities, *etc*.

Submit new abstract

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NOTES: MOL2NET is a year-round conference series with multiple associate workshops worldwide. As is the conference is running and open to submissions almost all the year, please read [Workshops/Sections Schedule]. We suggest you to **download and use** the [Template File] to write your communication. We also strongly recommend you to **read carefully** the [Instructions to authors] about publication model, copyright, authors responsibilities, *etc*.

[Facebook] [2015] [2016] [Welcome Videos] [Mandarin, 官话] [Hindi, हिन्दी] [Euskera] [Castellano] [Português] [Français]

Welcome Message from Chairs

Dear all we are glad to welcome you to the workshop in research sciences applied in medicinal chemistry 2017, organized by researches from Department of Chemistry, Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos (PgPNSB - Postgraduate Program in Natural Products and Synthetic Bioactive) and Hospital Universitário of Universidade Federal da Paraíba (UFPB). This year the WRSAMC *is dedicate to the* PgPNSB 40th Anniversary.

This is workshop also is associated to MOL2NET-2 International Conference on Multidisciplinary Sciences. Consequently, the present page of the conference will serve as both: (1) publication media for communications, posters, or plenary conferences (videos) and also (2) online discussion media to post comments, questions, and answers about the workshops, respectively. Participants on both events, **online discussions** at the end of the year and workshop along the year, are entitled to receive attendance certificates from both the WRSAMC workshop and the MOL2NET conference. The WRSAMC will be held from **11-14 December 2017,** online via the platform SciForum.
The online submission is now open and the publication of communications will be ASAP after acceptance.

Abstract/Paper submission until: **December 4, 2017**

Paper acceptance until: **December 5, 2017**

Notes to Participants

Submission process has the following steps: 1-Registration, 2-Submission of abstract, 3-Wait for abstract acceptation email, 4-Upoload of full communication, 5-Wait for communication approval/publication emails. After you successfully register, you can submit your paper online. Communications are expected to be short papers 1-3 pages. Please, donwload and use the following template WRSAMC template to submit your communication.

Be aware:

on step 1, you should select MOL2NET 2017, International Conference on Multidisciplinary Sciences, 3rd edition (Conference).

on step 2, you should select WSRAMC2017 workshop in research sciences applied in medicinal chemistry.

Before to submit your work be aware that the editorial publication model of this workshop is similar to a PREPRINT service. It means that works presented here have to be considered only as preliminary communications and not as final post-print versions of journal papers. In this sense, even when all the works published are revised by at least one member of the committee and/or external reviewer this level of revision checks only apparent scientific soundness and general scientific interest.

In a second level, collective post-publication review, the works published may receive comments (published in the form of posts) from all the participants registered in the conference. The authors are prompted to use all these opinions to write the full versions of their works and submit them to publication in a peer-reviewed scientific journals according to SciForum copyright rules. Some workshops will use specialized services to checked for possible similarity. In any case, **the authors are the only one responsible for** the veracity of the contents, checking similarity to other works, and proper citing or under-citing of previous works, etc.

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Prof. Marcus Tullius Scotti, Departamento de Química, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba - Campus I, Cidade Universitária, CEP: 58.051-900 João Pessoa - Paraíba - Brasil, Emails: mtscotti@gmail.com, mscotti@ccae.ufpb.br

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M.Sc. Yuri Mangueira Do Nascimento, Instituto de Pesquisa em Farmacos e Medicamentos, Universidade Federal da Paraíba - Campus I, Cidade Universitária, CEP: 58.051-900, João Pessoa - Paraíba – Brasil.

Advisory Chairman

Prof. González-Díaz H., IKERBASQUE Professor, Email: mol2net.chair@gmail.com
(1) Department of Organic Chemistry II, University of the Basque Country UPV/EHU , 48940, Leioa, Biscay, Spain.
(2) IKERBASQUE, Basque Foundation for Science , 48011, Bilbao, Biscay, Spain.
ORCID: https://orcid.org/0000-0002-9392-2797

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by Chonny Herrera-Acevedo , Luciana Scotti , Marcus Tullius Scotti *

Protein model built through molecular modeling by homology of a potential target of anti-leishmania drugs show abstract

by Mayara Maia *, Marcus Tullius Scotti *, Luciana Scotti , Chonny Acevedo

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by ÉLIDA CAVALCANTI *, MARCUS TULLIUS SCOTTI *, LUCIANA SCOTTI , VICENTE EMERECIANO

***In silico* evaluation of spiro-acrydine derivatives as potential inhibitors of the receptor of human epidermic growth factor (HER2)/ Epidermic Growth Factor receiver (EGFR)** show abstract

by Vanessa Serafim , Luciana Scotti *, Marcus T Scotti

14: UCHCEUW-14: 14th UCH-CEU Workshop on Multidisciplinary Sciences, UCH, Valencia, Spain, 2017

UCHW01: UCH Workshop on Multidisciplinary Sciences, Valencia, Spain, 2017

SUBMIT TO THIS WORKSHOP

Dear all we are glad to welcome you to the WUCH01: UCH Workshop Series on Multidisciplinary Sciences, Valencia, Spain, 1st edition. This is one scientific conference series for professors, researchers, and young scientists; with special emphasis on the active participation of graduated students of the CEU Cardinal Herrera University (UCH), Valencia, Spain. The main source of papers of this workshop are the results presented by the professors and students of the UCH on their 14th Students International Congress. This congress was hold in person on July 2016 at UCH, Montcada, Valencia, Spain.

However, the workshop also welcomes online participation (posts) and/or submissions (2-3 pages long communications) from all colleagues worldwide to be published online. The submission is open and the publication of communications will be ASAP after acceptance, all the year; to submit a communication use the Submission link. After you successfully register, you can submit your paper online. You need to register and send your abstract first. For more details please contact the chairperson of this workshop by email. This is workshop is associated to MOL2NET-03International Conference on Multidisciplinary Sciences, MDPI Sciforum, Switzerland, HQ UPV/EHU, Bilbao, Spain.

Be aware:

on step 1, you should select **MOL2NET 2017, International Conference Series on Multidisciplinary Sciences, 3rd edition**

on step 2, you should select Section: UCHW01: UCH Workshop on Multidisciplinary Sciences

Publication Model and Authors Responsibility:

Before to submit your work be aware that the editorial process is the same than for a **PREPRINT** service. Therefore, all works receive doi number and are indexed in databases (GoogleScholar, Publons, *etc*.). However, the works published here are **preliminary communications and not post-print journal papers**. In this sense, committee and/or external reviewers check only scope and apparent scientific soundness. The works may receive also comments from registered participants (public post-publication review). The authors are encouraged to submit their works to a peer-reviewed scientific journals of MDPI or other editorials during or after finalization of the conference, as per **SciForum copyright rules**.

In any case, **it is the responsibility of the authors,** to ensure the veracity of the contents, checking similarity to other works, and carry out a proper citation of previous works. The committee is not responsible of this previous aspects in this publishing modality. In this sense, we strongly recommend the authors to use online text-similarity checking services to avoid any form of plagiarism or copyright violation. Some workshops in this conference series use specialized services to checked for possible text similarity. For instance, MODEC is using the official account of their organizing university for URKUND web server. Please, be aware that the authors may be requested to modify (re-write their texts) the communication in the case that high similarity is detected and reported to the committee. In these cases, the manuscript could temporarily withdrawn until the

authors re-submit the proper version. The authors are also allowed to submit short reviews, comments, letters, or discussions of papers already published if they guarantee sufficient difference to previous public contents.

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METHODOLOGY FOR DESIGNING AN AGRICULTURAL COMMODITY LOGISTICS INFORMATION SYSTEM

Fabiola Sánchez Galván[1], Horacio Bautista Santos[2], Nicolás Francisco Mateo Díaz[3], Neify Patricia Robles Hernández[4] , Neyfe Sablon Cossio[5]

Abstract. **This project is born from the needs of Chontla Municipality which is located in Veracruz, México to allow small agricultural producers to have a tool for making decisions about their commercialization and production planning. It is proposed to design and validate statistically a measurement instrument in the form of a survey applicable to small agricultural producers. The results obtained will be used to design the logistics and management indicators that will serve as a strategic resource to empower the municipality and achieve a substantive contribution in the agricultural commodities information systems, thus contributing to local economic development. It is proposed to apply the Checkland systemic methodology, data statistical analysis and software development methodologies.**

Key words: **logistics information system, local economic development, agricultural logistic indicators**

I. INTRODUCTION

The information revolution as a source of technological innovation and improvement has spread throughout the economy. Exceptional reductions in the cost of obtaining, processing and transmitting information are transforming the way that companies are managed (Porter & Millar, 1985). The use of information systems is changing the way that companies currently work, achieving considerable improvements, automating operational processes, providing information to support decision making, and enabling the achievement of competitive advantages through its implementation (Cohen & Asin, 2000).

Peña Ayala (2006) defines business intelligence as the term that seeks to characterize a wide variety of technologies, software platforms, specifications and processes. The goal is to contribute to making decisions that improve the company´s performance and promote its competitive advantage in the market, under a three perspective approach: making better decisions faster, converting data into information and using a relational application for management.

A systems approach that considers the agricultural chain as a set of sequential and interrelated stages from the field until reaching the consumer has other variants such as supply chains, commodities systems, production chains and value chains. In any case, it has been established that these chains have highly evolved coordination forms and integration and participation rules (Vorley, 2001), which are systems' properties. The systems approach has established that vertical coordination, organization of participants in agribusiness, feedback mechanisms and tools for

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quality and safety assurance are part of the 3Cs (coordination, cooperation, and communication) and are key elements of the value chain's success (Hobbs, 2000).

The fundamental reason for making the present methodological proposal is precisely the real business need to interact with information and use it in the development of small agricultural producers, promoting local economic development.

In recent years, this concern and interest has been applied in companies to streamline their activities and improve their processes that are currently key elements for the management of local economic development programs (Vélez, 2011).

In this paper, a logistics information agricultural system is proposed according to the needs of Chontla municipality which is located in the North of Veracruz, México. In fact, that all kinds of information systems exist in the Computational Information Technologies (TICs) as strategic resource to speed up, reduce costs and multiply users among other potentialities. Information is an intangible good for making decisions and if presented in an orderly, easily locatable, user-friendly manner, then a substantive contribution can be made to information systems, specifically in the agricultural sector.

II. METHODOLOGICAL PROPOSAL

In order to design an agricultural logistics information system, the methodology is proposed in four stages: (1) data collection and data validation statistically, (2) agricultural logistic indicators design, (3) agricultural logistic information system design, and (4) agricultural information system implementation.

Stage-1: Data collection and data validation statistically

This stage will be carried out through interviews and surveys. Specific information will be collected from small agricultural producers. The reliability of the designed survey will be validated using Cronbach Alpha statistics that provides a reliability measure of internal consistency; it is the average of all possible division coefficients by half that result from the different divisions of the scale reagents. The calculation method requires a single measuring instrument application; measurement is applied and the coefficient is calculated (Hernández Sampieri, 2010, Mahotra, N., 2008).

Stage-2: Agricultural logistics indicators design.

The information obtained from the surveys, will be used to design the logistical and management indicators that serve as the quantifiable performance for evaluating the agricultural regime and contribute a tool to support decision making between the logistics parts chain of Chontla municipality. Measurement instrument validity will be made through exploratory and confirmatory factor analysis, which indicates into how many dimensions a variable can be integrated and what items make up each dimension. Factor analysis is a data reduction technique that serves to find homogeneous groups of variables from a large set of variables. Reagents that do not belong to a dimension, means that they are "isolated" and do not measure the same as the other items, therefore should be eliminated (Hernández Sampieri, 2010, Mahotra, N., 2008).

Stage-3: Agricultural logistic information system design

The components that will be integrated into the system will be analyzed under the seven stages of Checkland systemic methodology that will allow building a system conceptual in its root definitions. The seventh stage, named "Take action to improve the problem situation" proposes to design the system under Edward Yourdon's methodology which provides a way to design step-by-step systems and programs. It should be mentioned that some steps are involved in the analysis, others the development of the design and others the measurement and the improvement of the quality of the design and to combine that methodology with the agile methodologies for software development. The essential model of the system is a model of what the system must do to satisfy user requirements, saying as little as possible (preferably nothing) about how it will be implemented. Specifically, this means that when the analyst speaks to the user about system requirements, he should avoid describing specific process implementations (bubbles in a data flow diagram) in the system, i.e. he should not show system functions which are being performed by humans or by existing computer systems. The essential model consists of two main components: the environmental model and the behavioral model. The environmental model defines the boundary between the system and the rest of the world (i.e. the environment in which the system exists) and consists of a context diagram, a list of events and a brief description of the purpose of the system.

The behavior model describes the behavior that the system requires in order for it to interact successfully with the environment. The model consists of designing data flow diagrams, entity-relation, transition states and dictionaries, and process specifications.

Stage-4: Agricultural information system implementation

The system must be fed with information gathered from the small agricultural producers and should be structured in such a way that allows the user to communicate efficiently with the software developed. Once implemented, training should be provided to potential users, including municipal authorities, small and medium producers, entrepreneurs, and logistics operators, among others.

III. EXPECTED RESULTS

The global food supply chain is made up of a network of companies ranging from primary production to the sale to the final consumer; it is also made up of primary producers, processors, distributors, marketers and service providers. Each of these actors performs some buying activity, processing, selling products and/or services that add value to the product until it reaches its final destination. The coordination and collaboration of all these actors determine the efficiency and competitiveness of the chain as a whole. The necessary future demand for a different product flow than traditionally used will require food systems to be restructured to reduce waste, serve urban populations and efficiently use existing infrastructure. Information flow through the supply chain is fundamental to the link between supply and demand. The actors in the chain will need the best coordination system to meet the demand of the most efficient consumers (SAGARPA, 2010).

With the project realization, Chontla municipality will be given a territorial information system that characterizes the agricultural sector, allowing the corresponding authorities to execute

projects that enhance the local, socio-cultural and sustainable economic development of its rural localities. The technological impact of this project is based on the strengthening of the agricultural production sector, supporting at least 200 producers of the municipality's shared land in the marketing of their products, improving the income of the producers by concentrating the production information, goods, services and networks of marketing of agricultural products and inputs.

VI. CONCLUSIONS

Currently there is a great need for both public and private actors to make decisions on territorial variables in less time and in more complex environments, the Mexican agricultural supply chain system presents several constraints that make it uncompetitive and inefficient: heterogeneity in distribution channels, limited storage and transportation infrastructure, little use of quality standards, traceability and standards, market and information failures and high transaction costs among others. These are only some of the problems on which the country's agricultural policy should put special emphasis. Having a territorial diagnosis of the Chontla Municipality, where there are agricultural logistics indicators, will allow a boost in local economic development, making it easier to make decisions regarding agricultural planning in relation to crop types, production volume and quality or optimal harvest time, sale or distribution, among other aspects.

IV. ACKNOWLEDGEMENTS

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Computational study of aromatic compounds inhibiting *Trypanosoma cruzi* glyceraldehyde 3-phosphate dehydrogenase

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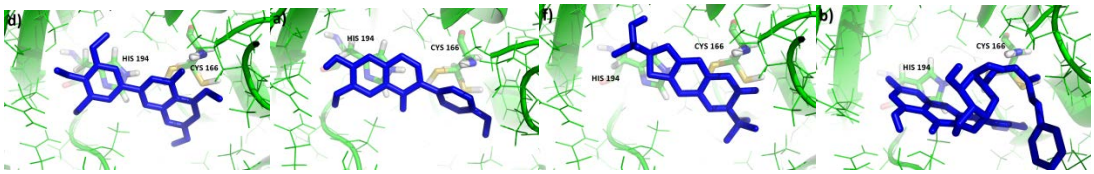
Abstract: Chagas disease is caused by the protozoan *Trypanosoma cruzi* and is widely distributed throughout Latin America. Because it is a pathology neglected by the pharmaceutical industry and because existing drugs have low efficacy and several side effects, interest in new drugs has been increasing. Due to the necessity of the discovery of new structures, the objective of this work was to relate the biological activity of natural and semi-synthetic aromatic compounds, inhibitors of glyceraldehyde 3-phosphate dehydrogenase enzyme, with descriptors calculated by molecular modeling, such as HOMO-LUMO frontier orbitals, partition coefficient (LogP), water solubility (LogS) and ionization potential, in addition to performing a molecular docking study, in order to obtain a better molecular view of the interaction of the aromatic compounds with the active site of the enzyme. It was observed that the compounds involved in the study interacted attractively with the enzyme, in accordance with experimental studies, and had adequate solubility for good pharmacokinetics. It was also possible to relate the pharmacological activity of some compounds with the energy of the LUMO orbital. The study showed that the methodology used in this work can be used to understand the interaction of active compounds with their respective targets, saving time and resources.

Keywords: Chagas disease, molecular docking, molecular modeling, aromatic compounds

Graphical Abstract:



Introduction:

Chagas disease is an infectious process caused by the protozoan *Trypanosoma cruzi*, which in turn is transmitted to humans through triatomine insects commonly known as "barbers" [1]. This pathology has spread throughout the world due to the migration of people from endemic to non-endemic regions. In addition to transmission by the barber, there are other means of transmission of Chagas' disease, such as blood transfusion, congenital transmission, accidental transplantation of organs, and oral and sexual transmission [2].

Chagas disease has been considered one of the main public health problems in Latin America and has been extremely neglected by the pharmaceutical industry because it represents a low-profit market [3].

A number of natural and synthetic compounds have been highlighted because they present high pharmacological activity against *T. cruzi*, through the inhibition of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme responsible for the conversion of glyceraldehyde-3-phosphate to 1,3 - diphosphoglycerate and which has structural differences with respect to the human enzyme. The infective forms of *T. cruzi* are dependent on the glycolytic pathway, which makes the enzyme a promising target for the creation of antichagasic drugs, since the inhibition of GAPDH will cause the inhibition of the *T. cruzi* glycolytic pathway [4, 5].

Several studies have shown excellent results for inhibition of the GAPDH enzyme by aromatic compounds, such as the tiliroside flavonoids [6, 7], 7-hydroxy-4',6-dimethoxyisoflavone [8], 3',4',5',5,7-pentamethoxyflavone [9], quercetin and guajaverin [6], and chalepin, which is a synthetic coumarin derivative [10] (**Figure 1**).

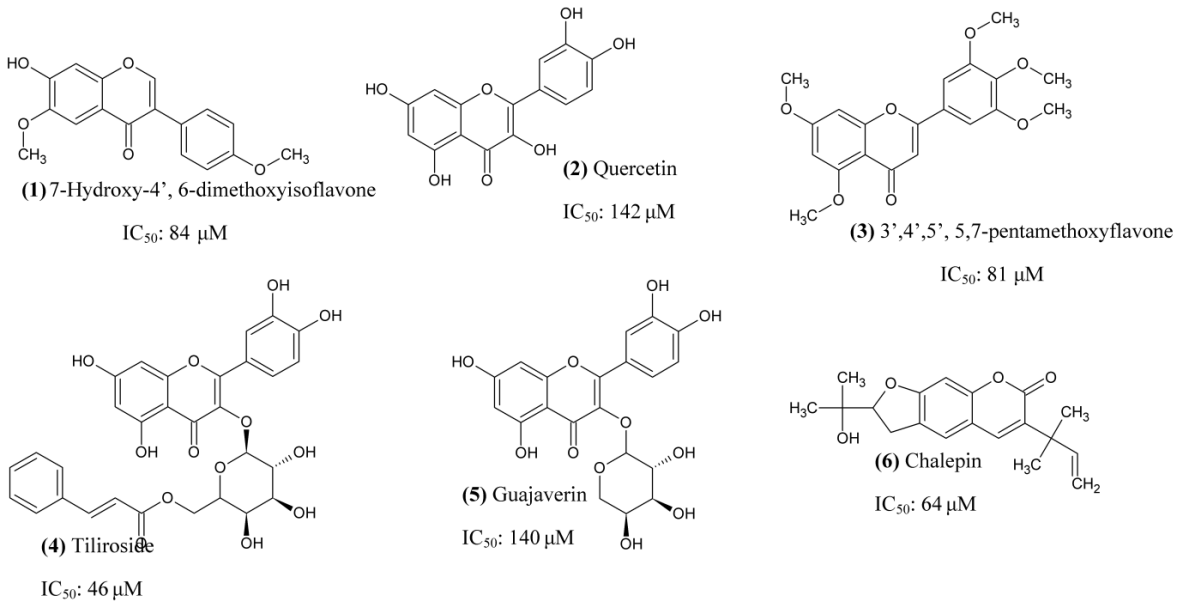


Figure 1. Glyceraldehyde 3-phosphate dehydrogenase inhibitors involved in the study

Flavonoids and coumarins are natural compounds found in several foods of plant origin and are characterized by two nuclei forming in some classes a heterocyclic [11]. These compounds have received much attention from the scientific community, including in the area of molecular modeling, not only for their anti-chagasic effect, but also due to several other pharmacological activities, such as antioxidant, antimicrobial, antithrombotic and anti-inflammatory, among others [12- 17].

In addition to the calculations of molecular descriptors such as partition coefficient, border orbitals and ionization enhancement, which are extremely used parameter in the quantification of the structure-activity relationship [18], molecular docking studies have been used to study the protein-linker interaction, which consists of joining a micromolecule (ligand) at the binding site of a macromolecule molecule (target protein), so that this junction has affinity for interaction between the Compounds, thus generating a greater number of ligand-receptor complexes [19].

Molecular modeling studies involving compounds with biological activity against the enzyme glyceraldehyde 3-phosphate dehydrogenase from *T. cruzi* contribute to a mechanistic proposal of the interaction of these compounds with the enzyme. The relevance of the present study lies in the understanding of the binder-enzyme interaction, which will enable future searches for antiparasitic substances with an inhibitory potential for the *T. cruzi* Glyceride-3-phosphate dehydrogenase enzyme, this being one of the main public health problems that Affects the low income population and who has been extremely neglected by the pharmaceutical industry, as well as elucidating the structure-activity relationship of the compounds, thus contributing to future molecular modifications.

Therefore, the objective of this work was to quantify the activity structure relationship of the aromatic compounds **1-6** inhibitors of the glyceraldehyde-3-phosphate dehydrogenase enzyme of *Trypanosoma cruzi* through calculations of border orbitals, ionization potential, logP and logS of the compounds, in addition To verify the interaction energy of the compounds in complex with the enzyme by molecular docking.

Materials and Methods:

Theoretical calculations were performed at the Materials Studies Laboratory (LEMAT), located at the Araguaia campus of the UFMT of Barra do Garças - MT.

The criterion used to include these substances in the work was the performance of other studies that involved proving the aromatic compounds in relation to inhibition of glyceraldehyde-3-phosphate dehydrogenase *in vitro*.

This work was carried out through the use of molecular modeling methods to evaluate the behavior of the compounds (ligand) in complex with the GAPDH enzyme, besides the solubility of the ligands.

2.1 Calculation of molecular descriptors

All structures of the ligands were drawn through *ChemSketch 11.0*. The molecular optimization and calculations of the border molecular orbitals, in addition to the ionization potential of the compounds, were performed by quantum mechanics using the semi-empirical method PM7, with software *MOPAC7* [20].

The *ALOGPS 2.1* software was used for calculations of partition coefficient (log P) and water solubility (log S) of the compounds [21] *ALOGPS 2.1* predicts the partition coefficient (log P) and water solubility (log S) of the compounds [21]. The *ALOGPS* was built on the *Associated Neural Network* (ASNN), which is a machine learning algorithm that combines neural network with k-neighbors [22]. The system implemented in *ALOGPS* for log P calculations was developed with 12908 molecules from the PHYSPROP database, using 75 E-state indices. Sixty-four neural networks were enabled using 50% of molecules chosen by coincidence from the whole set. The accuracy of the log P prediction presents an RMS value of 0.35 and mean standard error S = 0.26 [23, 24]. For the calculation of water solubility, *ALOGPS* was developed using 1291 molecules. The accuracy of the log S prediction presents RMS = 0.49 and mean standard error S = 0.38 [25].

2.2 Molecular Docking Study

The crystallographic structure of the enzymatic target GAPDH was obtained from the Protein Data Bank database [PDB ID: 1K3T]. This structure was chosen because it has already been used in other molecular docking studies. The enzyme was elucidated by X-ray crystallography, with resolution of 1.95 Å. Gasteiger loads and polar hydrogens required for power calculations were added considering the target structure, with the water molecules removed. The non-polar hydrogens of the linkers were suppressed and the rotational bonds of each linker were automatically set.

AutoDock 4.0 software [26] was used as the choice to conduct the studies in the GAPDH target. The *AutoDock* Tools module was used to prepare and analyze the computational simulations. *AutoDock* requires pre-calculated three-dimensional maps, arranged in a box composed of a three-dimensional grid of points, in a region defined in the macromolecule (target site). The *AutoGrid 4.0* software was used to generate the maps for the binders. The box was positioned in the catalytic region of the enzyme. The Lamarckian Genetic algorithm (GA-LS) was chosen to search for the best conformations [27]. The LGA combines an efficient generalized search from locations far from the minimum, which is performed by the genetic algorithm. However, the genetic algorithm is inefficient for near local search to the minimum. For this reason, we use a stochastic search method associated with a deterministic minimization method [28]. LGA begins the search for the random construction of an initial population with a number of individuals determined by the

operator, each individual being symbolized by a chromosome (possible solution for docking, that is, orientation and conformation of the ligand in the target). Each chromosome consists of fragments, called genes (values of variables or sets of variables, corresponding to rotational, translational and conformational degrees of freedom). The projection of the initial population is followed by successive generations of routes until the maximum number of generations or the maximum number of energy evaluations is obtained [26, 29]. 100 runs were performed for each binder (genetic algorithm with local search). The initial population was defined as 150 and the search process occurred through random initial conformations. The maximum value of energy assessments chosen was 25,000,000, while the maximum number of generations was maintained at 27,000, as well as the number of elitism was maintained at 1. The rates of genetic mutation and crossover established were respectively 0.02 and 0 , 80. After completing the calculations, 100 different conformations were obtained and grouped into different clusters, defined by energy proximity and RMS values ("Root Mean Square deviation"), according to the *AutoDock* default [26]. During the search process, the enzyme was kept rigid, while the binders were flexible.

Results and Discussion:

3.1 Molecular Descriptors

The present study sought to investigate the solubility of compounds **1-6** in the aqueous and lipophilic equilibrium phase, since it is known that the solubility of the compounds is of great importance for their pharmacological activity, due to the fact that the drugs need to cross the biological barrier Lipophilic [30].

In relation to the octanol / water partition coefficient (LogP) calculated by *ALOGPS 2.1*, the values found for compounds **1-6** can be visualized in **Table 1**. It can be observed that guajaverin and quercetin, compounds with higher experimental IC_{50} values (**Figure 1**), among the target compounds of the present study, were the compounds that presented the lowest logP value, which suggests that, even more soluble in organic solvents, the compounds have less efficiency in the permeabilida in hydrophobic biological barriers, when compared to Other target compounds of the present study.

Table 1. LogP and logS values of the compounds calculated by ALOPS 2.1

Compound	LogS (calc)	LogP (calc)	LogP (exp)
7-Hydroxy-4', 6-dimethoxyisoflavone	-3.85	2.71	-
3',4',5', 5,7-pentamethoxyflavone	-4.67	3.03	-
Quercetin	-3.06	1.81	1.82 [34]
Guajaverin	-2.50	0.70	-
Tiliroside	-3.52	2.97	2.71 [34]
Chalepin	-4.15	3.54	-

When using the *ALOGPS 2.1* software for the calculation of water solubility (LogS), it was observed that 3',4',5',5,7-pentamethoxyflavone

had the lowest value (less water soluble), while quercetin presented The highest value (higher solubility in water), in line with logP calculations.

The results show that all the compounds involved in this work have adequate solubility for a good bioavailability, because it can be said that compounds with logS values between -1 and -5 present hydrophilicity required for aqueous solubility and lipophilicity to interact with hydrophobic surfaces [31]. Very polar molecules with difficulty to permeabilize on hydrophobic surfaces have logS values above -1 [31].

According to this study compounds **1-6** have adequate solubility to meet pharmacokinetic requirements, presenting sufficient hydrophilicity to interact with biological fluids (plasma) and lipophilicity suitable to interact with hydrophobic (cell membrane) surfaces.

In order to predict the electronic characteristics of compounds **1-6**, the boundary molecular orbitals (HOMO and LUMO) were calculated by the semi-empirical quantum method PM7. The HOMO and LUMO energies are used as indexes of chemical reactivity and are commonly correlated with other indices, such as electron affinity and ionization potential. The energies of the boundary orbitals of compounds **1-6** are shown in **Table 2**.

It can be observed that triliroside, the compound with the highest inhibitory activity of the enzyme GAPDH, showed the lowest energy of LUMO orbital, which indicates that its stability to the active site can occur by the interaction of the LUMO of the triliroside with homo orbital of the enzyme.

Table 2. Descriptors used in analysis

Compound	**$IC_{50\ \mu M}$ (exp)**	**E_{HOMO} eV (calc)**	**E_{LUMO} eV (calc)**	**$\Delta E_{LUMO-HOMO}$**	**Molecular weight**	**Ionization potential eV**
7-Hydroxy-4', 6-dimethoxyisoflavone	84	-8,604	-0,728	7,877	298,294	8.604
3',4',5', 5,7-pentamethoxyflavone	81	-8.808	-0,696	8,112	372,373	8.499
Quercetin	142	-9.089	-1,114	7,975	302,239	9.090
Guajaverin	140	-9.528	-1.087	8,441	434,355	9.529
Triliroside	46	-9.150	-1.230	7,920	594,527	9.150
Chalepin	64	-8.949	-0.912	8,037	314,3804	8.948

Also shown in **Table 2** are the molecular weight and ionization potential values of compounds **1-6**. Low ionization potential values for active compounds may indicate possible mechanisms of charge transfer in the interaction of the ligand with the receptor, and may also indicate that the ionic form of the substance is the one with biological activity. The potentiation

values can also be used to measure the hardness and softness of the compounds. These values are expressed in terms of the ionization energy between the neutral atom and its anion. Thus, molecules with high ionization potential and high electronegativity have high absolute hardness, and the higher the hardness, the lower the softness of the molecule [32]. As observed in **Table 2**, guajaverin, the compound with the lowest inhibition performance among those included in the present study, was the one with the highest energy value of ionization potential, which characterizes a state of hardness higher than the other compounds and lower capacity of Transfer of charge during interaction with the enzyme.

3.2 *Docking* Molecular

Table 3 shows the results obtained through the docking study between compounds **1-6** with the enzyme glyceraldehyde 3-phosphate dehydrogenase. In addition to binding energy, Table 2 also shows details of the hydrogen interactions occurring between the binders and the macromolecule [33].

Table 3. Result of the docking study of the compounds with the enzyme glyceraldehyde 3-phosphate dehydrogenase.

Compound	Docking Free Energy (kcal/mol)	Donor H-bond	Aceptor H-bond	Distance H-bond (Å)
7-Hydroxy-4', 6-dimethoxyisoflavone	-6.69	SER 247 C: H	LIG: O	2.3
3',4',5', 5,7-pentamethoxyflavone	-7.13	CYS 166 C: H	LIG: O	2.25
		CYS 166 C: H	LIG: O	2.10
		SER 247 C: H	LIG: O	2.14
Quercetin	-5.62	LIG: H	THR 197 C: O	2.16
		LIG: H	THR 167 C: O	1.92
		CYS 166 C: H	LIG: O	2.04
		SER 247 C: H	LIG: O	1.87
Guajaverin	-6.17	CYS 166 C: H	LIG: O	1.90
		SER 247 C: H	LIG: O	2.07
		ASN 335 C: H	LIG: O	1.91
Tiliroside	-7.29	LIG: H	THR 199: O	2.17
		CYS 166 C: H	LIG: O	2.05
		SER 247 C: H	LIG: O	2.07
		ASN 335 C: H	LIG: O	1.91
Chalepin	-7.21	CYS 166 C: H	LIG: O	1.91

It can be seen that all compounds **1-6** interacted with the enzyme glyceraldehyde 3-phosphate dehydrogenase in an attractive way, and the compounds tiliroside, chalepin and 3', 4', 5',5,7-pentamethoxyflavone were those that

obtained Lower interaction energy, showing to be more stable in complexes with the active site of the enzyme (GADPH).

Figure 3 shows the more stable conformation of compounds **1-6** at the site of action of the GADPH enzyme.

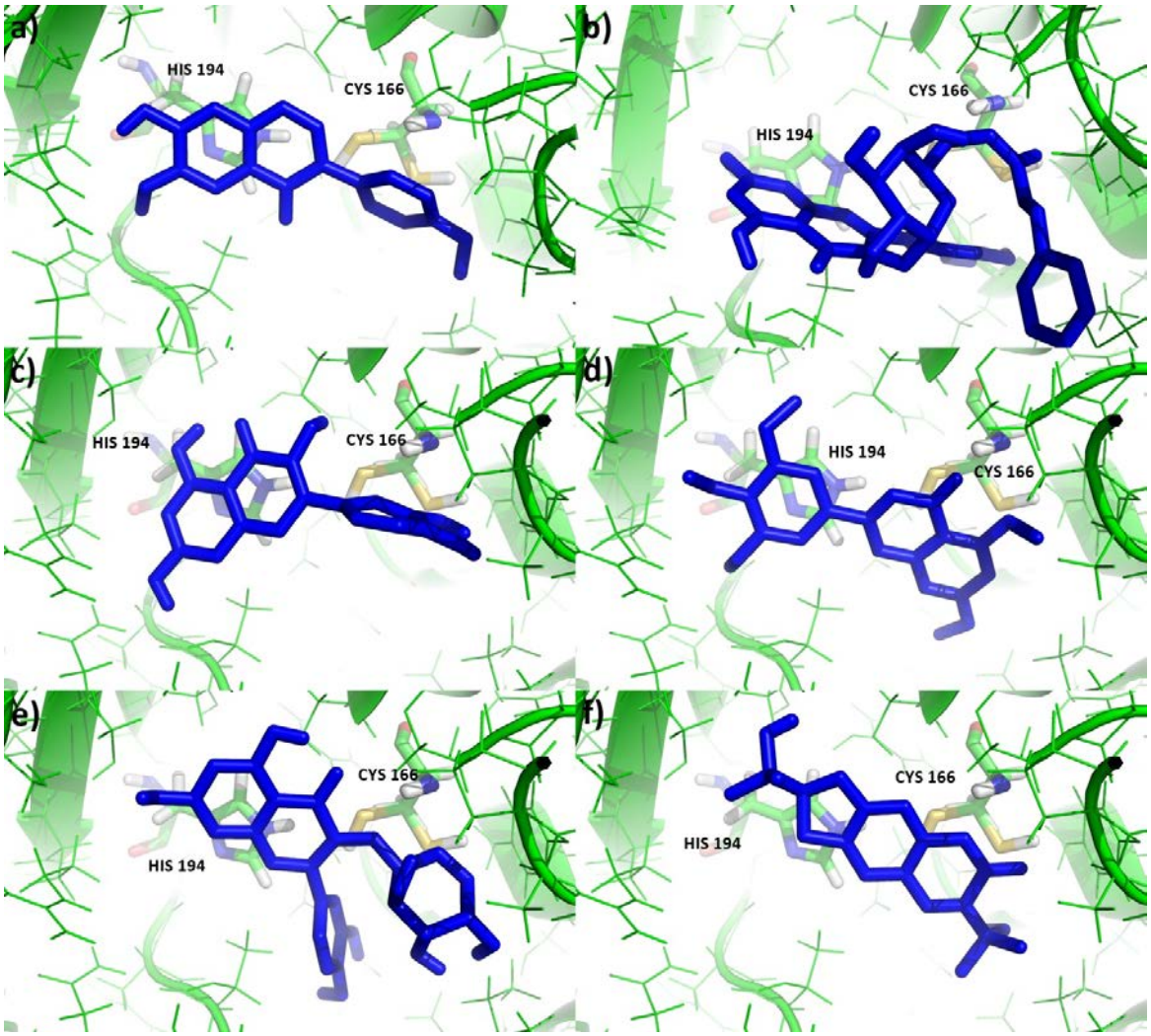


Figure 3. Compounds in the site of action of the enzyme GAPDH.a) quercetin; b) tiliroside; c) 7-Hydroxy-4', 6-dimethoxyisoflavone; d) 3',4',5', 5,7-pentamethoxyflavone; e) guajaverin; f) chalepin.

In **Figure 3** it can be seen that all the compounds have approached, through their ring systems, the amino acids HIS 194 and CYS 166, which are essential for catalytic activity of the enzyme, since this activity involves the nucleophilic attack of catalytic cystine (CYS 166) on the substrate. The HIS 194 is responsible for the activation of CYS 166 and also for the formation of the tetrahedral intermediate, which will later decompose by transferring a hydride to the NAD + cofactor, forming a highly energetic thioester, which after phosphorylation will release the product of the enzymatic catalysis, Or 1,3-bisphosglycerate. This result suggests that all ring structures of the compounds, besides the polar groups, are extremely important for the pharmacological activity of these compounds.

In **Figure 4** the overlap of all compounds 1-6 can be observed at the active site.

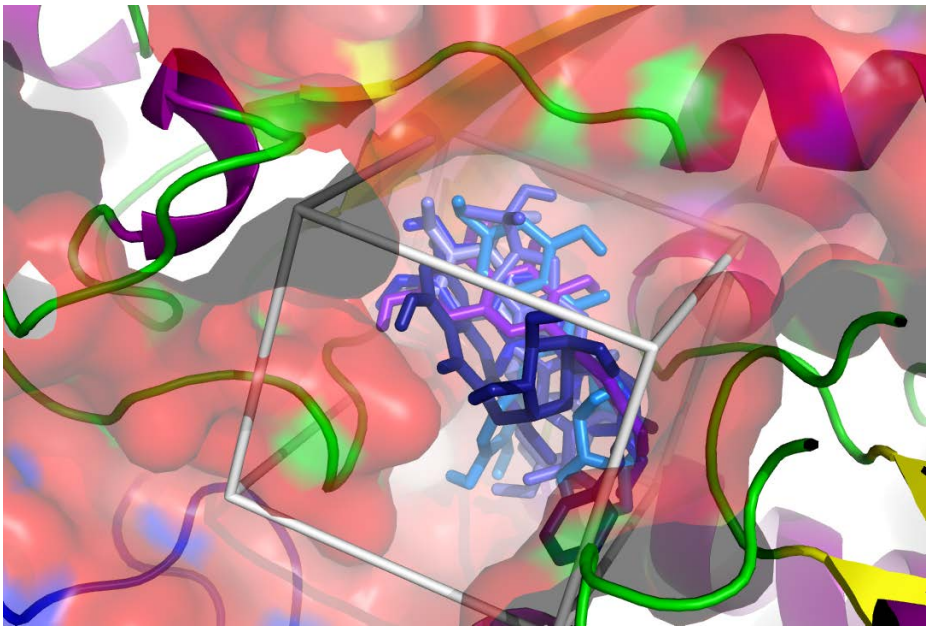


Figure 4. Compounds 1-6 superimposed on the active site of GAPDH

In **Figure 5** the linear regression is presented associating the values of IC_{50} with the values obtained by molecular docking. It is seen that, in part, the values obtained in the molecular docking study can be rationalized with the IC_{50} values obtained experimentally (R2: 0.86).

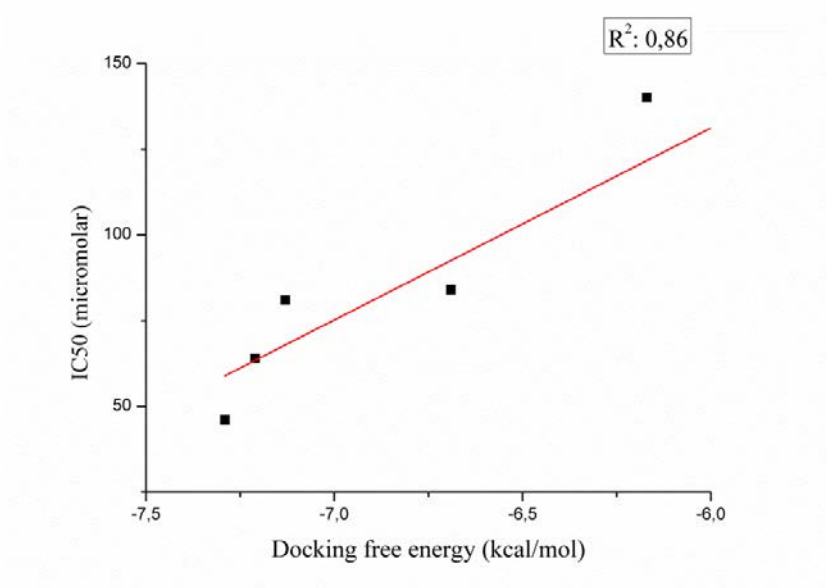


Figure 5. IC_{50} of compounds versus molecular docking energy

Conclusions:

The computational study carried out in this work allowed a better view, at a molecular level, regarding the interaction of compounds 1-6 with the enzyme, showing that the compounds that have lower IC_{50} also have more stable energy of drug-receptor binding. This result suggests that there is a more selective mechanism of interaction in GAPDH.

Conflicts of Interest:
The authors declare no conflict of interest

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New insights to understand the CoMFA and CoMSIA analysis within the framework of Density Functional Theory. Toward a generalized methodology.

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Abstract: Currently, the three-dimensional quantitative structure-activity relationship (3D QSAR) models have many applications; however due to the complexity to understand its results is necessary postulate new methodologies. In this sense, this work postulates a generalized version joining the quantum similarity field and chemical reactivity descriptors within the framework of density functional theory.

This generalized methodology can be applied to understand the biological activity on a molecular set taking a reference compound. In this sense, this methodology allows study the CoMFA and CoMSIA results in term of quantum similarity and chemical reactivity. In this form, is possible study steric and electrostatic effect on local substitutions. Considering that these methodologies can be used when the receptor is known or even when it is not known.

Keywords: Comparative Molecular Similarity Field Analysis (CoMFA), Comparative Molecular Similarity Indexes Analysis (CoMSIA), 3D-QSAR, Molecular Quantum Similarity (MQS), Chemical Reactivity Descriptors, Density Functional Theory (DFT).

Introduction: Taking into account that the CoMFA and CoMSIA analysis have many applications in the three-Dimensional Quantitative Structure-Activity Relationships (3D QSAR) studies **[1]**, in this work are presented a new considerations about these methodologies within the Density Functional Theory (DFT) context.

In the DFT framework, the MQS is a field very applied, was introduced by Carbó and co-workers **[2-6]**. In the MQS field the key variable is the density function **[9-11]**; therefore it reasonable think that

can be related with the chemical reactivity descriptors such as chemical hardness (η), softness (S), electrophilicity (ω) and Fukui Functions. Therefore, employing this hybrid methodology (joining the MQS and chemical reactivity) is hope show new insight on the understanding of the CoMFA and CoMSIA results within the DFT context. In this form, the main aim of this work is presents new relationship between the MQS and Chemical reactivity that can be applied on the CoMFA and CoMSIA analysis.

Results and Discussion:

2. New insights proposed.

2.1. Quantum object sets (QOS)

Considering the follow set: $Z=\{z_I \,|\, I=1,N\}$, being N the set cardinality, is the Cartesian product of two sets: $Z=\{P\times M\}$, where $P=\{p_I \,|\, I=1,N\}$ is the set of the objects and $M=\{m_I \,|\, I=1,N\}$ the set of tags. Therefore we can write: $\forall I=1,\ \mathrm{N}{:}z_I=(p_I;m_I)\in Z$ **[12,13]**. In this sense, a (QOS) is a tagged set: $Q=P\times S$ made by a set of submicroscopic objects and a set of quantum mechanical Density Function (DF): $S=\{\rho_I \,|\, I=1,N\}$, as elements of the tag set.

Of this form, we can use the Hilbert semispace tag set S and define a central averaged DF using the expression:

$$\rho_C=N^{-1}\sum_I \rho_I \rightarrow \langle\rho_C\rangle=N^{-1}\sum_I \langle\rho_I\rangle=N^{-1}\sum_I \upsilon_I=\upsilon_C \qquad \textbf{(1)}$$

with the DF Minkowski norms being define as:

$$\forall I=1,N:\langle\rho_I\rangle=\int_D \rho_I(r)dr=\upsilon_I \qquad \textbf{(2)}$$

Therefore, the centroid DF can be seen as a function describing the arithmetic average of the number of particles υ_C of all quantum objects involved. To relate the shape functions associated to the quantum set, we define tag set H associated to the DF set S, therefore we have:

$$S=\{\rho_I \,|\, I=1,N\}\rightarrow\forall\langle\rho_I\rangle=\upsilon_I\wedge\sigma_I=\upsilon_I^{-1}\rho_I\wedge\langle\sigma_I\rangle=1\rightarrow H=\{\sigma_I \,|\, I=1,N\} \qquad \textbf{(3)}$$

From this equation 3, we can write the shape centroid function as:

$$\sigma_C=N^{-1}\sum_I \sigma_I \rightarrow \langle\sigma_C\rangle=N^{-1}\sum_I \langle\sigma_I\rangle=N^{-1}N=1 \qquad \textbf{(4)}$$

2.2 Local Molecular Quantum Similarity Measure. A generalized version.

The quantum similarity measure Z_{AB} between compounds A and B, with electron density $\rho_A(r_1)$ and $\rho_B(r_2)$ respectively, can be understood using the minimizing of the expression for the Euclidean distance as **[12-15]**:

$$D_{AB} = \left(\int \left| \rho_A(r) - \rho_B(r) \right|^2 dr \right)^{1/2} = \left(\int (\rho_A(r_1))^2 dr_1 + \int (\rho_B(r_2))^2 dr_2 - 2 \int \rho_A(r_1) \rho_B(r_2) dr_1 dr_2 \right)^{1/2} = \sqrt{Z_{AA} + Z_{BB} - 2Z_{AB}} \quad \textbf{(5)}$$

Where Z_{AB} is the overlap integral between the electron density of the compound A and B into the **(QOS)**, Z_{AA} and Z_{BB} are the self-similarity of compounds A and B **[16]**.

In this researcher we have used the Carbó index due to that is very used in the quantum similarity context **[12-15]**:

$$I_{AB} = \frac{\int \int \rho_A(r_1) \rho_B(r_2) dr_1 dr_2}{\sqrt{\left(\int \rho_A(r_1) dr_1 \right)^2 \left(\int (\rho_B(r_2))^2 dr_2 \right)^2}} \quad \textbf{(6)}$$

As the main structural difference on the molecules used by our group in the previous work are local differences **[1]**; the similarity features can be associated from the local point of view, in this order of ideas is used the Hirshfeld approach to study the local quantum similarity.

With the aim to obtain a generalized Hirshfeld approach to our systems, considering the electron density $\rho(r)$ in contribution $\rho_{x^1}(r)$, where x=is an atom. These contributions allow define a concept of atom in a reference system and study its (dis)similarity on a molecular set (i.e.; substituent effect analysis). On the other hand, these contributions are proportional to the weight $w_C(r)$ of the electron density of the isolated compound in the so-called *promolecular density* **[17-20]**. The promolecular density is defined as:

$$\rho_{x^1}^{Prom}(r) = \sum_y \rho_y^0(r) \quad \textbf{(7)}$$

To calculate the contribution of an atom (x) in the electron density in a molecule A $\rho_A(r)$ we have:

$$\rho_{x^1}(r) = w_{x^1}(r) \rho_A(r) \quad \textbf{(8)}$$

In this form, the weight ($w_x(r)$) is obtained as:

$$w_{x^1}(r) = \frac{\rho_{x^1}^0}{\sum_y \rho_y^0(r)} \quad \textbf{(9)}$$

Here $\rho^0_{x^1}(r)$ is the electron density of the isolated carbon atom x^1, (i.e.; the reference electron density) **[21]**. In this sense, the contribution atomic of other carbon atom (x^2) in a molecule B is obtained as:

$$\rho_{x^2,B}(r) = w_{x^2}(r)\rho_B(r) \quad \textbf{(10)}$$

with

$$w_{x^2,B} = \frac{\rho^0_{x^2,B}(r)}{\sum_y \rho^0_y(r)} \quad \textbf{(11)}$$

So we can write the contribution of the asymmetric carbon atom products $\rho_A(r)\rho_B(r)$ as:

$$\rho_{x,AB}(r) = w_{x,AB}(r)\rho_A(r)\rho_B(r) \quad \textbf{(12)}$$

Using the equations (7-12) we can write the numerator Z_{AB} in the Carbó index (equation 6) as:

$$Z^{Local,x}_{A,B} = \frac{Z_{AB}}{\sqrt{Z_{AA}Z_{BB}}} = \frac{\iint w_{x,AB}\rho_A(r)\rho_B(r)dr_A dr_B}{\sqrt{\left(\int w_{x,A}(r)\rho_A(r)dr_A\right)^2\left(\int w_{x,B}(r)\rho_B(r)dr_B\right)^2}} \quad \textbf{(13)}$$

The equation 10 is a generalized Hirshfeld approach to our systems **[1]**, where x= is an atom, therefore we can write the global index (equation 6) as local contributions. In this context, is possible study the local similarity and the substituent effects on some reference compound into the (QOS).

2.3 Reactivity descriptors.

The CoMFA and CoMSIA analysis are understand in terms of physical-chemistry properties such as electrostatic, hydrophobic and hydrogen-bond donor or acceptor properties, these properties can be related with global chemical descriptors as chemical potential, hardness, electrophilicity index and local reactivity descriptors as the Fukui Functions **[1]**. In the DFT context, the global reactivity indexes give information about the reactivity or stability of a chemical system front to external perturbations.

The chemical potential (μ) can be understood as the tendency that have the electrons to exit of the electron cloud and is calculate according to the equation:

$$\mu \approx \frac{\varepsilon_H + \varepsilon_L}{2} \quad \textbf{(14)}$$

Where (ε_H) is the energy of the (HOMO) and (ε_L) is the energy of the (LUMO) **[22, 23]**. Using the equation 14 the chemical hardness is defined according to Pearson et. al. **[24]**.

$$\eta \approx \varepsilon_L - \varepsilon_H \quad \textbf{(15)}$$

From the equation (12), we obtain the softness **[25]** as:

$$S = \frac{1}{\eta} \quad \textbf{(16)}$$

Finally, using the equations 14 and 15 is defined the electrophilicity index (ω) **[25, 26]**. This index is understood as the measure of the stabilization energy of the system when it is saturated by electrons from the external environment and is calculated as follows:

$$\omega = \frac{\mu^2}{2\eta} \quad \textbf{(17)}$$

Finally, the Fukui Functions (equation 18 and 19, $f(r)$) are defined as the derivative of the electronic density with respect to the number of electrons at constant external potential:

$$f_k^+ \approx \int_k \left[\rho_{N+1}(\mathbf{r}) - \rho_N(\mathbf{r})\right] = \left[q_k(N+1) - q_k(N)\right] \quad \textbf{(18)}$$

$$f_k^- \approx \int_k \left[\rho_N(\mathbf{r}) - \rho_{N-1}(\mathbf{r})\right] = \left[q_k(N) - q_k(N-1)\right] \quad \textbf{(19)}$$

Where q_k refers to the electron population at k^{th} atomic site in a molecule. (f_k^+) governing the susceptibility for nucleophilic attack and (f_k^-) governing the susceptibility for electrophilic attack **[27-30]**.

2.4 Quantum Operators to calculate Local Similarity.

One the most operator used in quantum similarity is the Dirac delta distribution $\Omega(r_1, r_2) = \delta(r_1, r_2)$ **[31]** so called overlap molecular quantum similarity measure and relates the volume associated with the overlap of the two densities $\rho_A(r)$ and $\rho_B(r)$:

$$Z_{A,B}^{Local,x}(\Omega)=\frac{Z_{AB}(\Omega)}{\sqrt{Z_{AA}(\Omega)Z_{BB}(\Omega)}}$$
$$=\frac{\iint w_{x,AB}\rho_A(r)\delta(r_1-r_2)\rho_B(r)dr_Adr_B}{\sqrt{\left(\int w_{x,A}(r)\rho_A(r)dr_A\right)^2\left(\int w_{x,B}(r)\rho_B(r)dr_B\right)^2}}$$
$$=\frac{\iint w_{x,AB}\rho_A(r)\rho_B(r)dr_Adr_B}{\sqrt{\left(\int w_{x,A}(r)\rho_A(r)dr_A\right)^2\left(\int w_{x,B}(r)\rho_B(r)dr_B\right)^2}} \quad \textbf{(20)}$$

Using this equation 20, is possible obtain information about the electron concentration in the molecule and indicates the degree of overlap between the compared compounds.

Another operator very used in quantum chemistry is the Coulomb operator $\Phi(r_1,r_2)$, defined as $\Phi(r_1,r_2)=|r_1-r_2|^{-1}$, this operator represents the electronic coulomb repulsion energy between molecular densities $\rho_A(r)$ and $\rho_B(r)$ as:

$$Z_{A,B}^{Local,x}(\Phi)=\frac{Z_{AB}(\Phi)}{\sqrt{Z_{AA}(\Phi)Z_{BB}(\Phi)}}$$
$$=\frac{\iint w_{x,AB}\rho_A(r)\left(1/|r_1-r_2|\right)\rho_B(r)dr_Adr_B}{\sqrt{\left(\int w_{x,A}(r)\rho_A(r)dr_A\right)^2\left(\int w_{x,B}(r)\rho_B(r)dr_B\right)^2}} \quad \textbf{(18)}$$
$$=\frac{\iint w_{x,AB}\rho_A(r)\rho_B(r)dr_Adr_B}{\sqrt{\left(\int w_{x,A}(r)\rho_A(r)dr_A\right)^2\left(\int w_{x,B}(r)\rho_B(r)dr_B\right)^2}}$$

The Carbó index is restricted to the range (0,1) where C_{AB}=0 means dis(similarity) and C_{AB}=1 self-similarity, according to the Schwartz integral.

$$\left[\int \rho_A(r)\rho_B(r)dr\right]^2 \le \int \rho_A^2(r)dr\int \rho_B^2(r)dr \quad \textbf{(19)}$$

2.5 Quantum Similarity Matrix.

The quantum similarity Matrix can be associated to a $[N\times N]$ metric associated to a (QOS) tag set made of quantum mechanical density function $S=\{\rho_I\,|I=1,N\}$ as:

$$\mathbf{Z}=\begin{bmatrix}\langle \mathbf{z_1}|\\ \langle \mathbf{z_2}|\\ \mathsf{M}\\ \langle \mathbf{z_N}|\end{bmatrix}=\left[\langle \mathbf{z_1}|\langle \mathbf{z_2}|\mathsf{L}\ \langle \mathbf{z_N}|\right] \quad \textbf{(20)}$$

In the equation 20 there are equivalence between rows and columns. In this sense, we have:

$$\mathbf{Z} = \left\{ Z_{IJ} = \left\langle \rho_I \middle| \rho_J \right\rangle \middle| I, J = 1, N \right\}$$
$$\wedge \left| z_I \right\rangle = \left\{ Z_{JI} \middle| J = 1, N \right\} \quad \textbf{(21)}$$
$$\wedge \left\langle z_I \right| = \left\{ Z_{IJ} \middle| J = 1, N \right\}$$

Another property important of the matrix **Z** is its symmetry, according to:

$$\mathbf{Z} = \mathbf{Z}^T \rightarrow \forall I, J : Z_{IJ} = Z_{JI} \quad \textbf{(22)}$$

Taking in account these properties associated to the similarity matrix, we can express the local molecular similarity measures using the overlap and coulomb operators (equations 18 and 19).

2.6 Joining QS and chemical reactivity

According to Carbó et al. **[32]** it is possible to consider a set of specific vectors and to associate a center for this QOS. Therefore a QOS represented by Fukui Funtions can be defined as:

$$M = \left\{ \left| I \right\rangle I = 1, N \right\} \quad \textbf{(23)}$$

In equation 23, the first order densities can be constructed by a set of molecular orbital (MO) of shape funtions contributions as:

$$P = \left\{ \sigma_I = \left| I \right\rangle \left\langle I \right| I = 1, N \right\} \quad \textbf{(24)}$$

The P elements correspond to the squared MO modulos. Using these consideration we can relate the frontier orbital (HOMO and LUMO) on the QOS. Defining $\{w_I\}$ as the number of occupation in the MOs, we can construct a linear combination of P to the first order density functional as **[33]**:

$$\rho = \sum_I w_I \sigma_I \quad \textbf{(25)}$$

with

(i) ν is the number of electrons: $\sum_I w_I = \nu$

(ii) where the Minkowski norms of the elements of the shape function set P are normalized to unity, belonging to the MO set normalization $\left(\forall I : \left\langle \sigma_I \right\rangle = 1 \right)$.

Therefore we can define a centroid shape function using an average function.

$$\sigma_C = N^{-1}\sum_I \sigma_I \rightarrow \langle \sigma_C \rangle = 1 \quad \textbf{(26)}$$

In this sense, each elements of set P can be compared with the centroid function and can be built as:

$$\forall I : \theta_I = \sigma_I - \sigma_C \rightarrow Z = \{\theta_I \,|\, I = 1, N\} \quad \textbf{(27)}$$

Finally the Minkowski pseudonorm of the centroid shape function set **Z** can be written as:

$$\forall I : \theta_I = \langle \sigma_I - \sigma_C \rangle = \langle \sigma_I \rangle - \langle \sigma_C \rangle = 0 \quad \textbf{(28)}$$

Therefore the shifted elements have a null Minkowski pseudonorm. Where the shape function is comprised of N linearly independent elements. Using these relations we can make quantum similarity using the Fukui Functions on the QOS taking in account a reference compound.

$$Z_{AB}(f^{+/-}(r)) = \frac{\left[f^{+/-}(r)\right]_A \left[f^{+/-}(r)\right]_B}{\sqrt{\left[f^{+/-}(r)\right]_A^2 \left[f^{+/-}(r)\right]_B^2}} \quad \textbf{(29)}$$

Using the equation 29, we can build scales of convergence quantitative **[1]**, among other. This equation shows a possible join between quantum similarity and chemical reactivity and can be used to calculate the quantum similarity on the local chemical reactivity (Fukui functions). These measures can be related with the contour maps generated by the CoMFA and CoMSIA results. The advantage of the proposed methodology is that shows a possible way to quantify the biological activity of the compounds additional to the reported by the 3D-QSAR studies.

Conclusions: In this work are reported new insights about the relationship between quantum similarity and chemical reactivity in a generalized form. This hybrid methodology, allow us study the steric and electrostatic effects in form of the scales of convergence quantitative; also substituent effects among others (see *J 2015 J. Mol. Model. 21, 156* and *Mol2Net, 2015, 1 (Section B), pages 1-13, Proceedings*).

In this sense, the CoMFA and CoMSIA results can be modeled joining MQS and chemical reactivity; in this context these outcomes can be applied in QSAR correlations and docking studies to understand the biological activity of some molecular set. Taking into account that this methodologies can be used when the receptor is known or even when it is not known.

Conflicts of Interest: *The author declare no conflict of interest*

Acknowledgements: Thanks to the Universidad de Talca (CBSM)) for the continuous support to this investigation and finally to the **postdoctoral project N^0 3150035 (FONDECYT, CHILE)**.

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Prediction of mRNA expression in cow's milk using mRNA secondary structures and Machine Learning classifiers

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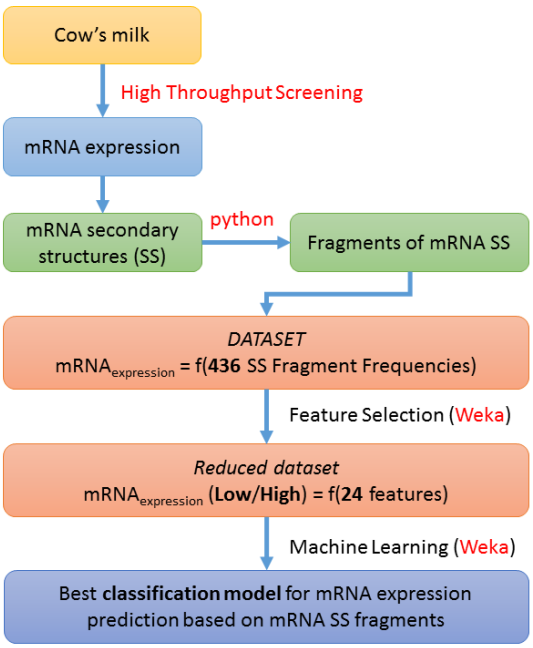
Abstract: The mRNA molecules expressed in cow's milk are important molecular biomarkers for different physiological and pathological conditions in cattle. The prediction of the quantity that a specific mRNA type could be expressed in cow's milk is a challenging theoretical task. The current study presents for the first time several different Machine Learning models to predict the mRNA expression using the mRNA secondary structure fragments. This unique methodology is based on a dataset of experimental mRNA expression data. Each mRNA molecule has a specific secondary structure represented as a string that can be used to read all the possible mRNA secondary structure fragments. This information is used as input for the Machine Learning methods from Weka software in order to obtain classification models that can predict low and high expression of new mRNA types in the cow's milk. The mRNA expression levels have been measured with High Throughput Screening techniques. The initial features included the counting of the mRNA secondary structure fragments for each expressed mRNA. The model features were transformed in frequencies and the expression levels were converted into low and high classes. In order to reduce the high number of possible features, a feature selection method has been applied. Thus, the best classification model was obtained with BayesNet method and is based on 24 features and 4067 cases. The model has the true positive rate for the low mRNA expression class of 0.78 (average true positive rate of 0.66). Further studies are needed improve the current results, using datasets with different feature sets and more advanced Machine Learning methods.

Keywords: *mRNA secondary structures, Machine Learning classifiers, mRNA expression*

Graphical Abstract:



Introduction: The mRNA expression in cow's milk is an important biomarker for the cattle conditions [1,2]. The current study proposes a method to predict the low or high expression levels of mRNA using mRNA secondary structure fragments and Machine Learning classifiers [3].

Materials and Methods: In the first step, the mRNA expression levels were measured using Illumina techniques. For each type of mRNA, there is a specific secondary structure (SS). Using python scripts, SS sequences were divided in fragments and their frequencies were calculated. The initial dataset had the output variable as two possible classes (low or high mRNA expression) and 436 frequencies of different mRNA SS fragments (4067 cases). In the next step, a feature selection method from Weka software [4] was applied in order to obtain a reduced dataset (only 24 features). Machine Learning (ML) techniques from Weka were used to find the best classification model that can predict mRNA expression levels.

Results and Discussion: The final dataset of 24 selected features was the input of different ML techniques from Weka, such as LibLINEAR, BayesNet, NaiveBayes, MultilayerPerceptron, RandomForest. The best model is a NaiveBayes classifier with the true positive rate (TPR) for the low mRNA expression class of 0.78 (average true positive rate of 0.66). These results demonstrate the necessity for better models in future works, with different types of ML technique and other sets of mRNA SS features.

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Cocoa polyphenols (*Theobroma cacao*) as natural Amazonian antioxidant in sausage fresh.

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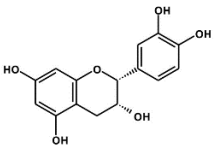
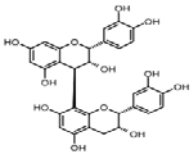
Graphical Abstract

Cocoa (*Theobroma Cacao)*

Polyphenols (*Theobroma cacao*) are used as natural



Epicatechin



Procianidin

Sausage prepared

ANALYSIS

Microbiological **Shelf life**
Organoleptic test

Abstract

Ecuador is considered one of the richest countries regarding biodiversity and its Amazon region guests several flora and fauna species. Cacao (*Theobroma Cacao*) is a tropical fruit with high commercial and biological importance due to the presence of polyphenols with antioxidant activity. In the present work cocoa polyphenols (*Theobroma cacao*) are used as natural Amazonian antioxidant in sausage fresh. Experimental units of sausage samples with different percentages (0, 2, 4, 6%) of cocoa polyphenols in the formulation with an experimental size of 5 kg were made; once the sausage was prepared, samples of 100 g were taken to perform sensory analysis and 100 g of samples destined for microbiological analysis. The microbiological analysis was performed on each sample of the formulated product, analyzes on the newly elaborated samples (1 day of elaboration) and an analysis with samples was developed after an estimated time (30 days) to assess the differences and active action of the antioxidant as a natural Amazonian preservative were performed. 15 people evaluated sensory characteristics to the sausage samples through a tasting test. As main results the natural antioxidants use allows prolonging the product shelf life, which results in an increase in color stability, since it prevents the transition from myoglobin to metamyoglobin, as well as maintaining its organoleptic conditions unalterable, slowing down oxidative phenomena such as product rancidity or increasing resistance to bacterial growth, since antioxidants of polyphenolic nature have antimicrobial activity.

Keywords: Polyphenols, antioxidant, functional foods, fresh sausage.

Introduction

For years, various strategies have been developed to prevent oxidative deterioration in products of meat origin through the use of antioxidants (Rostamzad *et al*., 2011). Most of these strategies have focused on limiting oxygen access to meat components susceptible to oxidation phenomena such as lipids and proteins. At the same time, new storage methods have been developed, such as vacuum packaging or packaging in a modified atmosphere in order to prevent the appearance of oxidation phenomena in the final product (Armenteros *et al*., 2012).

One way to reduce the occurrence of oxidation phenomena in meat and / or meat products is the use of antioxidants. The term antioxidant is generally attributed to any substance that is present at low concentrations, with respect to those of an oxidizable substrate and retards or prevents the oxidation of that substrate (Halliwell and Gutteridge, 1990). When antioxidant reacting with the free radical, it gives an electron oxidizing in turn and becoming a weak radical, with little or no toxic effects. In recent years it has been shown that a diet rich in plant polyphenols can improve health and decrease the incidence of cardiovascular diseases (Quiñones *et al*., 2012). In the present work cocoa polyphenols (*Theobroma cacao*) are used as natural Amazonian antioxidant in sausage fresh.

Materials and methods

Location and duration of the experiment

The present research was carried out in the Agroindustry's Laboratory, located in the Amazon State University, Km. 2 1/2 via Puyo to Tena (Paso Lateral), province of Pastaza, between coordinates 0º 59 '-1 "S and at a length of 77º 49' 0" W, it is found in the Amazonian Region of Ecuador in the west of the province of Pastaza, at about 924 m.a.s.l. Temperature 18 to 24 ºC.

Experimental units

Experimental units were formed for each sample of chorizo with different percentages of *Theobroma cacao* polyphenols in the formulation (0, 2, 4, 6%) and an experimental size of 5 kg of prepared dough. Once the sausage was prepared, samples of 100 g of each replicate sample were taken to perform sensory analysis and 100g of samples destined for microbiological analysis.

Microbiological analysis.

The microbiological analysis was performed on each sample of formulated product, analyzes were performed on the newly elaborated samples (1 day of elaboration) and an analysis with samples was developed after an estimated time (30 days) to assess the differences and active action of the antioxidant as a natural Amazonian preservative.

Sensory analysis and shelf life.

Through a tasting test, 15 people evaluated sensory sausage samples. An evaluation the product to know the shelf life in 30 days was made.

Results and discussion

Sensorial analysis

40% of the evaluated peoples that correspond to 6 persons, likes sausage samples without any addition of natural antioxidant; 9 from 15 people like the product with 2% natural antioxidant addition, 7 people who represent 47% have similarity in the sausage with 4% natural antioxidant incorporated, finally, 6 people equivalent to 40% like it and they like the product with 6% antioxidants. It should be noted that 4 people (27%) like the product a lot when they sensually find it optimal when 4% natural antioxidant is added (table 1).

Table 1. Percentage (%) of antioxidant in sausage samples.

Level of liking	0%	2%	4%	6%
I like very much	2	0	4	2
I like it	6	9	7	6
I do not like or dislike	5	6	3	4
I do not like	2	0	1	2
I dislike a lot	0	0	0	1
Total	15	15	15	15

Natural antioxidant and antimicrobial systems are set to become an important component in food preservation methodology. Wojciak et al. (2016) studied the effect of alternative natural preservatives (*Sinapis alba* L.-M, *Rosmarinus officinalis* L.-R, *Juniperus communis* L.-J) in combination with acid whey after the ripening period (21d) and over a prolonged storage period of sausage. An antioxidant activity of extracts exercise was performed. The antimicrobial, oxidative stability and sensory properties of these natural preservatives were compared to curing-control. Significantly lower rancid odor and rancid flavor were observed for R and M compared with the control sample. Incorporation of acid whey with rosemary extract will give the product a threefold effect: high quality (sensory acceptance), healthy benefit (elimination of nitrite and nitrate from meat products) and safety (improved microbiological and oxidative stability).

Marangoni and Moura (2011) determined sensory prolife of four samples of Italian salami using a methodology based on the Quantitative Descriptive Analysis. They select twelve individuals as judges and properly trained and used the following criteria: discriminating power, reproducibility, and individual consensus. The salami with coriander essential oil had lower rancid taste and rancid odor, whereas the control showed high values of these sensory attributes. Regarding brightness treated with coriander essential oil showed the best result.

By other hand, the addition of okra flour to an emulsified meat product (Frankfurter type sausage) was evaluated (Kitagawa *et al*. 2010) based on the physical, chemical, technological, and sensory characteristics of the final product. The results showed that the sausages containing okra flours A and B, as well as the control sausage, were accepted by the sensory panel. Moreover, there were no significant differences ($p \leq 0.05$) in the physical (color, objective texture, and emulsion stability) and chemical (pH and proximate composition) measurements of the sausages with and without the okra flour.

Microbiological analysis

The following table shows the significant reduction of microbial load in the stored sausages and that they contain polyphenols percentages incorporated in their formulation, while in the control product, since there is no protective agent, a microbiological growth can be observed (table 2). The use of natural antioxidants allows to prolong the useful life of the product which is in an increase of the stability of the color, since it avoids the transition from myoglobin to metamyoglobin, as well as maintains its

organoleptic conditions unalterable slowing down oxidative phenomena as the rancidity of the product or increasing resistance to bacterial growth, since antioxidants of polyphenolic nature have antimicrobial activity (Naveena *et al.*, 2008).

Consumers are becoming more aware of the toxicological implications of artificial additives in foods. Natural antioxidants, in addition to reducing the deleterious effects of lipid oxidation, are currently extremely highly valued. Santi *et al.* (2015) investigated the effect of addition of sun mushroom (*Agaricus blazei* Murrill) powder on the oxidative and microbiological stability of pork sausage during the shelf life. The results of the proximal composition and microbiological analysis for coagulase positive *Staphylococcus*, coliforms at 35 ºC and 45 ºC, *Salmonella sp* and sulfite-reducing Clostridium were consistent with those required by Brazilian legislation. The color of the products was of a decreased redness at the end of the storage period, on the 35th day, the TBARS values for the sausage with 4.0% powder was 0.509±0.12 mg MDA/kg sample and for the control was 1.131±0.12 mg MDA/kg sample. The sun mushroom powder had no effect on microbiological stability. It is concluded that sun mushroom was effective in terms of the oxidative stability of pork sausage when added in powdered form at concentrations of 1.0%, 2.0% and 4.0%.

Table 2. Total of microbial load in the stored sausages

General data			Indicators							
			Fresh samples				Sample with 30 days of preparation			
Type of sample	Code	Sample	Residual total coliforms	Mesophil bacteria count	Total coliforms	*E. coli*	Residual total coliforms	Mesophil bacteria count	Total coliforms	*E. coli*
Sausages	001	0%	<3	$2x10^4$	579	NA	<3	$2,2x10^4$	655	Nd
Sausages	002	2%	<3	$2,6x10^4$	723	NA	<3	$2,2x10^4$	567	Nd
Sausages	003	4%	<3	$2,2x10^4$	564	NA	<3	$1,8x10^4$	344	Nd
Sausages	004	6%	<3	$2,8x10^4$	490	NA	<3	$2,1x10^4$	302	Nd

Maximum Permissible Limits			
Total coliforms	Mesophil bacteria count	Total coliform	*E. coli*
0,3 – 1 < 1/g	M ufc/g	<2 NMP/100 ml	<0 NMP/100 ml Absence

Note:
ufc/g: colony forming unit per grams.
NMP/100ml: Most probable number of coliforms/100 milliliters of sample.
NA: Not Applicable

Conclusions

Cocoa polyphenols (*Theobroma cacao)* presents good qualities as an antioxidant in the use of sausages, allowing a better conservation and providing organoleptically unique qualities. Chorizo with 2% presents the best sensory characteristics and most welcome in the respondents. Meat products made with the addition of natural antioxidants from the Amazon provide, in addition to their basic nutritional properties, providing consumers with food that allows them to obtain better quality, health and life expectancy.

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Molecular Docking study of the flavonoids quercetin and artemetin front the angiotensin-converting enzyme

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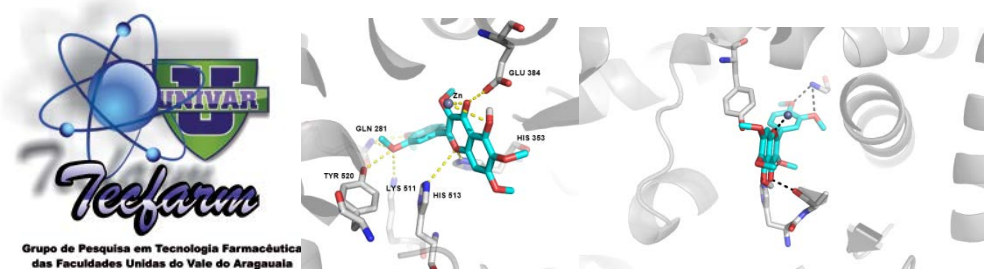
Abstract: Phenolic compounds, such as flavonoids, have aroused great scientific interest due to their diverse pharmacological activities, such as antioxidant, anti-inflammatory, anticancer and antihypertensive, among others. Several studies suggest the mechanisms responsible for the antihypertensive activity of flavonoids, and among them is the inhibitory activity of the angiotensin-converting enzyme (ACE). Thus, the objective of the present study was to perform a molecular docking study of flavonoids quercetin and artemetin against ACE, aiming at a better understanding of the interaction of these flavonoids with the enzyme. The crystallographic structure of the enzymatic target ACE was obtained from the Protein Data Bank database [PDB: 1UZE]. The molecular docking study was performed using Autodock 4.0 software. Gasteiger charge and polar hydrogens needed for the power calculations were added to the enzyme, with the water molecules removed. The grid was positioned in the catalytic region of the enzyme with dimensions on the X-, Y- and Z-axis at 32 Å 30 Å and 38 Å, respectively, spacing 0.375 Å. The Lamarckian Genetic algorithm was chosen to search for the best conformations with 100 runs for each binder. During the search, the enzyme was held rigid and the ligands were kept flexible. Both artemetin and quercetin interacted with the active site of the enzyme attractively. With docking energy at -6.89 kcal/mol, artemetin was more stable in complex with the active site of the enzyme ACE, whereas quercetin presented docking energy at - 6.63 kcal/mol. Both ligands interacted by hydrogen bonds with amino acids GLU 384, TYR 520, HIS 513, HIS 353, GLN 281, LYS 511 and the Zn ion. The study showed that the methodology used in the present study can be well used for the understanding of the interaction of pharmacologically active compounds with target enzymes, saving time and resources.

Keywords: Molecular docking; flavonoides; angiotensin-converting enzyme

Graphical Abstract:



Introduction:

Flavonoids are phenolic compounds that can be found in various plant foods such as fruits, teas, wine, chocolates and others. Flavonoids have gained great interest due to their diverse pharmacological effects, such as antioxidant, anti-inflammatory, anticancer and antihypertensive action, among others [1].

There are several studies suggesting the mechanisms responsible for the antihypertensive activity of flavonoids. Recent studies have shown that flavonoids have antihypertensive activity due to the inhibitory action of the angiotensin converting enzyme (ACE) [2]. ACE inhibitors prevent the formation of angiotensin II.

In a study by Häckl et al. (2012) [3] it was observed that quercetin (**Figure 1-a**) satisfactorily inhibited the angiotensin-converting enzyme, through a mechanism similar to that of captopril. In a study by Souza et al. (2010) [2] it was observed that artemetin (**Figure 1-b**), another flavonoid, has been shown to be effective in reducing blood pressure through inhibition of angiotensin converting enzyme.

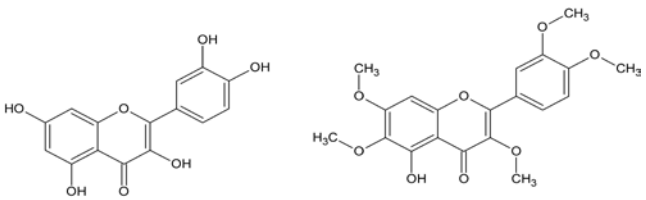


Figure 1. Molecular structure of the binders. A) quercetin; B) artemetin.

In this perspective, the objective of the present work was to perform a molecular docking study of the flavonoids quercetin and artemetin against the angiotensin converting enzyme in order to verify the interaction energy of the flavonoids with the active site of the enzyme and the functional groups of the ligands Responsible for interacting with ACE.

Materials and Methods:

The crystallographic structure of the enzymatic target ACE was obtained from the Protein Data Bank database [PDB: 1UZE]. The structures of the ligands were obtained through the PubChem base data and optimized by quantum chemistry calculations using the DFT (Density Function Theory) method, with B3LYP and base set 6-31G, through GAMESS software. The molecular docking study was performed using Autodock 4.0 software. Gasteiger and polar hydrogens needed for the power calculations were added to the enzyme, with the water molecules removed. The grid was positioned in the catalytic region of the enzyme with dimensions on the X-, Y- and Z-axis at 32 Å 30 Å and 38 Å, respectively, spacing 0.375 Å. The Lamarckian Genetic algorithm was chosen to search for the best conformations with 100 runs for each binder. During the search, the enzyme was held rigid and the binders were kept flexible. The method was validated by RMSD (Root-Mean-Square-Deviation), obtained through molecular re-docking of enalapril.

Results and Discussion:

Both artemetin and quercetin interacted with the active site of the enzyme attractively. With docking energy at -6.89 kcal / mol, artemetin was more stable in complex with the active site of the enzyme ACE, while quercetin presented docking energy at -6.63 kcal / mol. Both ligands interacted by hydrogen bonds with amino acids GLU 384, TYR 520, HIS 513, HIS 353, GLN 281, LYS 511 and the Zn^{+} ion, as shown in **Figure 2**.

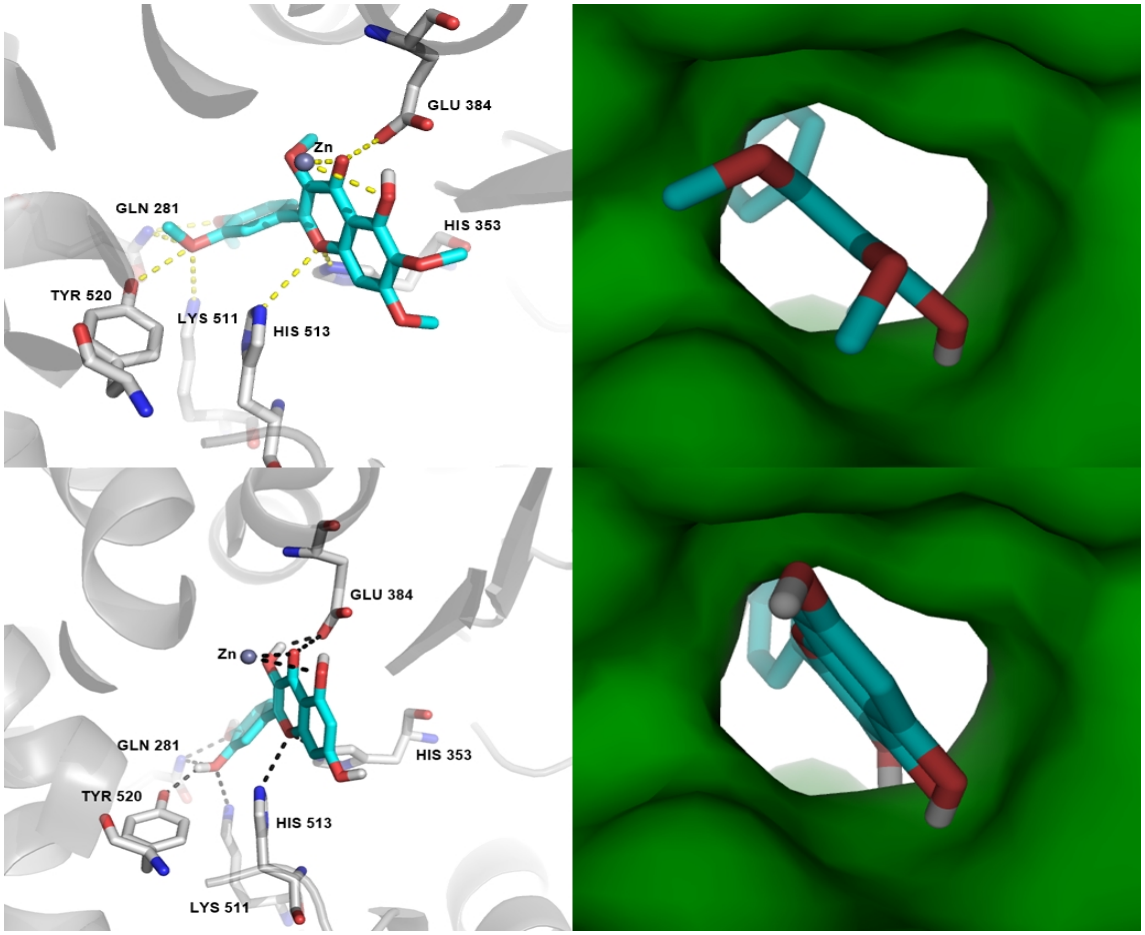


Figure 2. Flavonoid-Enzyme Complex. A) artemetin; B) quercetin

It is worth noting that hydrogen bonds were predominant in the interactions between the ligands and the amino acids of the active site of the enzyme, which shows that the phenolic hydroxyls of quercetin and the methoxyl groups of artemetin are of extreme importance for the activity of the ligands.

The redocking presented RMSD value = 0.89 Å, considering the most stable pose of the most populous cluster. This result is considered satisfactory when the RMSD (which measures the deviation) between the best pose and the complexed crystallographic ligand is less than 2.0 Å [4].

Conclusions:

It was observed that the interaction of flavonoids quercetin and artemetin with ACE occurred favorably, in accordance with the experimental studies. These interactions occurred mainly through the polar groups of the ligands. The study showed that the methodology used in the present study can be well used for the understanding of the interaction of pharmacologically active compounds with target enzymes, saving time and resources.

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DESIGN OF INNOVATIVE PRODUCTS FROM ELEMENTS OF THE ECUADORIAN AMAZON

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Abstract.
In the times of the fourth Industrial Revolution, product design reaches a new look. The objective of this work is to develop a methodology for the design of innovative products based on Amazonian elements, and the application of this in two practical case studies. Design methodologies were analyzed, common points were identified between them, a content matrix was constructed for the definition of design elements. The Amazonian raw material used is chontacuro (larvae of R. palmarum), a product rich in antioxidants and monounsaturated fatty acids (oleic), and two products are produced and result in: pâté and sausage with added value for human health. These products analyze the market, use the benchmarking technique, the form of industrial processing (formulations and processes) and define improvement actions. This work has a social value, because the larvae of this insect are part of the basic diet of the communities of the Ecuadorian Amazon.
Keywords: Food, amazon, Ecuador.

1. Introduction

The characteristics of the fatty extract of Rhynchosphorus palmarum (chontacuro) make it possible to raise potential use in the food industry; which contributes positively to the food sovereignty of the indigenous peoples of the Ecuadorian Amazon where the larvae are grown and traded (Sancho 2015).

The high prices and high demand of R palmarum ensure that productions are easily tradable and profitable; the knowledge on the use of this resource of the forest allows new productive activities, that tributar to the local development of the province of Pastaza of Ecuador (Sancho 2015).

The larvae are considered as a source of protein, fats, vitamins and minerals. Its fat content is abundant and consists mainly of polyunsaturated fatty acids (1.5%), saturated (36.8%), monounsaturated (60.4%) and unidentified fatty acids (1.3%). In addition to being a source of food, its sale has become an economic means for the Amazonian Indians who cultivate and sell the larva, its value varies between 30 and 50 cents on the local markets. There are people who sell them in bulk sporadically, their price then depends on the business between the buyer and seller. In typical Amazonian food establishments, the larvae are sold in different dishes: roasted has the value of $ 1 dollar per unit; in a maize dish consisting of larvae (4 to 5), tender palm stems wrapped in leaves of bijao and cooked to the embers, varies between $ 5 and $ 6 dollars; in other cases they are found to the plate served with green banana and yuccas boiled at prices similar to the maitos.

Its sale in the markets of the canton Pastaza has been frequent for approximately 15 years, being in the beginning marketed to a value close to 10 ctvs dollar. As it was something new and unknown for some people larva sales were directed towards the indigenous and mestizo settlers of the area, knowledgeable of its use as food and ancestral medicine. In the market there are 10 fixed places where you can always find larvae, weekly each of them have between 100-200 units of larvae and sell them without any problems at a cost of 50 cts., In the low sales weeks usually sell 3 units for 1 dollar (this case is not frequent).

The merchants of this animal are indigenous of the Amazon, they comment that can obtain 60 larvae of the palm of chonta, 100-150 of the palm of ungurahua and 100-200 or more in the palm of morete. However, the harvest of the larvae is at different times and in order to reach the maximum yield of each palm they must pass 3-4 weeks approximately.

The main limitation of this food is its easy decomposition when cooking because of the characteristics mentioned, so it becomes a challenge to obtain a product derived from this raw material, the objective of this work.

2. Materials and methods

The literature studied is analyzed, (Acuña, 2009; Allende, 2011; Ariza, 2009; Calomarde, 2000; Casp, 2012; Cortés, 2014 ; Cortez, 2000; Chauvin, 2014; Departamento de Organización de Empresas, 2007; Gaither, 2000 ; Jordi, 1989; Lockyer, /s.a/; Machado, 2007; Miranda, 2000; Ramirez, 2009; M. Salvador, 2004; V. Salvador, 2014; Sanchez, 2016; Suh, 1990; Turmero, /s.a/; Wodehouse, 2010), are defined as steps for product design, Figure 1:

- Idea generation
- Product selection
- Preliminary product design
- Construction of the prototype
- Testing and evaluation
- Definitive design of the product

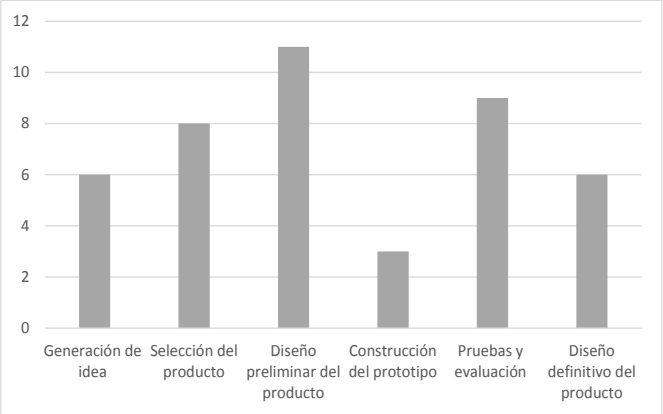


Figura 1: Product Design Steps.

3. Results and discussion

In this research larva oil is used as an enriching omega for the production of sausages. Rhynchophorus palmarum is the scientific name of the innovative ingredient of sausage, this larva contains unsaturated fats that are excellent for the body, more protein, which provides a unique added value that differentiate it from other brands. The Palmarun mark is designed as Figure 2. As the name that will represent the product is understood to come from a palm, for the same reason the image that was used is a palm abstraction in general and not specific, Figure 3. The sologan of the brand is EXÓTICAMENTE AMAZÓNICO.

Figure1: Mark the Palmarun

Figure 2: Label

In order to study the market acceptance of the product, the demographic segmentation of the population according to different variables is analyzed, Table 1.

Table 1: Variables of the market segment.

Targeting Base	Categories
Type of population	Urban and rural
Age	5-54 years
Sex	Female and male
Sexual Orientation	Heterosexual, homosexual, bisexual
Social class	High Medium and High
Civil status	Married, single, free marriage, widower, divorced, separated.
Race	Indigenous, white-European, Asian, black.
Nacionality	All

In the study of the demand is used the survey and interview to the traders and consumers, with focus to the larva Rhynchophorus palmarum or chontacuro, to know the possible acceptance of consumption of the same, in the province of Pastaza to undertake the possible industrialization of the Figure 2.

Figure 2: Survey to know the existing demand for a new Amazonian product.

Good afternoon, this is a survey conducted students of seventh Agroindustries. The information you provide us will be used to know the degree of market acceptance of a new Amazon product. The survey will not take more than 5 minutes. Thank you very much for your help.

Age: Year: Sex: F___ M___

1. Know the larva Rhynchophorus palmarum L. commonly called chontacuro or mallón? IF NOT _____ If yes, continue with the survey.

2. A consumed larva Rhynchophorus palmarum L. commonly called chontacuro or mallón? IF__ NOT ___

3. What price has paid for the larva Rhynchophorus palmarum L. commonly called chontacuro or mallón?

a) 0.50 cts. b) $ 1.00 c) $ 1.50 d) $ 2.00

4. How the larva Rhynchophorus palmarum L. has been consumed, commonly called chontacuro or mallón: Crude ___ b) Roasted ___ c) Maito __ d) Grilled ____ e) Other ___

5. Know the nutritional value of the larva Rhynchophorus palmarum L. commonly called chontacuro or mallón: YES ____ NO ____

6. Consume the larva Rhynchophorus palmarum L. commonly called chontacuro or mallón by its:

a) Taste b) Color c) Odor d) Nutritional value e) Medicinal use f) Other _____ which _____

7. Which of these processed products would you like to consume the larva Rhynchophorus palmarum L. commonly called chontacuro or mallón?

a) Sausage__ b) Conserva__ c) Oil__ d) Pate__ e) Others__

Results of the test

Of the 307 gender-related surveys 60% of 183 people are women and 40% are men. Of the 307 surveys concerning the city of origin, we conclude that the majority of respondents are from the Ecuadorian Amazon with 85%, Graph 1.

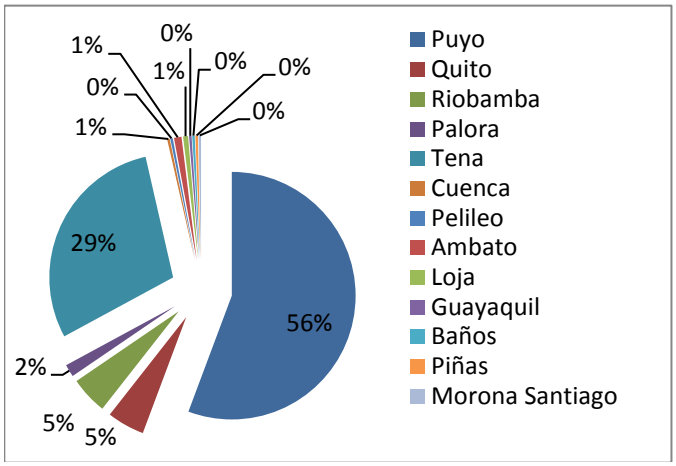
Of the 307 surveys concerning Rhynchophorus palmarum L., we concluded that approximately 99% of the people surveyed know the larva and only 1% do not. Of the 307 surveys concerning the form of consumption of the larva Rhynchophorus palmarum L. we conclude that; 36% consume it raw, 32% roast, 20% in maito and in lower percentages 10% fried, 2% has not consumed and with a person who has consumed the griddle.

Of the 307 surveys concerning which of these processed products would like to consume the larva Rhynchophorus palmarum L. we conclude that; 40% want to consume it in sausage, 31% in oil, 25% canned and in lower percentages of 1 and 2% pâté, oil and none. Graph 3.

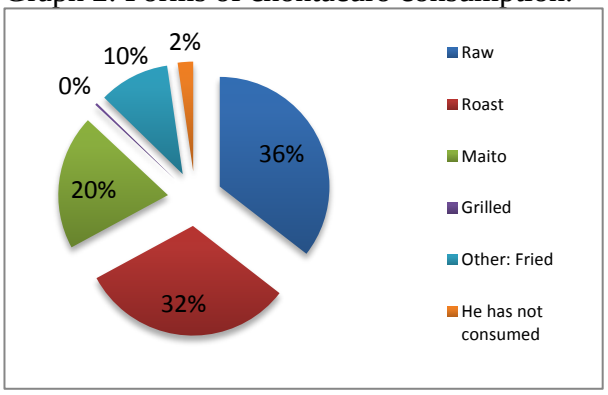
Of the 307 surveys on whether he knows the nutritional value of the larva Rhynchophorus palmarum L. we conclude that; 90% of people surveyed if they knew it while 8% did not and 2% did not answer the question.

Of the 307 surveys concerning what he likes about the larva Rhynchophorus palmarum L. we conclude that; 41% of the respondents liked the larva because of its flavor, 30% because of its nutritional value, 27% because of its medicinal use and in a lower percentage of 1% the color and did not respond, whereas with a non-response showing another and olor.

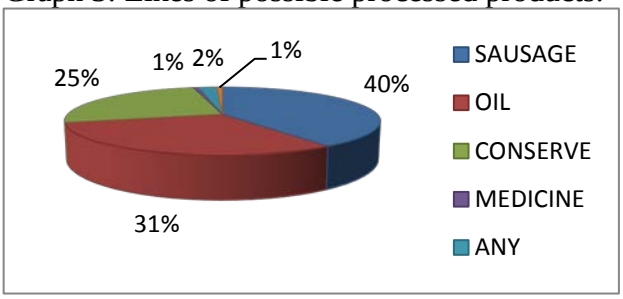
Graph 1: Source of respondents .



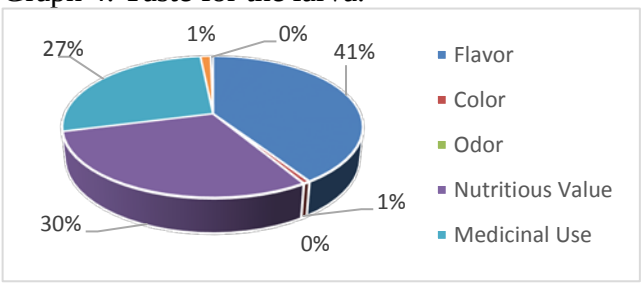
Graph 2: Forms of chontacuro consumption.



Graph 3: Likes of possible processed products.



Graph 4: Taste for the larva.



Testing and evaluation

The competition is analyzed the competition of the products in the market, to determine the characteristics that must be improved in each one of them. The benchmarking technique is used for this analysis. In which parameters such as brand, properties, composition, price, packaging, quantity, quality, packaging, the place where it is manufactured and the place of sale are studied.

Final Product Design

Formulation of the product

The ingredients of the sausage product, Table 2:

Table 2: formulation sausage PALMARUM

Ingredients	%
Pork Meat	24,00
Beef	30,00
Larvae of R. palmarum	16,00
Flour	22,78
Sugar	3,00
Salt	1,00
Curing salts	2,00
Polyphosphate	0,25
Monosodium glutamate	0,25
Flavorful	0,29
Colorant	0,49
Pork Meat	0,01
TOTAL	100

(Sarabia, 2012).

Obtaining Palmarum sausages consists of several operations:

- ✓ Reception: the raw material was selected cleaned and weighed
- ✓ Chopped: in the chopped, the pork and beef is cut into cubes of approximately 4 cm.
- ✓ Frozen: meat cubes as well as fresh R. palmarum larvae were frozen at minus 10 ° C for 12 hours in order to obtain a suitable texture.

- ✓ Chopped: it is done with a 5mm sieve and the meat ingredients are mixed.
- ✓ Curing: In this step the salt, sugar and curing salts were added leaving the mixture to cool at 2 ° C for six hours.
- ✓ I added dry ingredients and condiments: the mixture was kneaded for 20 minutes and where the flour and other seasonings were added.
- ✓ Texture: The texture is made in a cutter including the above mixture the ice, during this process it is verified that the temperature of the product does not exceed 8 ° C.
- ✓ Sausage: it was made in a hydraulic filler using 12 gauge synthetic casing dividing the sausages into portions of 10 cm in length
- ✓ Pasteurized: This was done by immersion in water at 70 ° C for 45 minutes ensuring that the internal temperature of the sausages is at 65 ° C, then cooled in ice water at 2 ° C.
- ✓ Packing: the packaging was done in vacuum in portions of 450 grams net weight.
- ✓ Storage: The finished product is stored in cooling at 4 ° C.

4. Conclusions

It is concluded that it is possible to produce an Amazonian product from chontacuro larvae that are possible to be rich in omegas and the taste of the clients. In relation to competition, there are similar parameters. The importance of this research is the application of scientific knowledge to the training of Agroindustrial students.

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The market of *Ilex guayusa*. Products, stakeholders and trends in the Ecuadorian Amazon Region

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Abstract.

Ilex guayusa is an important species for the economy of the Ecuadorian Amazon Region. The plant is well known as a traditional medicine and drink but is also a promising source of bioactive compounds for functional foods and cosmetic products. The aim of the study is to realize a review regarding the guayusa products, the involved companies and the future trends in the Ecuadorian Amazon Region. For this purpose have been analyzed 55 articles based on international and national studies. Relevant topics have been identified: innovation, energy drinks, sustainability, natural medicine and market. The study detected products and stakeholders. The new trend for *I. guayusa* is to develop innovative products with added value as phytocosmetics and nutraceuticals. Finally, to improve the I. guayusa value chain can enhance the incomes of local Amazonian communities of the Ecuadorian Amazon.

Keywords: Ilex guayusa, products, stakeholders, prototypes, trends

Introduction

The Ecuadorian Amazon region (EAR) represents 48% of the surface of Ecuador and covers only 0.2% of the Earth's surface, but hosts 70% of the world's known biological species [1], therefore Ecuador belongs to the 17 defined countries Megadiverse. In Ecuador have been identified the 7% of known vascular plant species, the 20% of orchids and 11% of ferns, [2]. Other authors mentioned that Ecuadorian biodiversity involves 18% of bird species, including 50% of those in South America and 7% of amphibians [3]. This important biological diversity is linked to a great cultural richness, represented by 14 ethnic groups or nationalities, of which ten live in the Ecuadorian Amazonian

Region (RAE) [4]. Among the plants of potential economic value is the guayusa (*Ilex guayusa*). Its high caffeine content places it within this group of plants with potential for the application of energy drinks, such as guaraná (*Paullinia cupana*), tea (*Camelia sinensis*) and coffee (*Coffea arabica*). The communities of the SAR usually consume guayusa daily in the form of infusion; however it is also used as raw material for other elaborations. Because of this it is possible to diversify the production of finished products, to obtain greater value, and to provide other family income.

The *I. guayusa* species, with its traditional and ritual uses, clearly represents how biological diversity is expressed between different cultures and is a relevant part of local populations [5,6].

It should be noted that the socioeconomic processes anchored to the guayusa are representative of a species very present in the ethnobotanical tradition, but that only in the last decades is assuming an organized production character for sale [7]. Derivatives of *I. guayusa* (infusions and homemade soft drinks) are part of the diet and traditional medicine [8], but the production of "guayuseros" begins to assume the characteristics of a chain of continuous value, Planned and capable of mobilizing constant volumes of production. The contribution of guayusa to the economy of producer families still requires a systematization of existing data, and also implies an analysis of the Amazonian socio-productive model, with special emphasis on the concepts of economy and market from the perspective of the native communities. A first financial evaluation based on information related to the conditions of its producers, the minimum price to not lose the investment in the production of a guayusa yard should exceed US $ 10.00, considering the sale of fresh leaves [9].

The objective of this article is to contribute to the improvement of the products of the *I. guayusa* value chain in the Ecuadorian Amazon, and its perspectives towards development.

Materials and Methods

The present research combines a systematic review methodology and exploratory type research in a descriptive stage. Were used several techniques such as: interviews, document review and analysis of indexed databases.

The interviews have been organized in order to obtain data regarding: identification of the product origin, localization of companies and products classification. Review methodology was performed adopting the following electronic databases: SciFinder, PubMed, Google Scholar, SciElo, Taylor & Francis and Scopus. Data were independently extracted from four reviewers and the final paper selections were completed avoiding duplication of data. The following keywords and their combination were used as key words for the research: *Ilex guayusa*, guayusa products, guayusa market. The reviewers selected articles were in English and Spanish language and were excluded data from patents. The above mentioned criteria allowed selecting eligible articles; we also considered some additional key papers for introduction and result chapters.

Results and Discussion

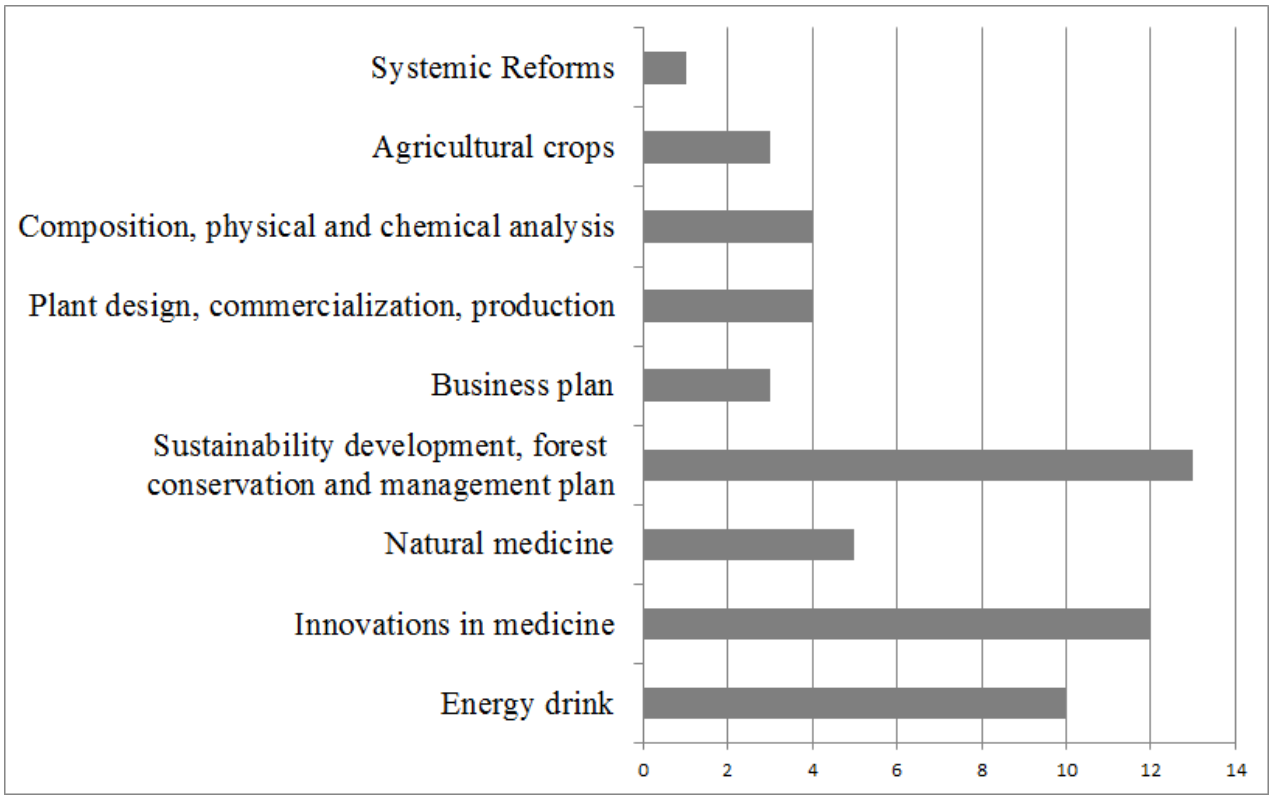
2.1. Investigation sources regarding *I. guayusa*

According to the result of the present research, the first article about *Ilex guayusa* was published in 1968 but there are bibliographic sources from missioners and scientist which are dated on XVII and XVIII centuries. Due to the knowledge about chemical composition and properties [10], several studies were carried out from the above mentioned period in many different sectors, such as:

- ✓ Energizing drink (Use)
- ✓ Innovations in medicine (Use)
- ✓ Natural medicine (Use)
- ✓ Sustainability conservation of forests and management plan (Studies)
- ✓ Business Plan (Studies)
- ✓ Plant design, marketing, production (Studies)
- ✓ Composition, Physical and Chemical Analysis (Studies)
- ✓ Crops (Studies)

From this information [11-48] we quantify the number of studies and uses referents to these topics, **Figure 1**. It is evident that the most treated topic is the conservation and management plan of the species and not as a value-added and diversified product.
Information about I. guayusa is available in: Ecuador, Perú, Argentina, Colombia, Bolivia, Brazil, Italy, México and Spain, as reported in **Figure 2**.
Finally, the *I. guayusa* supply chain can be observed in the **Figure 3**.

Figure 1. Uses and researches about *I. guayusa*.



Fuente:

Figure 2. Origin of information about I. *guayusa.*

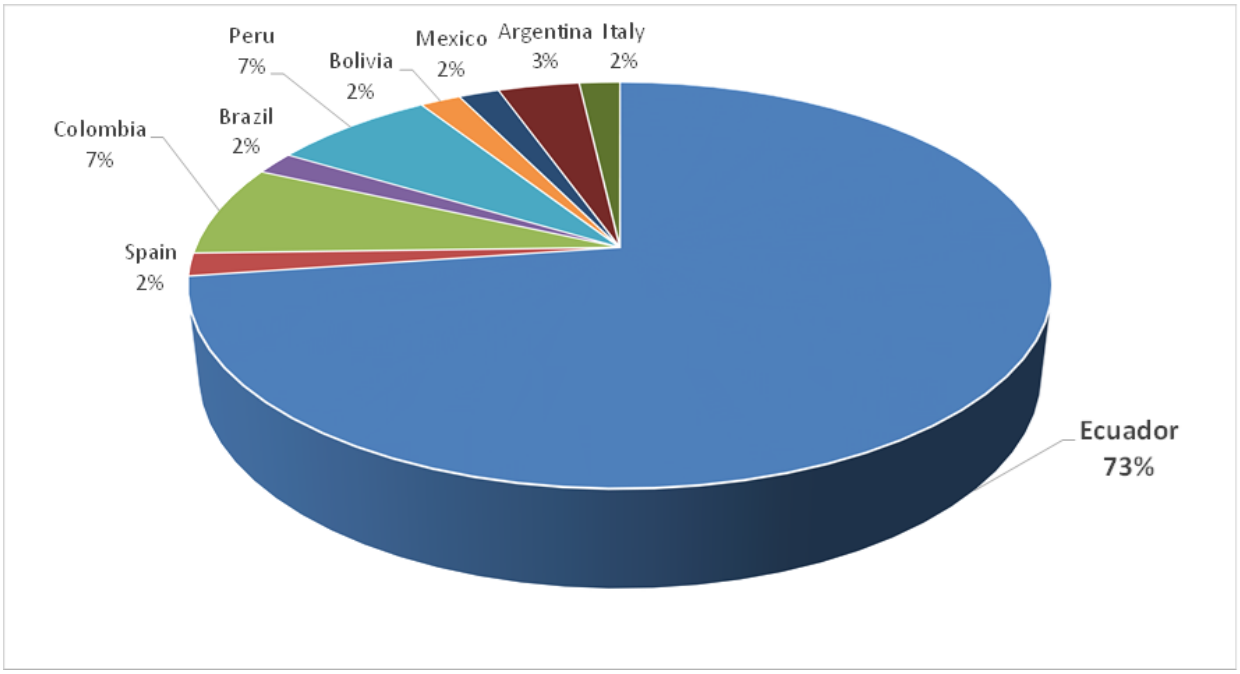
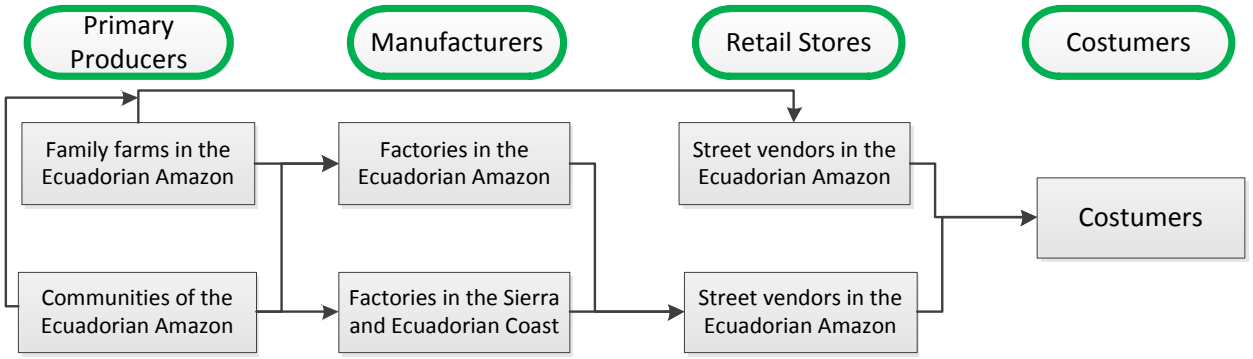


Figure 3. *I. guayusa* supply chain.



2.2 *I. guayusa* products and markets.

Regarding derived products, *I. guayusa* supply chain shows three essential links: primary produces, merchants and sellers; because the big market is focused on raw material.

The local market of *I. guayusa* is still based on traditional consumption as home-made infusion. Nevertheless, in the last decade several products have been developed including *I. guayusa* as raw material, as showed in **Table 1**. These products are manufactured in Ecuador. The data show that in Ecuador the varieties of *I. guayusa* products focus on tea, soft drinks and chocolate.

Regarding the national and the international market, **Table 2** shows which countries where actually the destination of sales activities.

Table 1. High add value products developed from I. guayusa.

Company	Product	Business area	Ref.
AROMAS DEL TUNGURAHUA	“Te de guayusa”	Tea	[43]
ECOCAMPO	“Guayusa TE’”	Soft drink	[44]
FUNDACION CHANKUAP	“Infusión de Guayusa e Ishpink”	Tea	[45]
	“Infusión de Hierba Luisa, Guayusa, Ishpink y Jengibre”		
GUAYUSA FRESH	“Bebida refrescante GUAYUSA FRESH”	Soft drink	[46]
PACARI	“Barra de chocolate PACARI con guayusa”	Flavored Chocolate	[47]
RUNA TARPUNA	“Runa Guayusa Amazónica Orgánica Original”	Tea	[48]
	“Runa Guayusa Amazónica Orgánica + Canela + Hierba Luisa”		
	“Runa Guayusa Amazónica Con Flor De Jamaica Y Naranja”		
	“Runa Guayusa Amazónica Orgánica con limón y menta verde”		
WAYKANA	“Hoja triturada de Té de Guayusa Verde”	Tea	[49]
WAJUKO	“Guayusa clásica”	Tea	[50]

Table 2. *I. guayusa* Markets

Company	Country	Ref.
AROMAS DEL TUNGURAHUA	Ecuador	[43]
ECOCAMPO	Ecuador	[44]
FUNDACION CHANKUAP	Ecuador	[45]
GUAYUSA FRESH	Ecuador	[46]
PACARI	U.S.A., Europe, Latin America	[47]
RUNA TARPUNA	Canadá, Colombia, Ecuador, Germany, Italy, U.S.A., U.K.	[48]
WAYKANA	Ecuador	[49]
WAJUKO	Ecuador	[50]

Conclusions

I. guayusa is a traditional plant from the Amazon region which represents a very important source of incomes for local communities. Beyond a socio economic impact on local communities, I. guayusa can be sustainable crop of Amazonian region but at the moment the supply chain presents a low level of integration and weakness of performance. The present systematic review highlights the need to promote innovative products which guarantee a higher return and an improvement in customer satisfaction.

Author Contributions

All the authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethnopharmacology, biological activity and chemical characterization of *Mansoa alliacea*. A review about a promising plant from Amazonian region.

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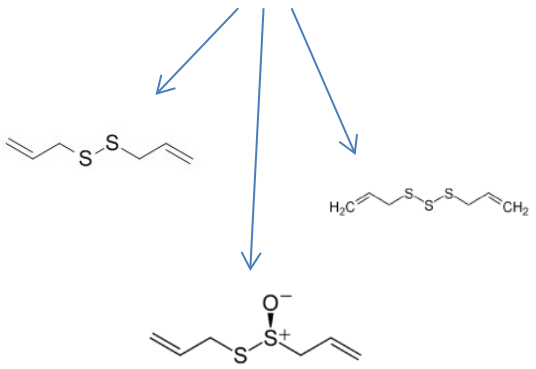
Graphical Abstract

Mansoa alliacea



Traditional medicine	
Magical and ritual uses	Cold, fever
Rheumatism	Food, spice
Antimalarial	Muscle pain

Biological activities	
Antioxidant	Antifungal
Antibacterial	Anti-inflammatory
Larvicidal	Antiplasmodial

Abstract.
Mansoa alliacea is a native plant from Amazonian basin and has great ancestral value for the local communities. *M. alliacea* is part of the traditional medicine for healers and shamans and has multiple uses due to the presence of several chemical constituents with important pharmacological properties. Plant derivatives are used as: antiseptic, diuretic, analgesic, antipyretic. Folk medicine is also related to the treatment of many diseases such as: reduction of blood pressure, against atherosclerosis, arthritis and rheumatism. Researches have also proven an appreciable antioxidant property, which revalue it for cosmetic purposes. Chemical composition of plant derivatives includes as main compounds: diallyl disulphide, diallyl trisulphide, alliin, allicin, propylallyl, divinyl sulfide, diallyl sulfide, dimethyl sulfide, daucosterol, beta-sitosterol, fucosterol, stigmasterol, iridoides and isothiocyanates, naphthoquinones, alkaloids, saponins, flavones. The present review includes ethnobotanical and pharmacological data that are related to the chemical composition of *M. alliacea*.

Introduction

Mansoa alliacea is a native plant to South America, exactly from the Amazonian basin, and has been recollected in Bolivia, Brazil, several Caribbean Islands, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, Nicaragua, Panama, Peru, Suriname [1].

M. alliacea is a native Amazonian plant belonging to the family of Bignoniaceae, its scientific name is *Mansoa alliacea* (Lam.) A. Gentry but has been classified with several synonyms [2].

M. alliacea is well-known with several common names in different countries, in Ecuador and Peru it is denominated “ajo de monte” or “sacha ajo”, in Brazil cipo-d'alho, cipo-alho, cipó-de-Alho, alho– da-mata, in Venezuela” bejuco de ajo”. *M. alliacea* grows in tropical areas of primary forest with rainfalls from 1800 to 3500 mm/year, in clay or sandy soils rich in organic matter, shaded or poorly shaded areas, temperatures between 20 to 26ºC, away from puddles because it is not resistant to floods. The name “ajo sacha” means false garlic, due to the characteristic garlic smell molecules present into the leaves [3]. As many other plants cited in traditional medicine [4,5,6], *M. alliacea* has been investigated in order to identify new potential useful drugs or a source of bioactive compounds.

Therefore, we aimed to compile an up to date and comprehensive review about *M. alliacea* studies that matches its traditional medicine uses, phytochemistry and pharmacology.

Materials and Methods

The present research was developed adopting the following electronic databases: Pubmed, ISI-Web of Science, Google Scholar, Scielo, Scifinder and Scopus. Data was independently extracted from four reviewers and the final paper selections were completed avoiding duplication of data. Four-teen scientific name (*Mansoa alliacea* and its 13 synonyms) were selected from the web page www.theplantlist.org and used as keywords. The whole list is: *Mansoa alliacea* (Lam.) A.H.Gentry, *Adenocalymma alliaceum* (Lam.) Miers, *Adenocalymma obovatum* Urb., *Adenocalymma pachypus* (K.Schum.) Bureau & K.Schum., *Adenocalymma sagotii* Bureau & K.Schum., *Anemopaegma pachypus* K.Schum., *Bignonia alliacea* Lam., *Pachyptera alliacea* (Lam.) A.H.Gentry, *Pseudocalymma alliaceum* (Lam.) Sandwith, *Pseudocalymma alliaceum* var. *macrocalyx Sandwith, Pseudocalymma pachypus* (K.Schum.) Sandwith, *Pseudocalymma sagotii* (Bureau & K.Schum.) Sandwith, *Pseudocalymma sagotii* var. *macrocalyx* (Sandwith) L.O.Williams.

The reviewers selected articles in English and Spanish languages avoiding data from thesis, patents, symposiums and congress.

The above-mentioned criteria allowed selecting 42 eligible articles and 7 additionally useful papers for the introduction, discussions and conclusions. 38 papers were rejected because did not satisfy the selection methodology or due to the lack of clarity in their procedures and methodologies.

Results and Discussion

Botanical description and traditional medicine

M. alliacea is an evergreen climbing shrub with semi-woody brunches that allows attaching on larger trees, used as growing supports. The plant reaches 3m tall and its leaves are bright green, slightly coriaceous, opposite and characterized by two ovate leaflets of about 15cm long. Flowers have funnelform corolla up to 6-9 cm long, with campanulate calyx, 5-8 mm long. They are violet colored and grow in terminal or axillary raceme inflorescences. Fruits are elongate capsules up to 25-35 cm long which contain transverse-oblong seeds characterized by wings broad. Leaves of *M. alliacea* are characterized by a pungent garlic-like smell when crushed [7].

M. alliacea is an emblematic plant for many Amazonian tribes; root, stem, leaves and flowers have been described as the parts of the plant which are useful for different traditional treatment. **Table 1** summarizes several traditional medicine uses which include ritual and magical application.

Table 1. Traditional medicine, magical and ritual uses of *M. alliacea*

Year	Country	Ethnic group	Traditional medicine, magical and ritual uses	Ref.
1984	Brazil	n.r.	Colds, fevers	[8]
2000	Bolivia	Tacana	Abdominal pain, fever, intestinal parasites, rheumatic pain, ritual uses	[9]
2002	Perú	Shipibo – Conibo y Ashaninka y mestizo	Anti-malarial	[10]
2008	Ecuador	Kichwa	Food, spice	[11]
	n.r.	n.r.	Analgesic, anti-arthritic, anti-inflammatory, antipyretic, anti-rheumatic, antitussive, depurative, purgative, vermifuge	[12]
2008	Surinam Brazil Guianas	n.r.	Analgesic, anti-rheumatic, anti-arthritic, antipyretic, colds, constipation, cough, epilepsy, fevers, food, headache, insecticidal, malaria, mystical and magical rituals, nausea, pneumonia, rheumatic pains, treatment of pains and muscular fatigue, tonic, useful for healthy pregnancy, vermifuge	[13]
2009	Peru	San Martin Quechuas or Lamas Quechuas	Rheumatism	[14]
	Peru	Yanesha	Fever, flu, rheumatic pain	[15]
2010	Panama	Téribe	Aggressive dementia	[16]
2011	Brazil	n.r.	Fly repellant (ethnoveterinary reports)	[17]
2012	Brazil	n.r.	Magical and ritual uses (evil eye)	[18]
2014	Brasil	Riverine communities	*Amoeba*, bath, cough, flu, pain of head	[19]
2014	South America (Brazil, Peru)	n.r.	Analgesic, antiarthritic, anti-inflammatory, antipyretic, antirheumatic, colds, constipation, depurative, nausea, pneumonia and respiratory disorders, purgative, vermifuge,	[20]
2015	Ecuador	Achuar	Cold	[21]
	Ecuador	Waorani	Magic rituals, Topical anesthetic	[22]
	n.r.	n.r.	Analgesic, anti-inflammatory, antirheumatic, body aches and pain, muscle aches, rheumatism, treatment for arthritis, injuries and pain	[23]
2016	Ecuador	Kichwa	Infections, muscular system disorders, respiratory diseases	[24]
	Ecuador	Kichwa and Mestizo	Anesthetic, cold, muscle pain, ritual use	[25,26]
	Brazil	Caruaru	Magical and ritual use “Limpeza do corpo” (Body cleaning); “Proteção” (Protection)	[27]
2017	Brazil	Riverine inhabitants	Magical and ritual use “Doença-do-ar” (air diseases); “espante” (fright); “vento caído” (fallen wind); “derrame” (leakage)	[28]
	Brazil	n.r.	Antifungal, antiviral, antimicrobial, anti-inflammatory, fever, rheumatism	[29]

n.r. – not reported

2.2 Phytochemistry and biological activity

Several authors focused their researches on the phytochemistry of *M. alliacea*, also adding some interesting study regarding the biological activity of its phytocomplex. Results are respectively reported in **Table 2** and **Table 3**.

Table 2 – Phytochemistry of *M. alliacea*

Plant part(s) used	Plant extract(s)	Main compound(s)	Ref.
Leaves	Essential oil	allyl methyl trisulfide, allyl propyl trisulfide, dithiacyclopentene, allyl propyl disulfide, allyl methyl trisulfide, allyl isobutyl sulfide, allyl isobutyl disulfide, diallyl monosulfide, diallyl disulfide, diallyl sulfide, diallyl trisulfide, diallyl tetrasulfide, 3-vinyl-1,2-dithi-4-en, allyl tri-sulfite, tetrasulfite, di-2-propinil, trisulfide, di-2-propenyl, 1-Octen-3-ol, 1-octen-3-ol, , allyl methyl disulfide, allyl methyl tetrasulfide, propenyl propyl trisulfide, , 3-vinyl-1,2-dithi-4-ene, 3-vinyl-1,2-dithi-5-ene, trithiacyclohexene, 2-methyl-2-pentenal, cis-dipropenyl disulfide, trans-dipropenyl disulfide, methyl salicylate, 3,4-dimethyl-2,3-dihydrothiophen-2-one, nonanethiol, diisoamyl disulfide	[8] [13] [20] [30]
	Petrol extract	n-alkanes C25-C35, n-alkanols, 24-ethylcholest-7-ene-3β-ol, fucosterol, 3β-hydroxyurs-18-en-27-oic acid, 32-hydroxyhexatriacontan- 4-one, 19-hydroxyhexatriacontan-18-one, 34-hydroxy-8-methylheptatriacontan-5-one, pentatriacont-1-en-17-ol, β-sitosterol, stigmasterol	[13] [31]
Flowers	Essential oil	diallyl disulfide, diallyl tetrasufide, diallyl trisulfide, 1-octen-3-ol	[8] [13]
	Methanol extract	Alliin, β-amyrin, apigenin, apigenin-7-glucoside, apigenin-7-glucuronide scutellarein-7-glucuronide, apigenin-7-glucuronyl glucuronide , apigenin-7-O-methylglucuronide, cyanidin-3-rutinoside, β-sitosterol, β-sitosteryld-glucoside, luteolin, 7-O-methylscutellarein , ursolic acid	[13]
Inflorescences	n.r.	benzaldehyde (54.8%), benzyl thiol (20.3%) dibenzyl disulphide (18.0%).	[30]
Wood (bark)	Dichloromethane phase of the methanol extract	9-methoxy-α-lapachone, 4-hydroxy-9-methoxy-α-lapachone	[13]
Plant	Ethyl acetate extract, Aqueous Infusion	*p*-coumaric acid, ferulic acid and resveratrol	[32]
	Dry extract	Betulinic acid	[33]
n.r.	n.r.	9-methoxy-*α*-lapachone	[34]
n.r.	n.r.	alliin, allicin, allylsulfoxide, diallyl sulfide, divinyl sulfide, propyl allyl disulfide, stigmasterol	[35]
n.r.	n.r.	alkaloid, ferulic acid, flavonoids, cumarin, p-coumaric acid saponin, resveratrol, sulfur compounds tannin, terpenes, caffeic acid	[36, 37]

n.r. – not reported

Table 3 - Biological activities of several *M. alliacea* extracts**.**

Plant part(s) used	**Biological activities**	**Ref.**
Leaves	Allosteric dose-depend effect on the muscarinic acetylcholine receptor M2 subtype	[34]
	Antimycotic effect against *Aspergillus flavus* and *Aspergillus niger*, antiaflatoxigenic effect. Non-phytotoxic effect.	[38]
	Antifungal activity against *Colletotricum gloeosporioides* Penz and *Botryodiplodia theobromae* Pat.	[39]
	Antifungal activity against *Microsporum gypseum*	[40]
	Antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*	[41]
	Larvicidal activity	[20]
	Partial insecticidal activity on *Bemisia tabaci* eggs, nymphs, and adults	[42]
	Inhibition effect of T3-HA cancer cells (tertiary liver metastatic tumor) at low concentrations and cytotoxic effect at higher concentrations.	[22]
	Antioxidant activity	[23]
	Antifungal activity against: *Alterniaria brassicae*, *Colletotrichum capsici*, *Curvularia lunata*, *Alternaria alternata*, *Alternaria brassicola*, *Alternaria carthami*, *Fusarium oxysporum*, *Fusarium udum* Antiviral activity against virus-mild mosaic. Antioxidant activity. Prostaglandin synthesis inhibition. Biocide activity against *Hipsiphyla Grandella* and *Anofeles*	[13]
Dried flowers	Blood cholesterol lowering effect in rats	[13]
Root and stem	Anti-inflammatory activity	[13]
Plant	Antiplasmodial activity	[43]
	Inhibition of the normal growth and development of the insect due to a prolonged and delayed larval and pupal duration	[20]
	Larvicidal activity against mosquito larvae (*Culex quinquefasciatus*).	[44]
	Sinergic larvicidal activity against *Anopheles stephensi* and *Culex quinquefasciatus* if used with using the synthetic insecticide temephos	[45]
n.r.	Antiallergic, antibacterial, antifungal, anti-inflammatory, antioxidant, antiviral, suppression of tumor growth	[36]

n.r. – not reported

Conclusions

Despite a big number of ethnobotanical data, the phytochemistry and biological activities of *M. alliacea* have been partially investigated and the main results have been obtained only in the last ten years. The presence of orgasulfur compounds in other species motivated a wide cluster of studies mainly focused on health promoting effects [46, 47, 48, 49]. These findings and the results presented in the present conference paper justify more pharmaceutical and nutraceutical researches as a new trend of investigation for *M. alliacea*. Finally, interesting preliminary results have been achieved also regarding the larvicidal activity and phytopathogen control.

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Author Contributions

All authors contributed extensively to the work presented in this paper.

Conflicts of Interest

The authors declare no conflict of interest.

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LATTICE DYNAMICS OF CARBON NANOTUBE INTERACTING WITH HYDROXYAPATITE

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Abstract:

One of the characteristics to be achieved by biomaterials is to have similarity to the host material. In cases of bone substitution, hydroxyapatite (HA) shows considerable similarities to human bone. However, it shows itself with low mechanical resistance, which in many cases makes it difficult to apply in areas subject to high mechanical stress. Carbon nanotubes (CNTs) have low density and strong covalent bonding between their atoms, which gives high mechanical resistance to the material. For this reason, the influence with the HA structure of single wall, pristine CNTs and functionalized with organic hydroxyl (-OH) and carboxyl (-COOH) clusters with functionalization concentrations of 5, 10, 15, 20 and 25% were studied by means of computational simulation. The software used to perform the calculations was GULP and the applied force field was DREIDING. The lattice dynamics revealed that pristine CNTs have a lower interaction with HA because of their high chemical stability. In contrast, the CNTs functionalized with -OH and -COOH interacted better with the HA matrix, indicating that the functionalization may be a factor that optimizes the interaction between these materials. The results of the Root Mean Square Deviation (RMSD), for all systems with functionalized CNTs, reach a stability around a point of equilibrium around 15ps, proving that the interactions are stable. The calculation of the Bulk modulus indicated that we can control the ability of this material to resist volume changes for a given applied pressure, modifying the amount of functionalization present in the CNTs. The functionalities of 20% of -OH and -COOH present greater difficulty in undergoing deformations (greater value of the Bulk module). On the other hand, the functionalizations of 15% of -OH and 5% of -COOH were the most vulnerable to deformation (lower value of the Bulk module). Finally, the Poisson ratio indicates that the theoretical model applied to the systems is reasonable since these coefficients were within the proposed theoretical range.

Keywords: *hydroxyapatite, Carbon nanotubes, lattice dynamics*

Introduction: The hydroxyapatite (HA), has similar physical and chemical properties with the mineral phase of the bones and human teeth. The characteristics of its structure allow its use in implants and bone grafts, presenting high compatibility with the living tissues of the hosts [1].

However, HA has a small mechanical resistance which may influence bone regeneration in the applications of some types of bone grafts, especially in areas subject to high mechanical stress [2,3]. One possible solution would be the introduction of carbon nanotubes (CNTs) to this material, perhaps the incorporation of this material improves the mechanical properties of hydroxyapatite.

Discovered by Iijima in 1991, the CNTs have several properties of interest such as large surface area, nanocapillary, and environmentally susceptible electronic properties that are exposed [4]. Even with all these properties, there is a discussion about a low reactivity of CNTs, due to its high chemical stability. However, many of the applications take into account a prior treatment, making oxidation that promotes better dispersion or introducing hydrophilic groups such as hydroxyl (-OH) and carboxylic (-COOH) which leads to the functionalization of the material, so we have a probable increase in the reactivity of the CNTs [5].

We use computational simulation based on lattice dynamics, to investigate HA interactions with single wall CNTs pristine and functionalized with the organic hydroxyl groups (CNT-OH) and carboxyl (CNT-COOH).

Materials and Methods: It departed from the cif data-26204-ICSD which contains all the information concerning the crystalline structure, to build the input files [6]. The unit cell of hydroxyapatite is mainly found in the spatial group P63/m with axes a=b=9,424 Å c=6,879 Å, this was repeated generating a supercell of 1xa, 4xb, and 6xc that would provide some flexibility of movement for CNTs. Periodic conditions were employed in the y and z directions. A vacuum of 20 Å was added in the x direction.

A CNT was introduced with a separation of approximately *3* Å in relation to the HA surface. The CNTs have chirality *n=10* e *m=0,* repeated in three units and passivated with hydrogen. Because pure CNTs exhibit high chemical stability, in practice, this material generally requires a treatment of its surface, making them more reactive. Due to that, we suggest the functionalization of CNTs with oxygenated organic radicals (-OH and -COOH). These groups act as mediators in the interaction between CNTs and other substances. The CNTs were used both as pristine and functionalized with -OH or -COOH, and the percentages of functionalization of CNTs were *(5,10,15,20,25) %.*

All calculations were performed using the software GULP. The force field used was the DREIDING. This field uses general force constants and the parameters are defined for all possible combinations of atoms [7]. The Canonical Ensemble (NVT) was used, which typically describes a system in contact with a thermal reservoir through a fixed and impermeable diathermic wall, the contacting systems will change their coordinates until they reach a common equilibrium state. The production time was 30 ps, specifying the simulation time to be spent collecting production data for further analysis.

Results and Discussion: In figure 1 is indicated the Root Mean Square Deviation (RMSD). For the HA system we have few changes and after 4 ps the structure remains in an equilibrium position. Already the Hydroxyapatite system plus pristine carbon nanotubes (HACNT) does not reach this stability, probably due to the pristine CNT being little reactive, indicating a high movement on the surface of the HA and not reaching a point of equilibrium. For all other HA systems plus CNT functionalized with -OH or -COOH a stability around a break-even point was reached from approximately 15 ps.

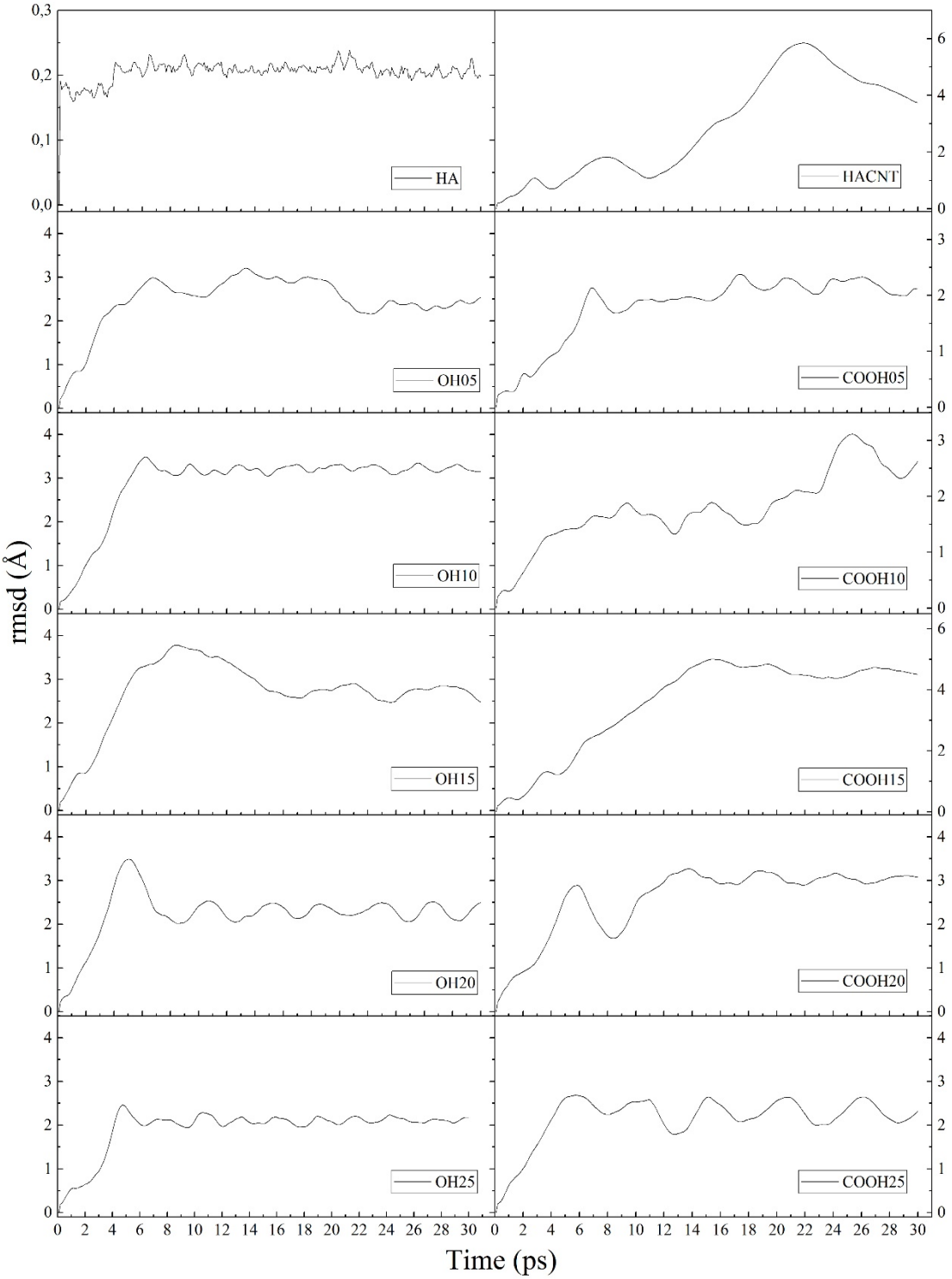

Figure 1- RMSD calculated for the HA and HA structures plus pristine CNT and functionalized with -OH(5,10,15,20,25) % and -COOH(5,10,15,20,25) %.

The measure of the ability of a material to withstand the volume change for a given applied pressure (Bulk module) is indicated in figure 2. The points -5 e 0 in the following graphs indicate the HA and HACNT systems respectively and the other points the percentages of functionalization of -OH or -COOH to which the CNTs were subjected. In the figure 1(a) we have the calculated Bulk module for the -OH systems. For these systems, we observe alternating variations and the extremes occurring for the functionalization of 15 and 20 % in -OH, being that of 15 % the easiest to undergo volume changes (lower Bulk module), already for the system with CNT functionalized with 20% of -OH we have the greatest difficulty of deformation (highest value Bulk module).

The result for the carboxylated system is shown in figure 1(b). As the percentage of functionalization increases, we have an increase in the Bulk module, achieving a partial stability to a percentage above 15 % in -COOH, being that for 5 % in -COOH the easiest to undergo deformation (lower value of Bulk module) and the 20% that is more difficult to deform (greater

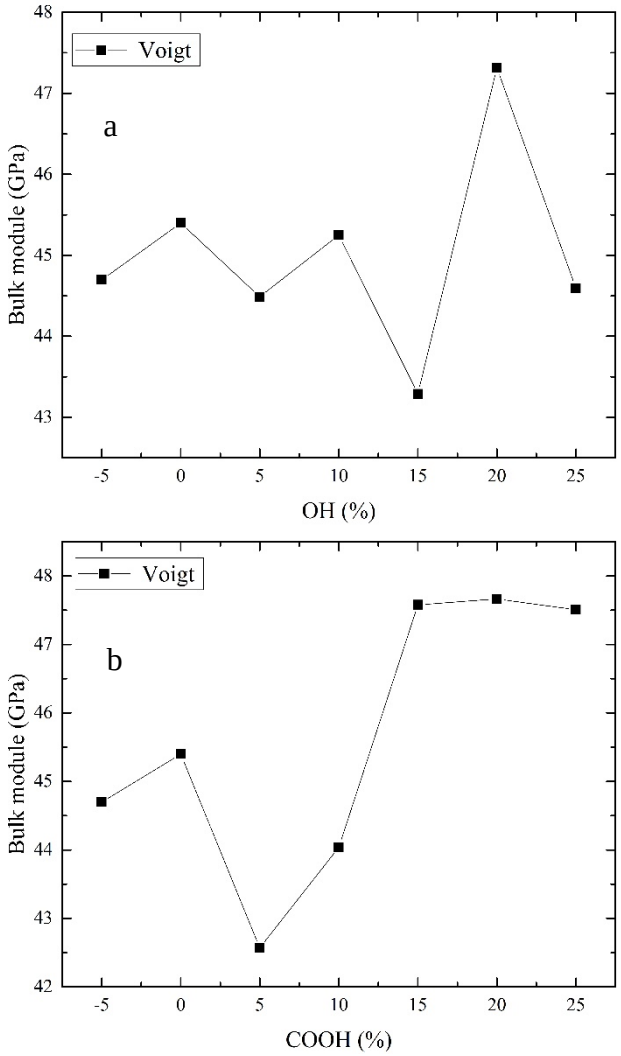

Figure 2- Bulk module versus functionalization percentage.

value of the Bulk module).

The Poisson ratio measures the deformation that is established between orthogonal deformations [8]. And through algebraic calculations, this interval for the Poisson coefficient is between $0 < \sigma < 1/2;\ -1 < \sigma < 0$ [9].

If we apply a force in the direction of x, we have that the relation between the lateral deformation and the longitudinal deformation of the force application does not present values typical of the Poisson ratio, these non-significant results were observed for both systems, -OH and –COOH. Probably the event is related to the fact that this was the direction where we created the vacuum for the introduction of CNTs.

As for the forces applied in the y-direction, the relation between the lateral deformation x and the longitudinal deformation of the force, we observe Poisson coefficients close to zero, indicating that the system suffers high deformations in the direction of x. For both systems when the force is applied in the direction of y and z, the highest coefficients were observed for the 20% of functionalization indicating that, for these directions, we obtained little deformation.

Conclusions: The calculation of RMSD showed that the pristine CNT does not establish strong enough bonds to attach to the HA structure, while the functionalized ones bind to the structure, reaching a position of equilibrium from 15 ps.

The Bulk modulus indicated the functionalization with the highest resistance to deformation and the Poisson ratio indicates that the theoretical model used is reasonable for the description of the systems since these coefficients were within the proposed theoretical range of (0-0.5), except when analyzing the direction of x because it presents the region of vacuum where it was introducing the CNTs generating a greater flexibility of movement of the atoms.

The results obtained from the theoretical calculations indicate that it is possible to change the mechanical properties of HA with the introduction of the CNTs and, for certain percentages of functionalization, we had significant changes in the properties related to the elastic constant of this material.

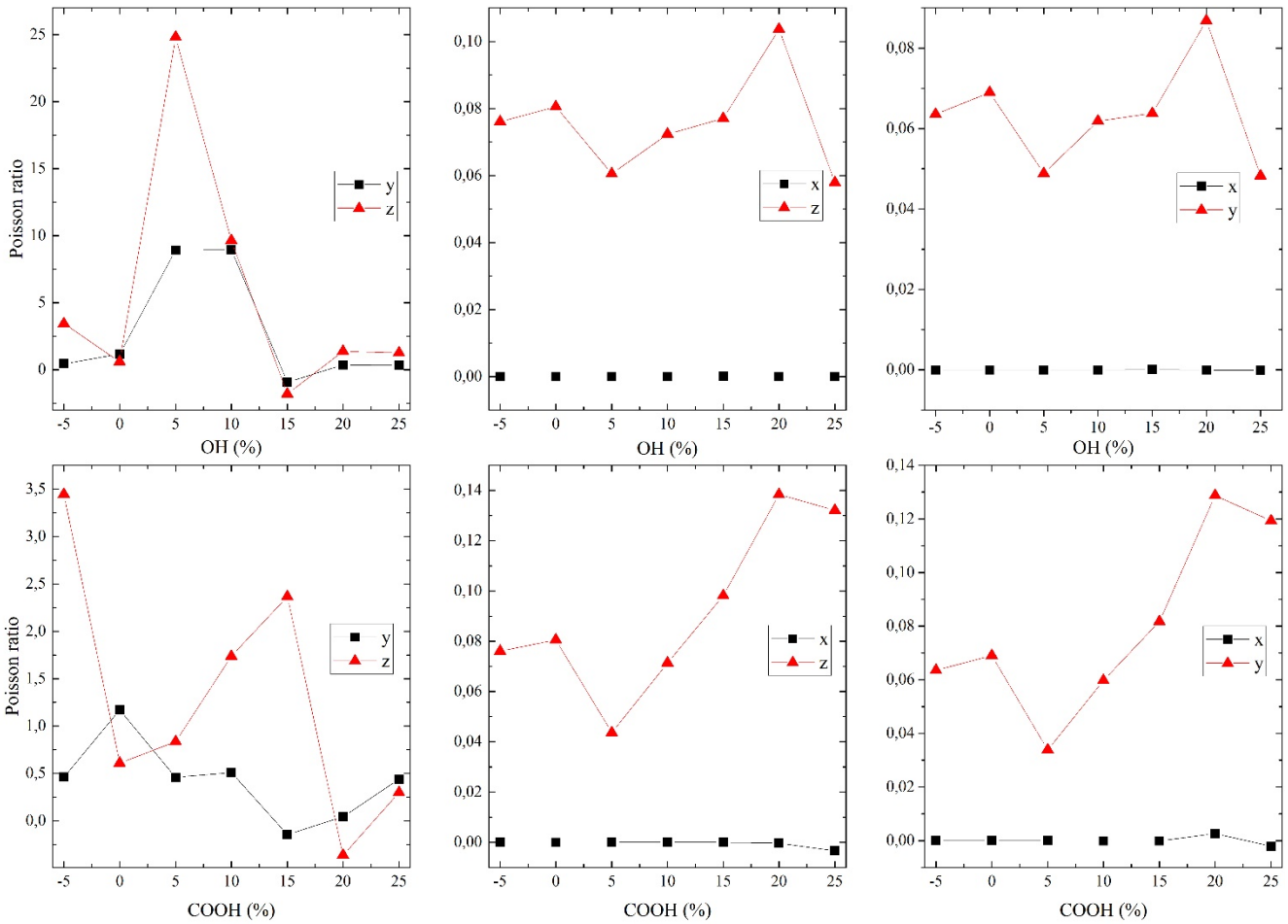

Figure 3- Poisson ratio for the HA point -5 systems, HACNT point zero, and HA with CNT(-OH ou COOH)(5,10,15,20,25) %.

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Control measures in the countries sending remittances and financial impact on the countries receiving them (Mexico and The United States)

Permanent migratory flows in OECD[1] countries have increased for the third consecutive year, according to preliminary data of 2016. In that year, around 5 million people emigrated on a permanent basis to countries of the OECD, a figure well above the previous maximum, observed in 2007 before the economic crisis.

In 2016 the employment rates of the migrant population of OECD countries remained relatively stable at 67.4%, which represents an increase of one percentage point compared to the previous year.[2]

The movement of people from one place to another is a phenomenon that has existed throughout the history of the world, which has brought a series of benefits for both, the country of origin and the country of destination. Remittances are the main economic source that in the last ten years have benefited thousands of families that depend on their relative residing in another country to meet their needs.

The main items that remittances cover are: food, health, housing and education.

These money transfers play a key role in the Mexican economy, since Mexico is the main recipient of remittances in Latin America. Today, more than a million Mexican households receive remittances from relatives living abroad, according to the information of the central bank of Mexico (Banxico). The total amount of family remittances in May of this year is 2,586.425023 million dollars.[3]

There is no doubt that the United States is the main sending country of remittances, and Mexico the largest receiving country; followed by Guatemala, El Salvador and the Dominican Republic, among others.[4]

The United States intentions to cut money transfers off or impose a tax on remittances has become a matter of concern to people who send money to their families in Mexico and could even have a great impact on the Mexican economy.

Mexicans residing in the United States as well as their relatives in Mexico live in a constant state of uncertainty. Indeed the United States' measures could make frequent money transfers complicated and more expensive.

Juan José Ling, an expert on the topic and chief economist of BBVA Bancomer, foresees an important rise in remittances sent to Mexico, at least in the short term. The increase may be motivated by the fear that Donald Trump carry out the threats made against mexican migrants during his campaign trail, including the halt of remittances. [5]

Since April 2016, the campaign staff of the current president of the United States, Donald Trump, assured that the construction of a border wall could be paid by cutting a portion of remittances off to Mexico, taxing money transfers, or by raising the fees for visas.[6]

On February 7, the Republican congressman from Alabama, Mike Rogers, announced that he would introduce an initiative to create a law to compel migrants to pay a tax of 2 percent in remittances, to cover the cost of the wall ordered by Trump.

Control measures implemented by financial institutions generate profits for the countries sending remittances at the expense of migrants who religiously send money to their families. Some of these measures are fees and limits for money transfers. Nevertheless, recipient countries are mostly benefit from these remittances since these incomes help mainly to the alleviation of poverty.

In case taxes are added to remittances, some measures must be taken: Firstly, the receiving families should anticipate the changes by looking for investment opportunities in their own country; thus contributing to the economic development of Mexico. Secondly, the Mexican government must guarantee the proper use of remittances by implementing a productive process that could create employments, therefore a sustainable economic and social development.

On the other hand, to open of a retirement savings account, called Afore in Mexico, is also recommended because it could be a productive way to take advantage of remittances, suggested Carlos Marmolejo, vice-president of operations of the national commission for the retirement savings system (CONSAR).

Mr. Mamolejo explained that one of the most important projects of this commission in 2017, is that in June a part of the remittances sent by migrants may be deposited in the Afore that they once had in Mexico or in one of a relative. He recalled that the intention is that a part of the 25 billion dollars a year sent Mexican migrants to the country be used to finance their retirement.[7]

According to the Mexico's central bank and the organism in charge of protecting and defending consumers of financial services (CONDUSEF)[8], the best way to send money is the service "Directo a Mexico" that charges only five dollars regardless the transfer's amount. This option is convenient, secured and offers the best currency exchange.

[1] The Organization for Economic Cooperation and Development (OECD) is an intergovernmental organization that brings together 34 countries committed to market economies and democratic political systems, which together represent 80% of world GDP.

[2] OECD (2017), *International Migration Outlook 2017*, OECD Publishing.

[3]Bank of Mexico 2017 Table Summary Income for family remittances consultation July 12, 2017 http://www.banxico.org.mx/SieInternet/consultarDirectorioInternetAction.do?accion=consultarCuadro&idCuadro=CE81

[4] Maldonado, Rene; Cervantes, Jesus. Remittances to Latin America and the Caribbean in 2015-2016, Accelerating growth. Center for Latin American Monetary Studies (CEMLA), Mexico City.

[5] The Universal, Copyright Grupo de Diarios AmÃ © rica-GDA / El Universal / Mexico. January 28, 2017, Mexico City

[6]Vlex Global, report written by Claudia Guerrero, February 13, 2017. Migration and Remittances Yearbook 2016

[7]Vlex Global, report written by Jessika Becerra, December 21, 2016

[8] National Commission for the Protection and Defense of Users of Financial Services

KEY WORDS: Migration, Remittances, Commissions, Income, Financial impact.

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Polyphenol extracts from Cocoa (*Theobroma cacao*) and Chuchuhuasi (*Maytenus macrocarpa*) as potential natural Amazonian antioxidants

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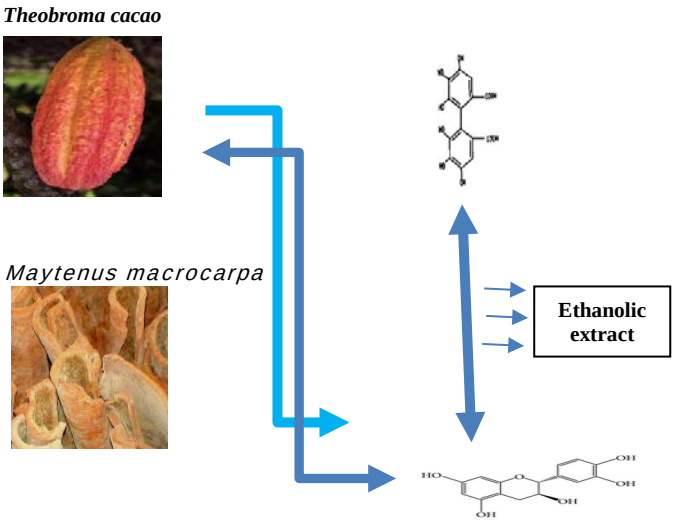
Graphical Abstract



Abstract.

The antioxidant activity, because of the presence and polyphenols chemical structure, has lead their interest in the promising valuable effects on health in foods and beverages with high content in polyphenols. Antioxidants protect the body from free radicals, which are highly reactive molecules that could damage it at the cellular level. This damage prompted by free radicals can increase the risk to the cancer development, cardiovascular diseases and other degenerative diseases. The present work aim is to achieve polyphenolic extracts from cocoa seeds (*Theobroma cacao*) and from Chuchuhuasi (*Maytenus macrocarpa*) cortex (bark) as potential natural Amazonian antioxidant source. The species were collected at the Research, Postgraduate and Amazonian Conservation Center, the botanist Dr. David Neill identified the specimens and they are to be found in the Ecuadorian Amazonian Herbarium (ECUAMZ). Polyphenolic activity was quantitatively determined in hydro alcoholic extracts by Folin Ciocalteau analytical method. Total polyphenolic concentration results based on gallic acid in the cocoa seeds (*Teobroma cacao*) extracts and chuchuhuasi (*Maytenus laevis)* cortex (bark) extracts were 24.44 and 19.90 $mg.mL^{-1}$, respectively. Thus, it was possible to conclude that the two Amazonian species under study provide relevant results in relation to the presence of total polyphenolic compounds, which allows the preliminary expectation of a promising antioxidant activity. This preliminary study allowed identifying, for the first time, new

	polyphenols sources in promising plant species of the Ecuadorian Amazon region.

Introduction

Ecuador is considered one of the most biodiverse countries on the planet. This biodiversity is not limited to the number of species per area but it also includes several natural environments or ecosystem types (Bravo, 2014).

The Amazon Region of Ecuador contains an important ecosystem variety; especially, its tropical rainforest is considered as one of the richest and one of the most complex habitats allover world for plants and animals (Matamoros, 2007). According to the book "Useful Plants of Ecuador", there are 5,172 useful plants in the country; this means that three out of ten species of plants growing in the country have some utility for people (De la Torre et al., 2008). From the species with edible uses, only 131 are cultivated (8%). The others are wild species or in the domestication process. From the total edible species, 80% are fruits or seeds, 12% are leaves and, on the other hand, 80% are consumed in raw form, 13% are prepared as drinks or juices, tea or aromatic waters or macerated with alcohol; 8% are used as sweet preserves and 5% are used as soups and stews.

In the Amazon forest there are ancestral plants with medicinal properties; cocoa is a tropical fruit, its crops are mostly found in the coasts and in the Amazonian region; it is a tree with small flowers that are observed in the branches and produce a cob that contains grains covered of some pulp rich in sugar, the grains have a high biological activity due to the occurrence of antioxidants like polyphenols that belong to the most extensive group of non-energetic substances existing in foods of plant origin. In recent years it has been shown that a diet rich in plant polyphenols can improve health and decrease the incidence of cardiovascular diseases (Quiñones et al., 2012).

Antioxidant activity, as a consequence of the polyphenolic content, has centered the interest on the promising beneficial effects on human health of foods and beverages rich in polyphenols (Scalbert et al., 2000). Antioxidants protect the living organisms from free radicals, which are highly reactive molecules that can damage the tissues at the cellular level. This damage inflicted by free radicals may increase the risk of developing cancer, cardiovascular diseases and degenerative diseases (Vinson et al., 1998).

The objective of the current work is to obtain polyphenolic rich extracts from cocoa (*Theobroma cacao*) seeds and Chuchuhuasi (*Maytenus macrocarpa*) bark that could be used as natural antioxidants.

Materials and Methods

Prior to the field operations, an extensive bibliographic search focused on recent publications concerned with the two the species under study was carried out. The search was using the following databases: Scopus, Scielo, PubMed and Scifinder. The scientific names for species under study were adopted as keywords. The articles found were detached into two groups: relevant (R) and non-relevant (NR) research, respectively. Studies focused on the bioactivity and the secondary metabolites characterization of the target species were identified in the first group (table 1). The articles considered to be irrelevant, although retaining their scientific value, were classified in this way because they address issues not related to phytochemistry, such as botany, genetics or conservation of the species under studied.

Table 1: Bibliographic research results

Species	Scopus		Scielo		PubMed		Scifinder	
	NR	R	NR	R	NR	R	NR	R
Teobroma cacao (cacao)	10	2	20	5	12	5	5	1
Maytenus laevis (chuchuhuasi)	2	0	3	0	5	0	0	0

The species (Table 2) were collected in the Amazon Region of Ecuador, especially at the Center for Research, Postgraduate and Amazonian Conservation (CIPCA), km 44 via Puyo-Tena and the Jartún Sacha Biological Station, which specimens were identified by the botanist specialist Dr. David Neill, and they rest in the Amazonian Herbarium of Ecuador (ECUAMZ).

Table 2: Botanical description of species under study

Common name	Scientific name	Botanical family	Collector	Nº collection	Origin
Cacao	*Teobroma cacao* L.	Malvaceae	D. Neill	18246	CIPCA
Chuchuhuasi	*Maytenus macrocarpa* (Ruiz & Pav.) Briq.	Celastraceae	D. Neill	18244	Jartun Sacha Biological Station

The plant material was washed with tap water and dried in a laboratory stove (Barnstead International, USA) with air recirculation at a temperature of 45 °C and further pulverized in a knife mill (Thomas Scientific, USA). Finally, it was sieved in order to guarantee a particle size less than 0.5 mm, considered suitable for subsequent extraction (Azwanida, 2015; Ph. Eur., 2017). The extracts obtained from the two

plants were made by means of a 9:1 ethanol: water mixture, with a ratio of 400 mL of solvent per 50 g of pulverized sample. Extractions were done in triplicate at 35 °C for 1 hour, the mixture was subsequently filtered on a Gooch filter and the crude extract obtained was concentrated with rotary evaporator (Büchi, Germany) at a temperature of 45 °C and a reduced pressure of 600 mmHg to 50 mL. For the implementation of the Folin-Ciocalteau test (Proestos and Varzakas, 2017, Yoshioka et al., 2017, Mansour et al., 2017; Apostolou et al., 2013), the previous standard calibration curve by successive dilutions from a concentrated solution (stock solution) of 1000 mg. L^{-1} gallic acid (reference standard) was made (table 3).

Table 3. Standard gallic acid curve preparation from the 1000 $mg.L^{-1}$ stock solution. Final volume: 10 mL (distilled water).

Components added	Gallic acid concentration of ($mg.L^{-1}$)				
	5	10	15	20	25
Gallic acid standard (µL)	50	100	150	200	250
Folin-Ciocalteu Reagent (µL)	500	500	500	500	500
Sodium carbonate solution 10% (µL)	500	500	500	500	500

For the sample preparation, 40 µL of each extract and 500 µL of Folin-Ciocalteau reagent were placed in a 10 mL volumetric flask, after shaking it was allowed to stand for 8 minutes protected from light; later, 500 µL of 10% sodium carbonate solution were added and the volumetric flask was flushed with distilled water to a volume of 10 mL. The resulting solution was homogenized by manually shaking and kept in the dark at room temperature for 2 hours. The absorbance values of prepared samples and standards were measured at 765 nm against the reagent blank. As reference, a sample of Chilean red wine Cabernet Sauvignon was analyzed.

Results and Discussion *(optional), no page limit*

The absorbance values recorded for the calibration curve are shown in table 4 and in figure 1. The total phenolic and polyphenolic antioxidants concentration results, based on gallic acid, in both Amazonian plants extracts analyzed, demonstrated that they could be an important source of polyphenols with antioxidant potential (tables 5 and 6).

Table 4. Calibration curve concentrations ($mg.L^{-1}$) and average absorbance values obtained.

Concentration $mg.L^{-1}$	5	10	15	20	25
Absorbance	0.297	0.747	1.193	1.497	1.657

The mathematical linear model obtained, as a result of the regression analysis, which allowed the further quantitative calculation was the following: C = (A + 0.0312) / 0.0778.

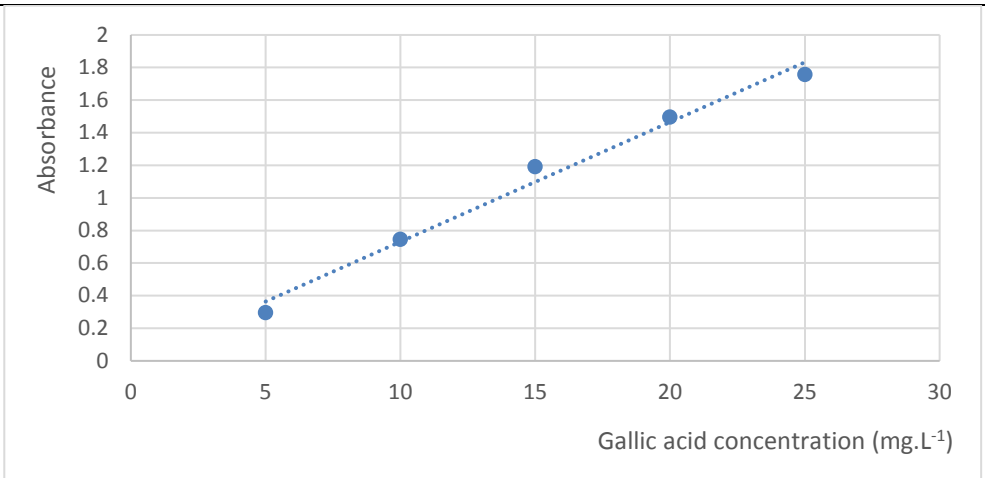


Figure 1. Gallic acid standard curve.

Table 5. Total polyphenols concentration based on gallic acid in the analyzed extracts.

Sample analyzed	Absorbance values				**Concentration**	**CV**
	A1	**A2**	**A3**	**Ā**	**(mg.mL^{-1})**	**(%)**
Teobroma cacao (cacao)	1.860	1.950	1.800	1.870	24.44	0.570
Maytenus laevis (Chuchuhuaso)	1.450	1.560	1.540	1.517	19.90	0.340
IIlex guayusa (Guayausa)	0.068	0.062	0.077	0.069	1.288	0.006
Chilean red wine	0.655	0.657	0.691	0.668	8.983	0.040

Table 6. Concentration (mg/100g of dry matter) for total phenolic and polyphenolic compounds in powdered solid samples.

Sample of pulverized solid analyzed	Concentration (mg/100g)
Teobroma cacao (cacao)	2443.70
Maytenus laevis (Chuchuhuasi)	1989.50
IIlex guayusa (Guayausa)	128.800
Chilean red wine	898.300

After analyzing the Amazonian species extracts under study, it is possible to encourage that, they provided relevant results in relation to the presence of phenolic and polyphenolic antioxidant compounds, which allows to carry out a preliminary statement about its promising antioxidant activity.

This research work supported an innovation element in the bibliographical research as it has been verified the lack of scientific information on the field.

In the application of the analytical method (Folin-Ciocalteu), an acceptable linearity for the calibration curve, with a correlation coefficient value of 0.9925 was obtained. In spite of the relatively complex process of obtaining the extracts, the precision of the polyphenol concentration results was adequate, with coefficients of variation in all cases lower than 5%.

Conclusions

The Amazonian species under study provided relevant results in relation to the phenolic and polyphenolic antioxidant compounds presence, which allows preliminary prediction of a promising antioxidant activity. There is an element of innovation in bibliographical research given by the lack of scientific information about this topic. On the other hand, the lack of phytochemical information about the species under study justifies new research work. This preliminary study allowed the identification, for the first time, of new sources of polyphenols in promising plant species of the Ecuadorian Amazon region.

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Study of microbiological laboratory techniques for the etiological diagnosis and guidance in the treatment of invasive candidiasis

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Invasive candidiasis is an important medical problem with a high mortality (30-50%) associated with both the health status of patients who are usually people with immunodeficiencies, and with the virulence of the fungus. The fungal species *Candida albicans* causes half of these diseases. Other species such as *Candida parapsilosis, Candida glabrata or Candida tropicalis* are important etiological agents because of their increasing frequency and the potential resistance to the antifungal drugs used in the therapy of candidiasis. *Candida auris* is an emerging species of recent association with human disease with cross resistance to antifungal drugs and the ability to persist in the hospital environment [1]. The early diagnosis of invasive candidiasis is difficult: There are not specific biomarkers for diagnosis and identification by conventional and molecular methods of the species causing the infection is based in the culture of this fungus in suitable mycological media.

Aims: To become familiar with the microbiological laboratory techniques used for the etiological diagnosis of invasive candidiasis and to guide in the treatment of invasive fungal infections.

Methodology: The current project performs several phases related to the diagnosis and treatment of invasive candidiasis. In one of the phases, studying in vitro antifungal susceptibilities for supporting treatment of candidiasis, we have studied the activity of SCY-078, a new antifungal drug for treating infections caused by species of *Candida* resistant to conventional treatments, including *Candida auris.* This compound SCY-078 is a

new oral and intravenous drug for the treatment. We have evaluated the in vitro action of this drug SCY-078 and other antifungal drugs (azoles and echinocandins) against isolates of different *Candida* species isolated from blood cultures, such as *Candida albicans, Candida parapsilosis, Candida tropicalis*, *Candida auris* or *Candida glabrata* using the EUCAST method [2]. This method is the European protocol for studying the in vitro activity of antifungal drugs. EUCAST is a microdilution method for determining the Minimum Inhibitory Concentration (MIC) of the different antifungal agents and their usefulness for the treatment of invasive mycoses.

Conclusion: The main action of azoles is to inhibit lanosterol 14-α-demethylase coupled to cytochrome P-450. This action causes an alteration of fungal cell membranes increasing permeability and producing inhibition of cell growth and cell replication. Echinocandins inhibit 1,3-β-D-glucan synthetase, the enzymatic complex that forms β-D-glucan polymers in the cell wall of the fungus. The cell wall provides rigidity to the cell and its rupture causes the cell death. The compound CSY-078 is derived from enfumafungin (formerly MK-3118) and it is an inhibitor of the 1,3-β-D-glucan synthesis. This drug is the first in its class: triterpenic antifungal drugs. SCY-078 has demonstrated in vitro activity against many *Candida* species and against the multidrug-resistant pathogen *Candida auris*, which has been classified by the Centers for Disease Control and Prevention (CDC) as a Serious Threatens a global level for health In clinical development to treat candidemia and candidiasis [1,3,4]. During my stay, we have verified the excellent in vitro efficacy of the echinocandins caspofungin and micafungin and the new drug SCY-078 against many of the clinical isolates of *Candida* that were resistant to fluconazole. Moreover, SCY-078 was active against many Candida blood isolates with decreased susceptibility to caspofungin and micafungin.

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Estudio comparativo de la Candidiasis Invasiva en México y España

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Resumen

El género *Candida* incluye más de 150 especies, de las cuales solo unas cuantas causan enfermedad en seres humanos. Con raras excepciones, los patógenos para seres humanos son *Candida albicans*, *Candida guilliermondii*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, *Candida lusitaniae*, *Candida dubliniensis* y *Candida glabrata*. De naturaleza ubicua, estos microorganismos se encuentran en objetos inanimados, alimentos, animales y son comensales normales de los seres humanos. Habitan en el tubo digestivo (lo que incluye boca y orofaringe), en el aparato reproductor femenino y en la piel. Con la introducción de antimicóticos, las causas de infecciones por *Candida* han variado desde el casi dominio completo de *C. albicans* hasta la participación común por *C. glabrata* y otras especies.

La candidiasis invasiva (CI) es la enfermedad fúngica más común entre los pacientes hospitalizados en países en vía de desarrollo. Comprende tanto candidemia como candidiasis de tejidos profundos. Generalmente se ve como el tipo más común de la enfermedad y representa la mayoría de los casos incluidos en ensayos clínicos. La candidiasis profunda se presenta ya sea por diseminación hematógena o por inoculación directa de las diferentes especies de cándida a un sitio estéril, como la cavidad peritoneal. La mortalidad entre los pacientes con candidiasis invasiva alcanza un 40% y en neonatos y niños hasta un 15%, incluso cuando los pacientes ya han recibido terapia antifúngica. Además, la incidencia creciente de las especies *Candida no-albicans* es preocupante, al igual que su resistencia a los fármacos antifúngicos.

Abstract

Candida genus includes more tan 150 species, including some human pathogens such as Candida albicans, Candida guilliermondii, Candida kruseii, Candida parapsilosis, Candida tropicalis, Candida lusitaniae, Candida dubliniensis and Candida glabrata. These fungi are found in non-living objetcts, food, animals and some of them are part of the human microbiota without causing disease. Gastrointestinal tract (including oral cavity and pharynx), female genitals and skin are common environments for Candida. Since the introduction of antifungal drugs,

the aetiology of invasive candidiasis is a changing from a complete predominance of C. albicans to a more diverse participation from other species, such as C.glabrata or C. parapsilosis (Longo, Fauci, Kasper & Hauser, 2012).

Invasive candidiasis is the most common fungal disease in hospitalized patients. This disease comprises different clinical presentation such as candidemia or different organ or deep tissue infections. Candidemia is the most common presentation. Deep candidiasis is caused by bloodstream dissemination or direct inoculation of the fungus in organs and tissues. Mortality among patients with invasive candidiasis is high (15-40%) (del Palacio, Villar & Alhambra, 2009) even when the patients have been treated with antifungal therapy. This growing incidence from the non-albicans species is worrying because many species are resistant to current antifungal drugs (Kullberg & Arendrup, 2015).

Objetivos

Discernir las diferencias en la etiología, epidemiología y manifestaciones clínicas de las infecciones por *Candida* haciendo énfasis en la Candidiasis Invasiva que se observa en México y España.

Metodología

Se realizó una revisión sistemática de la literatura médica, para identificar y resumir todas las publicaciones relacionadas con la Candidiasis Invasiva, así como sus características clínicas y epidemiológicas. Se realizó selección de artículos relevantes, guiados por el título y resumen hasta obtener estudios confiables. Se efectuó la búsqueda de información en la base de datos Pub-Med/Medline de la National Library of Medicine (NLM/NCBI) de 2009 a 2017. Las estructuras de búsqueda se diseñaron usando palabras texto identificadas en artículos relevantes, palabras clave según la base de datos consultada. La estrategia de búsqueda fue mediante los términos “*Candida*” “*Candida* and Mexico” “*Candida* and Spain” “Invasive Candidiasis” “Candidaemia” “Epidemiology and incidence Cándida” “Cándida and children”. Finalmente se generó un reporte en Microsoft Word con

asesoría del Dr. Guillermo Quindós en la Facultad de Medicina y Enfermería de la Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU).

Antecedentes

Las levaduras del género *Candida* forman parte de la microbiota del aparato digestivo, superficies mucocutáneas como la piel, orofaringe y vagina. Los huéspedes susceptibles se pueden infectar por *Candida* a partir de su propia microbiota donde ocurre un cambio en la relación entre la levadura y el huésped (infección endógena) o por infección horizontal por contacto (transmisión exógena)[1].

Teniendo en cuenta la reproducción asexuada de las levaduras se las incluye en las subdivisiones *Ascomycotina, Basidiomycotina* y *Deuteromycotina*. Su clasificación taxonómica es:

- *Dominio: Eucarya*
- *Reino: Fungi*
- *División: Eumycota*
- *Subdivisión: Deuteromycotina*
- *Clase: Blastomycetes*
- *Familia: Cryptoccocaceae*
- *Género: Cándida*
- *Especies: albicans, glabrata, krusei, parapsilosis, tropicalis, etc[2].*

Las especies pertenecientes al género *Candida* representan los patógenos fúngicos patógenos más frecuentes, actualmente, se sabe que estos microorganismos colonizan la mucosa gástrica y pasan al torrente circulatorio mediante un proceso de translocación gastrointestinal o a través de catéteres intravasculares contaminados, interaccionan con las defensas del huésped y abandonan el compartimiento intravascular para invadir tejidos profundos de distintos órganos diana como hígado, bazo, riñón, cerebro, etc. Entre las características del microorganismo que podrían contribuir a su potencial patógeno se encuentran la capacidad de adhesión a tejidos, el dimorfismo levadura-micelio y la hidrofobicidad de su superficie. Se cree que la capacidad de adhesión a distintos tejidos y

superficies inanimadas es importante en las fases iniciales de la infección por *Candida*. La capacidad de adhesión de las distintas especies de este género presenta una relación directa con su nivel de virulencia según diversos modelos experimentales[2].

Patogenia

El proceso de infección comienza con la adherencia del microorganismo comensal a las células de la mucosa o queratinocitos que interactúan en la relación de la pared fúngica de polisacáridos (mananos) con un receptor en la célula epitelial. Se han reconocido como adhesinas putativas los mananos, manoproteínas y quitina. Aunque in vivo, la situación es más compleja que en estudios experimentales, se han postulado los siguientes mecanismos de virulencia:

- Capacidad de adhesión
- Producción de enzimas proteolíticas, en especial proteasas y fosfolipasas, las cuales facilitan la penetración y degeneración de queratina y colágeno.
- Transformación morfológica de levadura en hifa, lo que también favorece la penetración y permite evadir el sistema inmune, debido a que la hifa libera mayor cantidad de fosfolipasas y es más resistente a la fagocitosis.
- Efectos inmunorreguladores de determinantes fúngicos que contribuyen a disminuir la actividad de defensa del huésped
- Cambios fenotípicos, los cuales permiten al hongo adaptarse a condiciones diferentes o cambiantes[3].

La pared celular de *Candida* está constituida por β-(1-3)-D-glucano, manano, quitina, proteínas y lípidos. Estudios con microscopía electrónica muestran diferencias en la organización y composición de la pared celular en las dos diferentes fenotipos de esta levadura. La adhesión depende de condiciones ambientales, pero también es influida por factores del huésped, como hidrofobicidad, mimetismo de las proteínas de superficie que puede afectar la unión a filos y, por lo tanto, la fagocitosis; el tipo de medio para su crecimiento y condiciones del mismo, así como las alteraciones hormonales e inmunitarias. Además de lo anterior, *Candida* es capaz de formar biopelículas (biofilms), mediante

polímeros que les permiten una fuerte unión y les confieren capacidad defensiva y mayor resistencia a antifúngicos. Las forman principalmente *C. albicans* y *C. parapsilosis*; la biopelícula de *C. albicans* es de las más estudiadas y se sabe que están formadas por una compleja red de células levadurifomes, hifas y pseudohifas interconectadas con una matriz extracelular formada por múltiples biocapas[4]. El mayor componente de la matriz extracelular son los carbohidratos (el 32% de ellos es glucosa), proteínas, hexoaminas, fósforo y ácido úrico. La formación y desarrollo de estas biopeliculas fúngicas depende de muchos factores, unos relacionados con el medio (presencia o ausencia de suero, saliva, orina, etc) y la superficie en la que se desarrollan (abiótica o biótica) y otros relacionados con la especie de *Candida* implicada, e incluso con el aislamiento y el origen clínico del mismo (infecciones invasivas, cutáneas, ambientales, etc[3].

Candidiasis

Es una infección primaria o secundaria, causada por levaduras del género *Candida*, con manifestaciones clínicas extremadamente variables de evolución aguda, subaguda, crónica o episódica, en las cuales el hongo puede causar lesiones cutáneas, mucocutáneas, profundas o diseminadas[5].

Candidiasis Invasiva

Las enfermedades fúngicas invasivas (EFI) son un problema médico creciente desde la década de 1960, sobre todo en pacientes críticos e inmunodeficientes. En las unidades de cuidados intensivos (UCI) representan entre el 10-15% de las infecciones hematológicas nosocomiales y se asocian a la gravedad del paciente y a la duración de su estancia en ésta. Además, son una complicación frecuente del tratamiento quimioterapéutico de las neoplasias y/o trasplantes, principalmente de las progenies hematopoyéticas. Las Candidiasis Invasivas (CI) son las EFI más frecuentes en los últimos años, aunque se han observado cambios significativos etiológicos y epidemiología debido a que la profilaxis primaria con fluconazol en los pacientes oncohematológicos ha reducido el número total de EFI al disminuir las CI por *Candida albicans* y *Candida tropicalis*, sin embargo, se observa un incremento etiológico de *Candida glabrata, Candida parapsilosis* y *Candida krusei*[6].

Candidemia

Es un tipo de Candidiasis Invasiva, se le define como el aislamiento de *Candida* de uno o más cultivos de sangre periférica o central[7]. La comprensión correcta del término Candidemia, es fundamental para el abordaje diagnóstico y terapéuticos correctos, ya que ésta no representa en sí una entidad específica; es el marcador principal de tres distintas formas de presentación que deben ser diferenciadas mediante la clínica: a) Candidiasis diseminada aguda; b) Candidiasis diseminada crónica; c) Candidemia transitoria[5].

Candidiasis diseminada

La Candidasis diseminada en los tejidos puede ser, a su vez, aguda o crónica. Estas formas ocupan los extremos de un síndrome que se manifiesta sobre todo en pacientes neutropénicos[8].

Candidiasis diseminada aguda

Ocurre de manera típica en niños granulocitopénicos. Se manifiesta por la presencia de candidemia, fiebre o hipotermia, inestabilidad hemodinámica y trombocitopénica. Puede haber lesiones viscerales y ocasionalmente cutáneas, esta forma clínica tiene elevada mortalidad a pesar de la terapia antimicótica. La presencia de *Candida* en un hemocultivo debe de ser considerada en prácticamente todos los pacientes neutropénicos[9].

La candidemia no tratada en pacientes sin granulocitopenia, que no recibe tratamiento puede causar complicaciones en el 10-20% de los casos, tales como endoftalmitis, meningitis, osteomielitis, artritis, pericarditis, endocarditis, candidiasis renal, flebitis supurada y abscesos. En 1-10% de los casos puede causar afectaciones cutáneas[10]. La candidiasis diseminada en neonatos y pacientes granulocitopénicos a menudo tiene una elevada mortalidad de más del 80% si se retrasa el tratamiento[11].

Su diagnóstico requiere el aislamiento del hongo en hemocultivos o en biopsia de tejidos afectados. Se han realizado muchos estudios para comparar las diferentes técnicas de hemocultivo para la detección de *Candida*, algunos resultados indican

que las técnicas de lisis-centrifugación y la de lisis-filtración son más sensibles que otras[11].

La positividad de un hemocultivo depende de varios factores, como el número de levaduras por mililitro de sangre durante la fungemia, la viabilidad de las levaduras en pacientes que ya han iniciado terapia antimicótica, una fungemia resistente, la frecuencia con la que se cultivan las muestras, el número total de muestras cultivadas y la forma clínica de la candidiasis[8].

Candidiasis diseminada crónica (hepatoesplénica)

La candidiasis crónica también conocida como hepatoesplénica, es un proceso indolente de candidiasis diseminada, a menudo sin presencia de fungemia en un paciente hemodinámicamente estable. Tiene sobrevida elevada si se da tratamiento antifúngico oportuno[8].

Los casos de candidemia diseminada crónica en niños descritos en la literatura son escasos, la mayoría son pacientes adultos. Es una entidad de difícil diagnóstico, sobre todo cuando no se tiene experiencia con las diferentes formas clínicas de la candidiasis diseminada[12]. La diseminación a las visceras es por vía hematógena a través de la circulación porta en pacientes neutropénicos. La fiebre puede ser la única manifestación, pero un hemocultivo positivo para *Candida* sugiere que hay una infección invasiva en ellos[8].

El diagnóstico de candidiasis hepatoesplénica no puede realizarse hasta que la cuenta de neutrófilos retorna a lo normal; este factor sugiere que el síndrome de respuesta inflamatoria es determinante tanto en las características de la lesión como en la candidiasis hepatoesplénica. Tanto el diagnóstico como el tratamiento temprano de esta complicación influyen en relación directa con la superviviencia, el diagnóstico requiere un cultivo positivo para levaduras o una determinación positiva de antígeno manano de *Candida* en suero y estudios de imagen compatibles con candidiasis hepatoesplénica o la demostración de levaduras o pseudohifas en la biopsia hepática. Por lo tanto, el diagnóstico se sustenta en parámetros clínicos, inmunológicos, microbiológicos e imagenológicos[12].

Los datos ultrasonográficos más importantes son: a) Presencia de anillos dentro de anillos, patrón que se observa en etapas tempranas de la enfermedad, b) Imagen en *ojo de buey* (Figura 1), estas lesiones miden de 1 a 4 mm de diámetro, c) Lesiones uniformemete hipoecóicas, d) Presencia de pequeños focos ecogénicos con grados variables de sombra posterior de 2 a 5 mm de diámetro, que se observan en la forma tardía de la enfermedad. Además de la resonancia magnética (RM) que ha cobrado imporrtantancia como elemento de apoyo diagnóstico, superior a la tomografía axial computarizada[8].

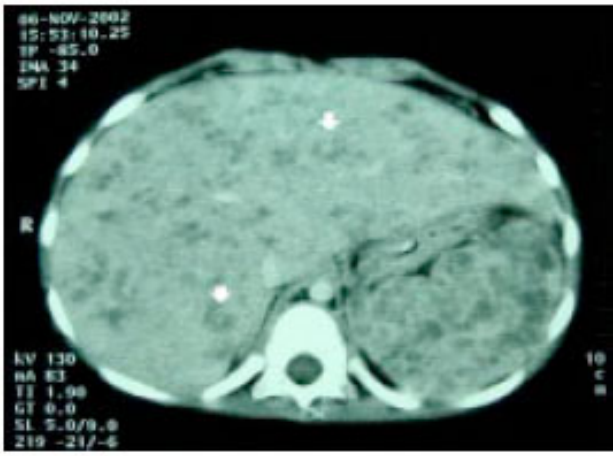

Figura 1. Imagen tomográfica de hígado y bazo que muestra abscesos hepáticos y esplénicos de patrón tipo 2 ('ojo de buey', flechas) y tipo 3 en una paciente con candidiosis crónica diseminada.

Todavía no existen criterios universales sobre la duración del tratamiento antifúngico (anfotericina B, anfotericina coloidal o liposomal, fluconazol). Sin embargo, existen estudios retrospectivos donde se ha observado que la duración promedio ha sido 112 días, con límites que van de 42 a 175 días[13]. El criterio de curación importante en la candidiasis hepatoesplénica de acuerdo con algunos autores se basa en el estudio radiológico, se considera como respuesta positiva completa, la desaparición de todos los signos y síntomas de infección con erradicación de todas las lesiones, basado en el examen físico y los datos radiológicos sin que aparezcan nuevas lesiones[8].

La respuesta parcial es la desaparición de todos los signos y síntomas de infección y la normalización de las pruebas de funcionamiento hepático y ultrasonográficos,

pero con persistencia de las lesiones identificadas por ultrasonido, tomografía computarizada o resonancia magnética[8].

Se considera que el tratamiento ha fallado si subsisten o progresan los criterios clínicos, bioquímicos o radiológicos de infección, si aparecen nuevas lesiones, fiebre persistente o si se deterioran las condiciones clínicas del paciente[14].

Epidemiología

Es una infección cosmopolita. Su incidencia ha aumentado considerablemente en los últimos 20 años en seres humanos. Las levaduras son causantes del 7,45% de las micosis, el 25% de las micosis superficiales y entre el 75-88% de las infecciones fúngicas nosocomiales. Afecta a individuos de cualquier edad, sexo o grupo étnico[8].

Las levaduras del género *Candida* existen en la naturaleza, suelo, agua dulce, vegetales, frutas, exudado de árboles, granos y en general toda sustancia rica en carbohidratos simples. Además, son habitantes habituales del aparato digestivo, respiratorio y regiones mucocutáneas del hombre y animales domésticos. En el adulto, dos factores regulan el número de levaduras en el intestino, 1) otros miembros de la flora intestinal que ejercen un control sobre la densidad de población de las levaduras a través de factores microbianos, inhibidores de la adherencia y potenciales de óxido-reducción y competencia por los nutrientes disponibles y 2) la dieta, ya que la ingestión excesiva de frutas frescas, dulces u otros materiales fermentables darán lugar a un aumento considerable en el número de levaduras intestinales, principalmente *C. albicans*[8].

Además de *C. albicans*, otras especies que pueden colonizar la mucosa oral y del tracto gastrointestinal humano como *C. glabrata, C. parapsilosis, C. tropicalis, C. dubliniensis, C. krusei*. La piel normal también puede presentan flora de levaduras residentes, que incluye *C. parapsilosis, C. guillermondii, C. krusei*. Otras especies como *C. albicans* y *C. tropicalis* no se encuentran con regularidad en la piel normal, salvo en la región perianal y alrededor de la boca. En la mucosa vaginal normal se puede aislar *C. albicans* y, con menor frecuencia, *C. glabrata, C. tropicalis, C. parapsilosis* y *C. krusei*[8].

Factores de riesgo

Existe una gran variedad de publicaciones sobre los factores de riesgo de CI y es objeto de debate si estos factores de riesgo tienen una relación que específicamente dan lugar a CI por distintos mecanismos fisiopatológicos o sólo son marcadores de la gravedad de la enfermedad, que predisponen al enfermo a la CI. El factor de riesgo más importante de CI es la estancia prolongada en UCI. En la primera semana de estancia, la incidencia de CI es baja, pero empieza a aumentar y su incidencia es mayor el día 21[15]. En los últimos años se ha descrito que la pancreatitis aguda también es un factor independiente de CI, ya que la incidencia de esta última es elevada, en torno al 25-35%. Además, la infección por *Candida* contribuye a que haya una alta mortalidad en estos enfermos[16]. Los factores de riesgo de CI en el enfermo crítico no neutropénico se han estudiado mucho en los últimos años y hasta la fecha son bien conocido, y actualmente es necesario desarrollar y validad de forma prospectiva estrategias de profilaxis y tratamiento correcto para los pacientes críticos. Los factores de riesgo en el paciente de UCI se resumen en la Tabla 1[1].

Tabla 1. Factores de riesgo de CI en el enfermo crítico de UCI

Estancia >3 días en UCI
Antibióticos de amplio espectro
Hemodiálisis
Catéteres Venosos Centrales
Gravedad de la enfermedad por la cual está en UCI
Nutrición Parenteral Total (NPT)
Perforación gastrointestinal o cirugía
Pancreatitis
Corticoterapia u otros inmunosupresores
Transfusiones
Diabetes

Sin embargo, no sólo los pacientes críticos de UCI tienen riesgo de contraer algún tipo de Candidemia, existen otros factores de riesgo para otro tipo de pacientes que se resumen en la Tabla 2[5].

Tabla 2. Factores de riesgo predisponentes a Candidemias.

Locales	Maceración, contacto con agua, mala higiene.
Fisiológicas	Prematuridad, recién nacidos o vejez, embarazo.
Endócrinas	Diabetes Mellitus, Hipotiroidismo.
Alteración de la flora normal	Antibioterapia
Enfermedades hematológicas	Linfomas, leucemias, anemia aplásica, agrunulocitosis, neutropenia, hipo y agamaglobulinemia,
Factores iatrógenos	Corticoterapia, quimioterapia, inmunosupresores, agentes citotóxicos, alimentación parenteral, trasplantes, cirugía abdominal, utilización de sondas o catéteres, radioterapia, prótesis, hemodiálisis.
Enfermedades debilitantes	Neoplasias, VIH, inanición, quemaduras graves, drogadicción, tuberculosis y otras infecciones graves.

Aunque globalmente *C. albicans* sigue siendo la especie más importante, se observa un aumento de especies de *Candida no albicans*, algunas de ellas resistentes a fluconazol, por lo que es preciso conocer de forma periódica la epidemiología en nuestro medio. La emergencia de especies de *Candida* está relacionada con factores bien definidos como la neutropenia, cirugía abdominal, tratamientos invasivos y antibioterapia de amplio espectro[17].

La detección de *Candida* en sitios diferentes a la sangre constituye un desafío ya que puede ser considerado como una colonización, infección local o infección invasiva. La importancia de la detección radica en la selección de los pacientes que se beneficiarán con un tratamiento antifúngico evitando la progresión a enfermedad invasiva y disminuyendo la mortalidad asociada[17].

En España en 2014 se realizó un estudio de vigilancia en 2014 para conocer la etiología de las Candidiasis Invasivas en hospitales, los resultados indican que las infecciones del torrente sanguíneo de *Candida* oscilan entre 8,6 por cada 100,000 habitantes al año. De 766 muestras de hemocultivos que presentó el estudio, *C. albicans* fue la especie predominante con un 45%, seguido de *C. parapsilosis* (25%), *C. glabrata* (13%), *C. tropicalis* (8%), *C. krusei* (2%) y otras especies raras engloban un 7% (Figura 2)[18].

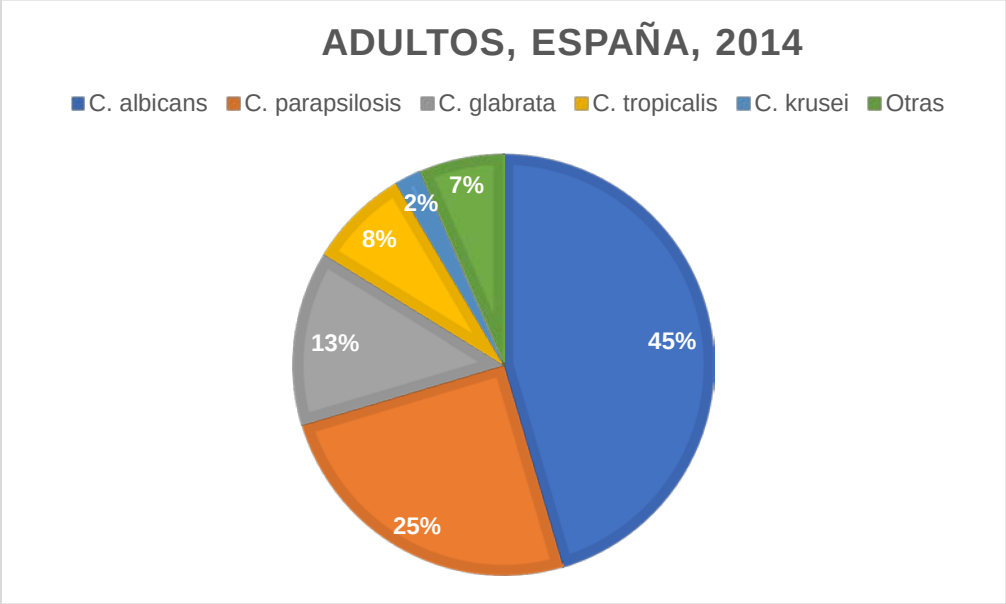

FIGURA 2. Agentes etiológicos de las CI en España, 2014.

En México, en los últimos años, el agente etiológico involucrado en la mayoría de los cuadros clínicos de candidiasis es *C. albicans* (62%), por lo que se considera a esta especie como politrópica, debido a su distribución topográfica, sin embargo, en algunas formas clínicos se han aislado otras especies con menor tropismo: *C. parapsilosis* (7,3%) en paroniquias, endocarditis y otitis externa*; C. tropicalis* (7,5%) en vaginitis, enfermedad intestinal, infecciones broncopulmonares, sistémicas, nerviosas y articulares; *C. krusei* (2,7%) en algunos casos de endocarditis, infección de mucosas y vaginitis, y *C. glabrata* con una frecuencia del 12% en todas las infecciones por *Candida* sistémica (Figura 3)[8].

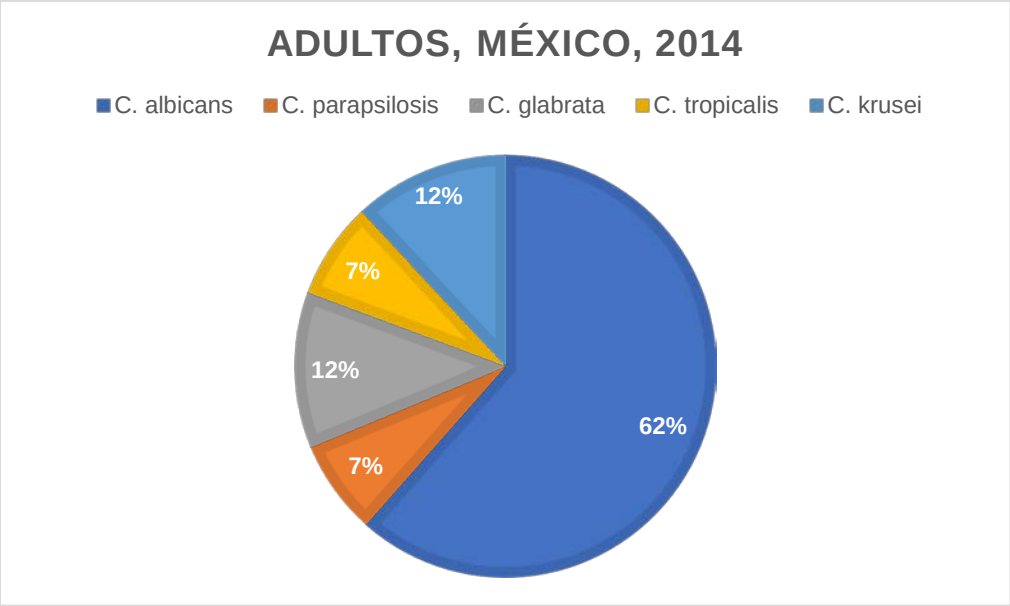

FIGURA 3. Agentes etiológicos de la CI en México, 2014.

Candidemia en población pediátrica

Por otra parte, en la población pediátrica española, el estudio que tomamos para análisis en este *review* fue realizado en 2013, donde muestran algunos resultados bastante interesantes. Se recogieron 200 episodios de candidemia en 12 comunidades autónomas del país. La candidemia infantil fue más frecuente niños que en niñas (2/1). Globalmente, la especie más identificada fue *C. parapsilosis* con 86 aislamientos (43%), seguida de *C. albicans* con 72 (36%), *C. tropicalis* con 12 (6%), *C. glabrata* con 8 (4%), *C. krusei* con 2 (1%) y otras levaduras con un 1.5% (Figura 4). Sin embargo, en el grupo neonatal, predominó *C. albicans*, mientras que, en lactantes, preescolares y escolares, la especie más frecuente fue *C. parapsilosis*. *C. albicans* fue la más frecuente en Baleares, Cataluña y Canarias, en preescolares y escolares no hubo diferencia significativa en aislamientos de *C. albicans y C. no albicans*, sin embargo, la edad neonatal se asoció significativamente con candidemia por *C. albicans*, se asegura (Figura 5)[19].

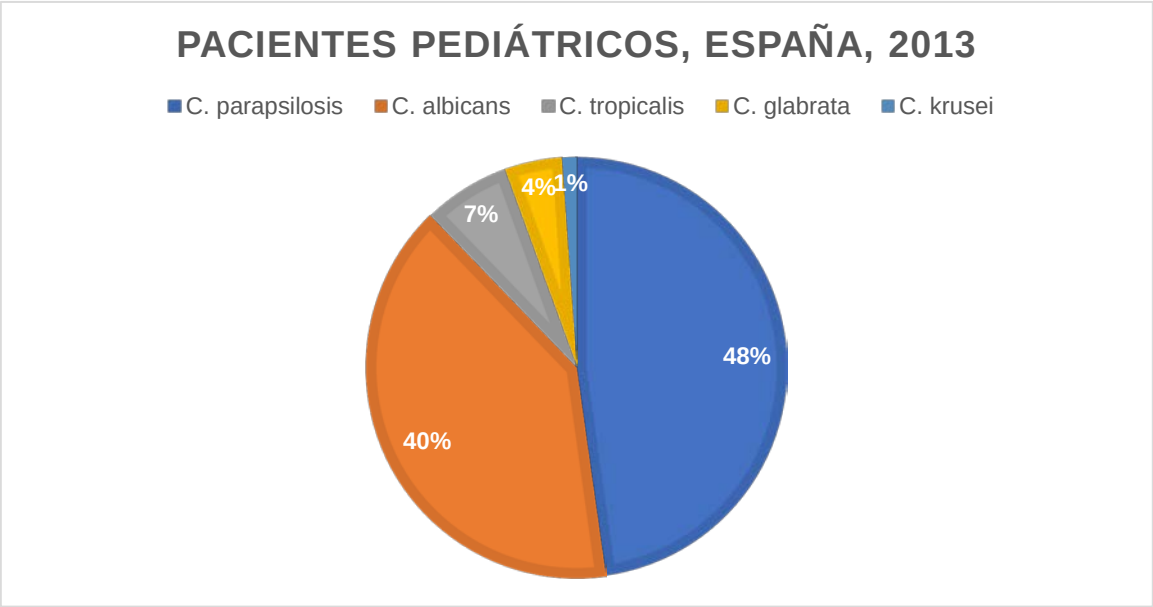

FIGURA 4. Agentes etiológicos de CI en población pediátrica en España, 2013.

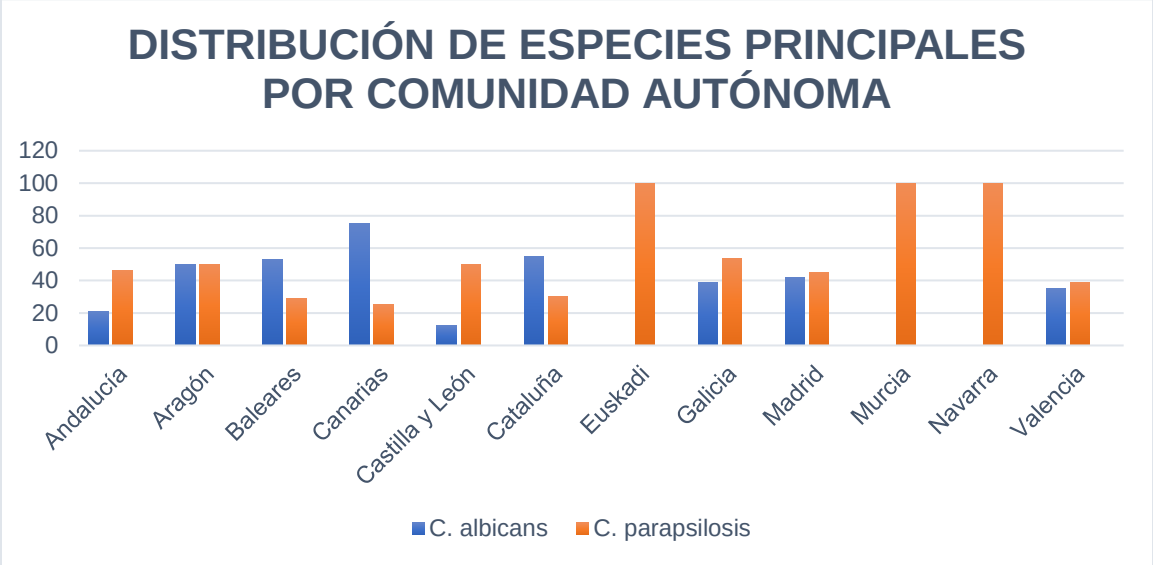

FIGURA 5. Distribución de agentes etiológicos principales en las principales comunidades autónomas de España, 2013.

En este estudio, el número de episodios de candidemia en función de las distintas unidades de hospitalización fue: neonatología con 63 episodios (31,5%), pediatría general con 50 (25%), UCI pediátrica con 38 (19%), UCI neonatal con 29 (14,5%), hematología con 11 (5,5%), cirugía con 5 (2,5%) y oncología con 4 (2%). En las

áreas de cuidados intensivos, incluyendo UCI neonatal, *C. albicans* (46,3%) fue la especie causan de candidemia más significativa (Figura 6)[19].

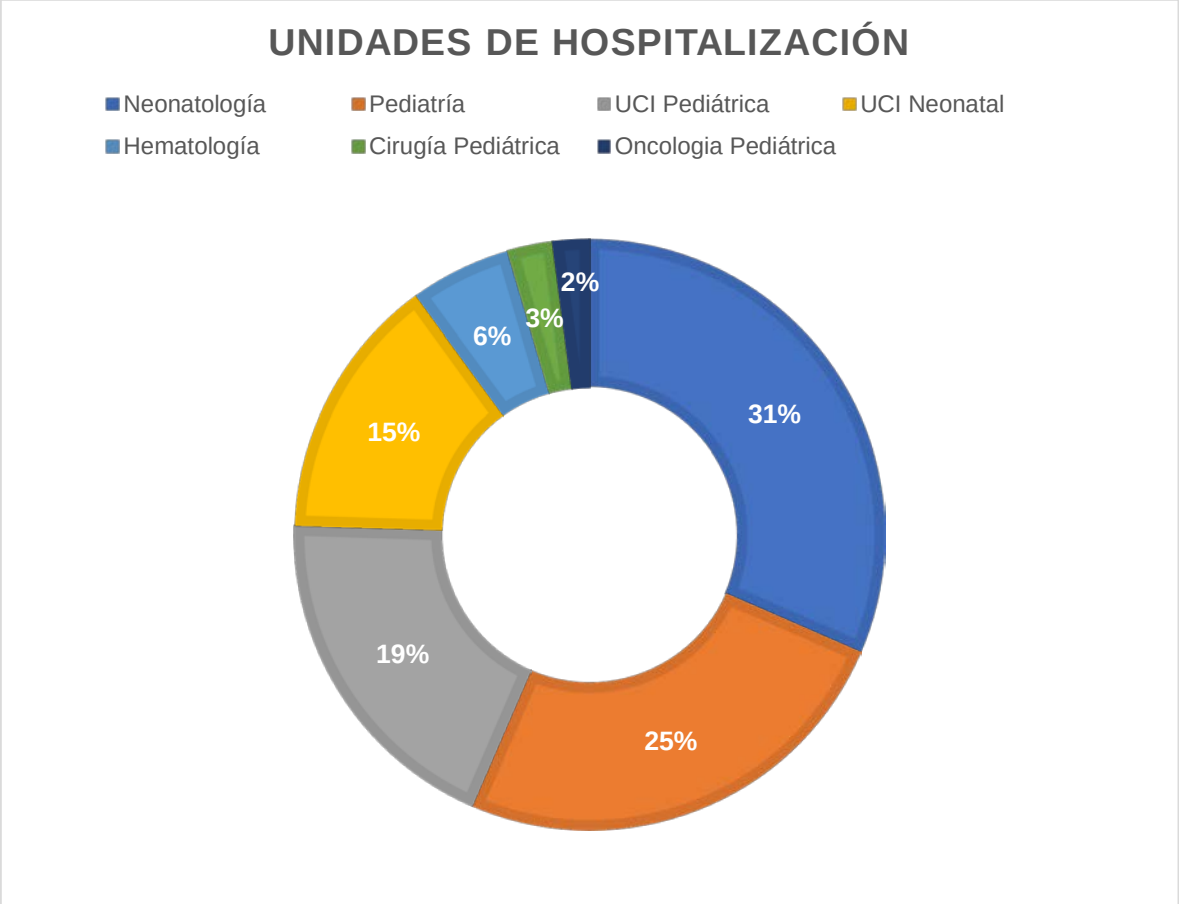

FIGURA 6. Frecuencia de presencia de CI en las diferentes unidades de hospitalización pediátrica de España, 2013.

La distribución etiológica en las comunidades españoles predominó *C. parapsilosis* en 8 comunidades y *C. albicans* en 3[19].

Los factores predisponentes de candidemia analizados fueron cirugías, quemaduras, VIH, neutropenia, trasplante de preocursores hematopoyéticos, trasplante de órgano sólido, catéter venoso central (CVC) y prematuridad. Sin embargo, la presencia de catéter y la prematuridad fueron los 2 únicos factores de riesgo que resultaron significativos[19].

En la población pediátrica mexicana, en un estudio de 2013, en un estudio en 10 diferentes hospitales de la República Mexicana, donde recogieron 342 muestras de neonatos, preescolares y escolares con múltiples episodios de fungemia en el

periodo de estudio. La distribución de especies en estos hospitales varió ligeramente respecto a los pediátricos españoles. La especie *C. albicans* se contabilizó en 127 muestras, representando un 38,08%, mientras que las especies *no albicans* representó el 56,53%, específicamente, *C. parapsilosis* con 127 muestras contabilizadas representa el 36,08%, *C. tropicalis* con 72 muestras, un 20,45%. En México, la especie predominante es *C. albicans* (Figura 7)[20].

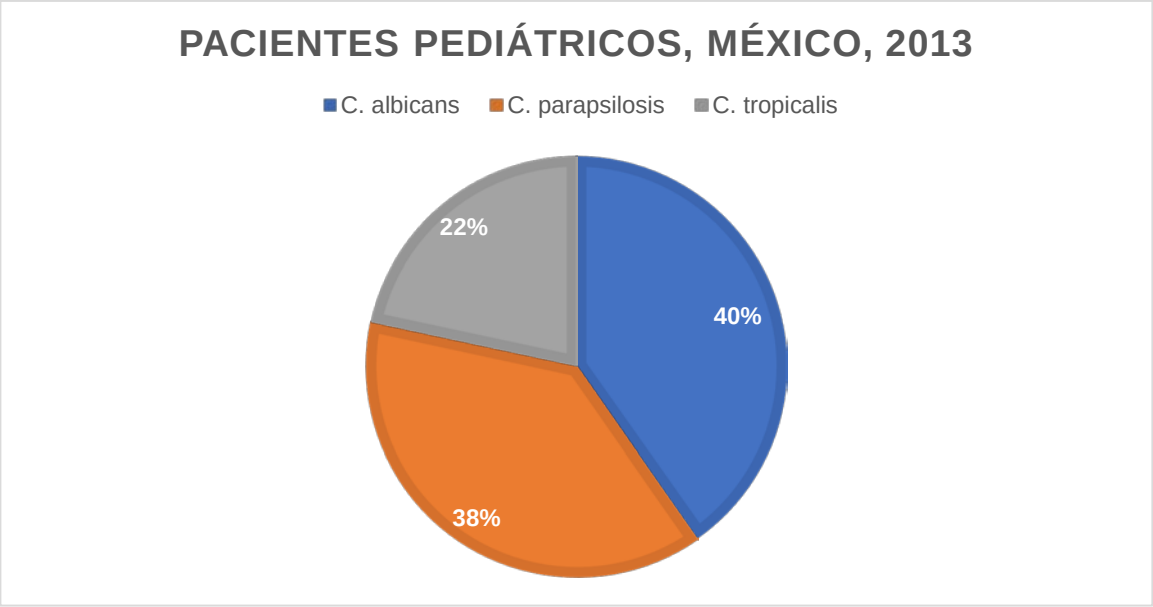

FIGURA 7. Agentes etiológicos de CI en población pediátrica en México, 2013.

En otro estudio en el 2015, en México, se realizó un estudio en 23 hospitales de atención terciaria, donde se estudiaron 302 casos consecutivos de candidemia, 89 de estos (29%) ocurrió en neonatos (<28 días) y 213 (71%) en niños de etapa preescolar y escolar (Figura 8). La media de edad de los pacientes fue de 16 días en los neonatos y 2 años en niños[21].

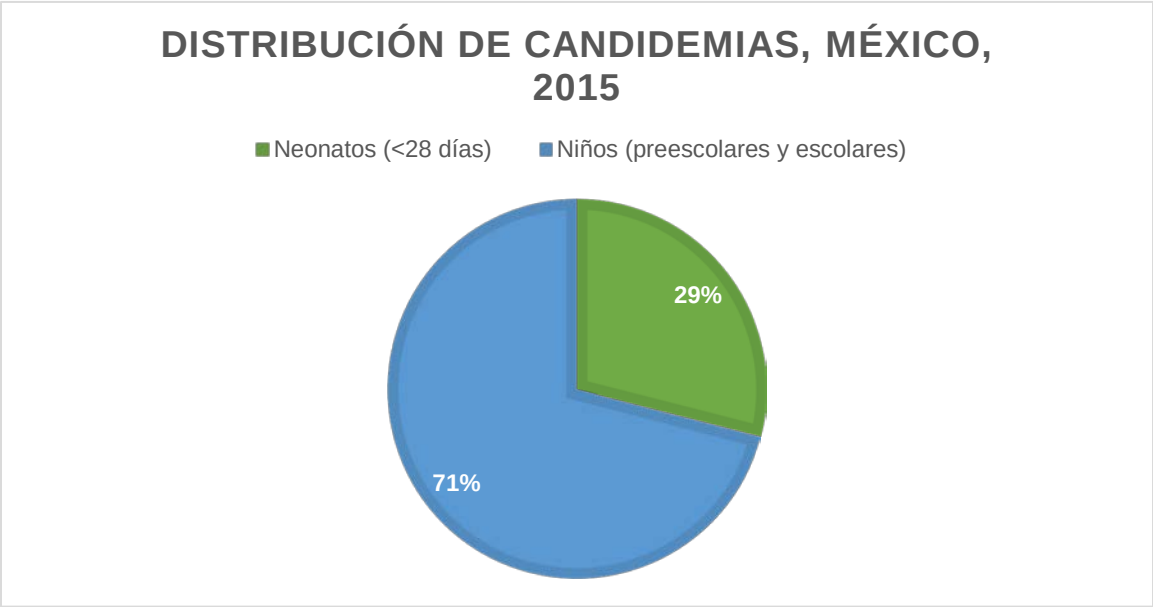

FIGURA 8. Distribución de Candidemias por grupo etario, México, 2015.

Las principales especies aisladas en neonatos y niños respectivamente fueron: *C. albicans* (43,8 y 35,7%), *C. parapsilosis* (27 y 26,3%), *C. tropicalis* (14,6 y 14,5%) y *C. guilliermondii* (4,5 y 12,7%), *C. glabrata* fue el más infrecuente en ambos grupos etarios (3,4 y 3,3%) (Figura 9)[21].

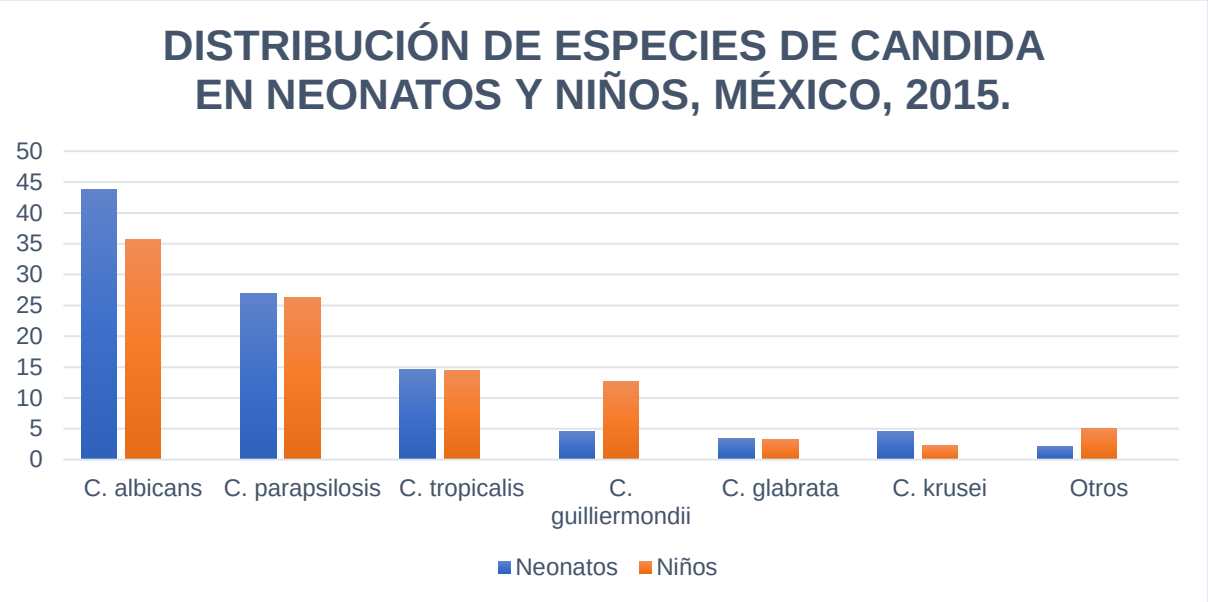

FIGURA 9. Distribución de agentes etiológicos de CI en diferentes grupos etarios pediátricos, México, 2015.

En este estudio se analizaron las condiciones concomitantes vistas en estos neonatos y niños. La gran mayoría de los neonatos eran prematuros (52.8%), los hospitalizados en UCI eran el 79%, los alimentados totalmente por vía parenteral representan el 48.3%, con enfermedad respiratoria el 30.3% y con ventilación mecánica el 67.4%. Por otra parte, las condiciones de los niños más grandes eran: Alguna tumoración maligna (25.4%), neutropenia un 17.8%, enfermedad neurológica un 18.3%, previo uso de corticoesteroides un 31.9%. Las condiciones concomitantes que estos dos grupos tenían en común eran el uso de catéter venoso central (Neonatos 70.8% vs Niños 62.4%), y previa antibioterapia (Neonatos 95.5% vs Niños 95.8%) (Figura 10)[21].

FIGURA 10. Condiciones y factores de riesgo predisponentes a las CI en pacientes pediátricos, México, 2015.

Clínica

La candidiasis diseminada, ya sea aguda o crónica, tiene manifestaciones clínicas variadas, que en muchas ocasiones se superponen a la clínica de la enfermedad concomitante, Cuando se han producido alteraciones orgánicas específicas pueden expresar alguna clínica más focal. La clínica va a estar presente en el órgano que se encuentre afectado, un ejemplo es la endoftalmitis que ocasiona *Candida*, lesión

indicativa de posible diseminación hematógena con afectación multiorgánica que hace recomendable la exploración frecuente en fondo de ojo en aquellos pacientes con alto riesgo de sufrir una candidemia. Otro ejemplo es la peritonitis candidiásica que son más frecuentes en los pacientes en diálisis peritoneal ambulatoria crónica o después de alguna cirugía abdominal[6].

Las candidiasis con afectación cardiaca se asocian a la colonización de una válvula cardiaca dañada o protésica durante la diseminación hematógena, sin embargo, pueden afectar el miocardio o pericardio, y se han descrito endocarditis por colocación de válvulas protésicas contaminadas con *C. parapsilosis*. Las candidiasis osteoarticulares suelen ser secuelas tardías de las candidemias; la espondilitis es la lesión más frecuente y se manifiesta como un dolor local acompañado de fiebre[6].

Existen lesiones cutáneas secundarias que se producen hasta en un tercio de los pacientes con candidiasis diseminadas, pero son similares a otras lesiones bacterianas o víricas. Debido a que la sintomatología de una CI es similar a la de cualquier septicemia, es necesaria la sospecha clínica ante todo paciente con enfermedades subyacentes o con factores predisponentes cuya febrícula no remite a pesar de una antibioterapia mayor a 3 días[6].

Es importante tener en cuenta que los pacientes oncohematológicos, la respuesta febril puede ser nula debido a la neutropenia y en muchos casos el aislamiento de *Candida* en los hemocultivos es el único hallazgo en el curso de una candidiasis diseminada. En los pacientes graves no neutropénicos deberá sospecharse en aquellos con una estancia hospitalaria mayor a 5 días que reciban nutrición parenteral y desarrollen algún cuadro de shock séptico seguida de alguna cirugía abdominal. Cuando la candidemia está asociada a presencia y colonización de un catéter intravascular pueden presentarse complicaciones como trombosis séptica, meningitis, entre otras. En estos casos es aconsejable la retirada y sustitución del catéter en medida de lo posible[6].

La presentación más común en la candidiasis neonatal es la afectación sanguínea asociada a meningoencefalitis que puede condicionar la aparición de secuelas

neurológicas permanentes y éstas pueden afectar a un porcentaje importante de sobrevivientes. Como ya mencionamos, la candidiasis hepatoesplénica es casi exclusiva de pacientes oncohematológicos, su cuadro clínico consta de fiebre en un 100% de los casos; el dolor abdominal en el cuadrante superior derecho ocurre en el 65% de los casos y se acompaña de síntomas gastrointestinales en el 34% de los pacientes ésta cursa además con hepatoesplenomegalia y elevación de la fosfatasa alcalina[22].

Diagnóstico

El diagnóstico de la candidiasis invasiva a menudo se efectúa tardíamente, lo que causa una demora en el inicio de la terapia antimicótica. El diagnóstico tardío puede ser el resultado de signos y síntomas clínicos inespecíficos[23] como la precisión variable de las pruebas diagnósticas disponibles, la demora en el crecimiento de los cultivos de *Candida*, de que los hemocultivos no sean positivos hasta las etapas avanzadas de la infección, del inadecuado volumen de muestra para los hemocultivos, de los resultados falsos negativos debido al uso de agentes antimicóticos en la profilaxis[24]. La superación de estas dificultades para el diagnóstico puede mejorar el tiempo requerido hasta instituir el tratamiento de la infección.

Métodos para detectar la infección hematógena por Candida

Se puede alcanzar el diagnóstico a través de métodos convencionales como el hemocultivo y la detección de marcadores serológicos como el 1-3-β-D-glucano (BDG), el manano o anticuerpos antimanano. Los métodos diagnósticos más nuevos incluyen la reacción cadena de la polimerasa (PCR) en muestras de sangre o biopsia de tejido y el enzimoinmunoensayo ELISA para detectar *Candida*[25].

Hemocultivo

Es el *gold standard* para el diagnóstico de la candidemia, sin embargo, es un procedimiento que requiere mucho tiempo. Esto puede afectar el momento de inicio de la terapia y contribuir a un incremento de la morbimortalidad. El tipo de medio utilizado para el cultivo también puede influir sobre el tiempo hasta la detección[26].

Serología

Actualmente se encuentran en desarrollo varias pruebas serológicas para la detección de candidiasis. Estos métodos incluyen el ensayo BDG y los ensayos de detección de manano y de anticuerpos antimanano[27]. Una reciente revisión de métodos serológicos para el diagnóstico de candidemia en pacientes críticos mostro una mayor sensibilidad diagnostico con el uso combinado de antigenos y anticuerpos. BDG al ser un componente estructural de la pared celular de *Candida* podría ser de utilidad como biomarcador de candidemia, estudios que utilizaron el ensayo sérico de BDG para el diagnóstico de candidemia informaron de tasas del 60 al 100% de sensibilidad y del 40 al 90% de especificidad, superiores a las de los hemocultivos[28].

Sin embargo, debido a la falta de disponibilidad y a los elevados costes es probable que muy pocos centros en México utilicen este ensayo[25].

Detección de infecciones profundas

La ecocardiografía ha demostrado ser una herramienta efectiva para el diagnóstico de la endocarditis inducida por especies de *Candida*[29] y las imágenes de resonancia magnética (RM) y de tomografía computarizada (TC) han mostrado ser efectivas como herramientas no invasivas para la identificación de infección hepatoesplénica[30]. Aunque la RM y la TC son sensibles para detectar microabscesos micóticos asociados a la candidiasis diseminada crónica, sin embargo, el requerimiento de imagen repetidas durante el curso de una infección limita su empleo. La ecocardiografía asistida por ordenador ha mostrado ser exitosa para detectar microabscesos en el hígado o bazo. Y puede ser repetida tantas veces como sea necesario, lo que la convierte en un método útil para la detección de la candidiasis diseminada crónica y el seguimieneto de pacientes con la mencionada candidiasis[31].

Biopsia

El diagnóstico de infecciones por *Candida* a partir de muestras de tejidos puede ser difícil. Estos microorganismos colonizan comúnmente la piel y las membranas

mucosas de los seres humanos y, por lo tanto, el aislamiento de muestras superficiales como esputo, secreciones traqueales y orina no es necesariamente una demostración de invasión. La candidiasis diseminada crónica se diagnostica a través de la identificación de estructuras micóticas en el microscopio o el crecimiento de hongos en materiales de biopsia dado que los cultivos de sangre son positivos en menos de 20% de los pacientes con candidiasis diseminada[32]. En pacientes en riesgo de desarrollar lesiones cutáneas se utiliza la biopsia para confirmar el diagnóstico de infección diseminada, al igual que una biopsia pulmonar confirmará el diagnóstico de neumonía mediante la identificación de elementos micóticos invadiendo el tejido pulmonar.

Nuevas perspectivas

Recientemente se desarrolló un ELISA para la detección de un antígeno de 65 kDa producido por *C. albicans, C. tropicalis* y *C. parapsilosis*. En este estudio esta nueva prueba diagnóstica detectó este antígeno en el 80% de los pacientes con candidemia[33]. La aparición de la PCR para la detección de ácidos nucleicos ha brindado un método alternativo más rápido y más preciso que el hemocultivo para la detección de especies de *Candida* en las muestras de sangre[34]. Además, se ha utilizado la PCR para la detección de *Candida* en muestras de biopsia en pacientes con candidiasis diseminada crónica.

Tratamiento

La conducta a seguir con pacientes con candidemia se resume en algunos puntos en general:

i. Por lo menos un hemocultivo positivo para *Candida.*

ii. Evaluación clínica rigurosa a fin de descartar compromiso visceral.

iii. Retirar, en medida de lo posible, el catéter endovenoso (si lo hay) y cultivar el extremo

iv. Controlar la evolución del paciente, ya que han aparecido candidiasis orgánicas semanas o meses después de episodios de candidemia aparentemente aislados[5].

Los criterios para indicar tratamiento antimiótico son;

i. Síndrome febril persistente y/o hemocultivos positivos después de extraer el catéter.
ii. Evidencia de compromiso orgánico.
iii. Neutropenia ≤500 PMN/mm_3[5]

Terapia empírica para pacientes neutropénicos

La terapia antifúngica empírica es considerada el tratamiento estándar en los pacientes neutropénicos con fiebre persistente a pesar de una terapia antibiótica apropiada y habitualmente se intenta que sea eficaz contra *Candida*. Su aplicación exclusivamente para CI es ocasional, y se considera su uso únicamente en pacientes que no hayan recibido profilaxis, tengan fiebre persistente, además de mucositis severa. Para estos casos, se recomienda la terapia antifúngica empírica con fluconazol, a una dosis de carga inicial de 800 mg (12mg/kg) y para continuar con una dosis de 400 mg diarios (6mg/kg)[36].

La decisión de efectuar el tratamiento empírico se basa usualmente en la sospecha de que el paciente tiene riesgo de estar infectado tanto por *Candida* como por alguno moho. Existen otras alternativas aceptables que incluyen el tratamiento con equinocandinas como la caspofungina (dosis de carga de 70 mg, posteriormente 50 mg diarios o la micafungina (100 mg diarios). Incluso una formulación lipídica de anfotericina B liposomal (3mg/kg diarios) o anfotericina B complejo lipídico (5mg/kg diarios) O voriconazol con una dosis de carga de 6 mg/kg 2 veces al día y después 3 mg/kg 2 veces al día[36].

Terapia para candidiasis hematógena confirmada

Para pacientes no neutropénicos, las equinocandinas es la recomendación de primera línea, se recomienda el uso de una equinocandina para el tratamiento inicial de la candidemia en adultos no neutropénicos. Las equinocandinas son inhibidores no competitivos de la síntesis de BDG. Su esquema de administración es cómodo (1 vez al día) y su actividad es fungicida contra todas las especies de *Candida*[35]. Varios factores avalan el uso de las equinocandinas frente a los azoles en el

tratamiento inicial de la candidemia; en primer lugar, los cambios en la sensibilidad antifúngica, como la creciente prevalencia de *C. glabrata* y su relación con el uso de fluconazol, así como la progresiva disminución de la sensibilidad de *C. krusei* frente a los azoles, lo que sugiere que el uso de las equinocandinas debe tener preferencia sobre estos para el tratamiento inicial[37,38]. Por último, hay que mencionar que el fluconazol es el agente antifúngico más utilizado como terapia primaria para la candidemia en México, pero con un alto reporte de mortalidad en todos los niveles[39].

Si se inicia un tratamiento con una equinocandina y durante el seguimiento se conoce que en los cultivos iniciales se aisló un microorganismo sensible al fluconazol, si la evolución del paciente es favorable, es posible bajar un nivel o desescalar el tratamiento, cambiando éste a fluconazol. Sin embargo, se recomienda considerar cuidadosamente la posología según la función renal del paciente. No se conoce la duración óptima de la terapia con una equinocandina antes de bajar un escalón terapéutico[39].

En un estudio de 159 pacientes con candidemia o CI se cambió el tratamiento con anidulafungina por fluconazol o voriconazol, se obtuvo una respuesta terapéutica global efectiva del 80.2% en los pacientes cambiados a fluconazol y del 93.6% en los pacientes cambiados a voriconazol[40].

Para el tratamiento de las infecciones fúngicas oculares se recomiendan los triazoles antes que las equinocandinas. Para las infecciones fúngicas cerebrales y ocurales se recomienda el voriconazol más que una equinocandina[36].

En pacientes neutropénicos, considerando los riesgos de toxicidad renal asociados al uso de anfotericina B desoxicolato, se recomienda con énfasis que se evite el uso de este agente para el tratamiento de la candidemia. Al igual que en los pacientes no neutropénicos se puede considerar una equinocandina como fármaco de elección para el tratamiento inicial de la candidemia en pacientes neutropénicos. Las alternativas al uso de una equinocandina incluyen el uso de una formulación lipídica de anfotericina B, vorizonazol o fluconazol. Sin embargo, el uso de estos azoles puede estar limitado por: a) la mayoría de los pacientes neutropénicos que han recibido previamente fluconazol como profilaxis, y b) la candidemia por *C*.

glabrata es más frecuente en este grupo de pacientes. El esquema para pasar a un agente oral como un azol puede ser adelantado cuando se dispone de información sobre la identidad de las especies y la sensibilidad antifúngica, siempre que el paciente esté mejorando[41].

Después de confirmar el diagnóstico de candidiasis hematógena, además de comenzar la terapia, se deben realizar una serie de exámenes, sin embargo, las guías actuales para el tratamiento de la candidemia brindan información limitada sobre recomendaciones para evaluar al paciente después del diagnóstico. Para la evaluación del paciente después de la terapia, se recomienda la repetición de los hemocultivos iniciales el día 3 y 5, o hasta que los hemocultivos no muestren crecimiento. El tratamiento de la candidemia en pacientes neutropénicos debe continuar durante 14 días después del primer hemocultivo negativo, siempre que se haya producido la resolución clínica de la infección[41].

En pacientes pediátricos, la candidemia diseminada secundaria a catéteres intravasculares diseminados exigen la retiración de éstos y dosis de anfotericina B (0,5-1mg/kg/día V.I), éste ha sido el soporte principal del tratamiento de la candidiasis sistémica. La duración del tratamiento varía ampliamente en función de la extensión de la infección, la respuesta clínica y la toxicidad del fármaco. La dosis total recomendada es de 20-30 mg/kg. La nefrotoxicidad es frecuente en neonatos y se manifiesta generalmente por oliguria, azoemia e hiperpotasemia. Las fórmulas de complejos lipídicos de anfotericina B (5mg/kg/día) estpan recomendadas en neonatos con función renal comprometida, incluyendo la duplicación de la creatinina sérica por tratamiento con desoxicolato de anfotericina B. El fluconazol es útil en el tratamiento de las infecciones invasoras por Candida en neonatos, especialmente las infecciones urinarias. El fluconazol es inactivo frente a todas las cepas de *C. krusei* y aproximadamente el 20% de las cepas de *C. glabrata*. Tales cepas son sensibles a voriconazol e itraconazol, sin embargo, presentan resistencia cruzada a otros azoles.

La caspofungina presenta una excelente actividad frente a la mayoría de las especies de *Candida* y se ha utilizado con éxito en enfermos con microorganismos

resistentes a azoles. Se deben retirar los catéteres vasculares, si los hay, asociados a la fungemia, administrando a continuación tratamiento antifúngico intravenoso durante 2-3 semanas. Los trombos infectados intracardiacos o intravasculares tienen que extraerse, aunque se han observado casos que han evolucionado bien sin cirugía.

En pacientes pediátricos inmunocomprometidos, la anfotericina B continúa siendo el tratamiento de elección en las CI, tanto en solitario como asociada a fluconazol, el cual es especialmente útil en infecciones del sistema nervioso central y del parénquima renal. Las fórmulas de complejos lipídicos de anfotericina B (5mg/kg/día) están recomendadas en pacientes con alteración de su función renal aunado a su inmunodepresión. Ni el voriconazol ni la caspofungina han sido suficientemente estudiados en niños[42].

Conclusiones

Las infecciones micóticas en el torrente sanguíneo y órganos en general están aumentando con frecuencia, en parte, debido al aumento del uso de dispositivos intravasculares y de antibioterapia de amplio espectro de manera no controlada. Los pacientes, ya sea adultos o pediátricos, con factores de riesgo concomitantes deben de ser prioridad del sector salud en ambos países, la mortalidad de éstos crece con el paso de los años, el riesgo no está en los agentes etiológicos que varían en dichos países, ya que es normal por la ubicación geográfica y el clima que existe tanto en México como en España, el riesgo muchas veces está en la mala profilaxis que existe por parte del personal de salud. En esta revisión bibliográfica se encontró como mayoría de factores de riesgo los agentes iatrógenos, es decir, los que dependen del ya mencionado, personal de salud; mal uso de antibioterapia, inmunosupresores, mala higiene y falta de esterilización de dispositivos que se colocan en los pacientes que, previamente, están con salud crítica. Es importante avanzar en tecnología para erradicar estas cepas de los hospitales y centros de atención primaria, pero es más importante, insistir en los pacientes y en el personal hospitalario sobre buenas medidas de higiene, para disminuir a corto, mediano y largo plazo la resistencia de estos hongos a los antifúngicos que conocemos, ya

que, como encontramos en esta revisión, también va creciendo a lo largo del tiempo. Además, creo importante, los médicos siempre deben de estar actualizados en cuanto a los nuevos tratamientos y medidas que se han ido estudiando en los últimos años para no caer en incentivar la resistencia de los hongos, antes de que éstos sean más resistentes que nuestros pacientes. No existen diferencias significativas entre agentes etiológicos en ambos países, sin embargo, es importante resaltar que sí existen semejanzas en cuanto a factores de riesgo se trata. Esto quiere decir que no importa qué cepa se manifieste, si no mantenemos una prevención efectiva, el hongo, sea cual sea, va a manifestarse en los pacientes más indefensos.

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CONTAMINATION OF SOILS IN HOMESTEAD

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Abstract

The term pesticide is a compound word that includes all chemicals used to destroy or control pests. In agriculture, herbicides, insecticides, fungicides, nematicides and rodenticides are used. A key factor in the Green Revolution has been the development and application of pesticides to combat a wide variety of insects and herbaceous pests that would otherwise reduce the volume and quality of food production. The use of pesticides coincides with the chemical age, which has transformed society since the 1950s. In places where intensive monoculture is practiced, pesticides are the usual method of pest control. Unfortunately, the benefits of chemistry have been accompanied by a number of harms, some of them so serious that they now pose a threat to the long-term survival of important ecosystems as a result of predator-prey relations and biodiversity loss. In addition, pesticides can have important human health consequences.

.*Keywords*: Contamination, Soil, Pollution, Pesticide, Miami, Farming.

CONTAMINATION OF SOILS IN HOMESTEAD

Seoánez Calvo, M., 1999a, states that soil is a vital resource and is the physical support on which all living beings settle. It is also the primordial source of raw materials and constitutes one of the basic elements of the natural environment. For centuries mankind has used the soil to develop and improve its living conditions, since it is carried out all the processes of man's production, such as agriculture, industry, urban infrastructure, among others.

It was in 1992 at the Rio Summit that the importance of the protection of soils and their potential uses in the context of sustainable development was recognized, particularly against pollution from anthropogenic actions or activities.

The ground's pollution the quality of soil is associated with the presence of chemical substances defines how the increase in the concentration of chemical compounds, of anthropogenic origin, causes detrimental changes and reduces their potential use, both by human activity and nature.

Causes of soil pollution

The causes of soil pollution are associated with certain winds such as: the atomic tests, such as those made by the British in Australia, which cause the soil to be subjected to decontamination for thousands of years; Nuclear accidents like Chernobyl show the incredible and enormous contamination of soils, water and atmosphere, as a result of lack of common sense or restrictive laws to possible sources of contamination; On the other hand, among the most common causes of soil pollution are: the use of agricultural technology in the harmful use of sewage or polluted waters of rivers; Indiscriminate use of pesticides, pesticides and hazardous fertilizers in agriculture; Lack or improper use of urban waste disposal systems; Industry with anti-regulatory waste disposal systems; Contamination of the soil by pouring plastics, among others.

Consequences of soil contamination

Among the consequences of soil contamination we can mention the alteration of the biogeochemical cycles, among which are the cycle of carbon, oxygen, phosphorus, sulfur and nitrogen, among others. These elements and derived processes go from land to air and water and also circulate between different living things and due to artificial contaminants, can be modified. On the other hand, groundwater pollution, groundwater accounts for 97% of all fresh water on the planet and can be contaminated by soil contamination of soil contaminants as pesticides used in excess in agriculture .The excess nitrogen deposited in the soil with fertilizers is stopped by filtration to the groundwater contaminating them. Another factor of importance is the interruption of biological processes, in which contaminated soil prevents the development of wildlife, without food or clean water, the species migrate or suffer irreparable damage to their procreation chain. This process then suffers what is called "degradation of the landscape" and therefore a loss of soil.

Some solutions to this problem may be: Eco-agriculture is gradually gaining ground in artificial agriculture, especially in European countries and in some poor communities seeking alternative food production. Ecoagriculture does not use pesticides or agrochemicals; The recycling of plastics, batteries, glasses or oils for cars and kitchens because they are elements that take many years to degrade, so this action contributes to keep our soil free of contaminants; The actions focus on improving recycling plants to reduce soil pollution and, at the same time, water pollution, proper recycling of waste and treatment of waste and promotion of renewable energy.

Pesticides. Pesticides are substances or a mixture of substances that are used intensively to control agricultural pests and insects vectors of diseases in humans and animals, as well as for the control of insects and mites that affect the production, processing, storage, transport Or marketing of food, agricultural products, wood and animal feed (FAO, 2003). However, it is

recognized that they are chemically complex substances which, once applied in the environment, are subject to a series of physical, chemical and biological transformations (adsorption and absorption phenomena on soils and plants, volatilization, photolysis and chemical degradation the microbial). In addition they can also be carried away by the currents of air and water that allow their transport to great distances; it must be added that the volatile residues pass into the atmosphere and return with rain to other places (López-Geta et al., 1992). These transformations can lead to the generation of fractions or to the total degradation of the compounds that in their various forms can affect the different levels of an ecosystem (Garrido et al., 1998).

Pesticides, heavy metals and other impurities are considered by the Environmental Protection Agency (EPA, 1992) as contaminants of aquifers due to their high toxicity, persistence and mobility, as well as affecting important hydraulic loads such as ponds and canals Of irrigation; And their physicochemical properties, are resistant to biological degradation (Hirata, 2002).

At present, one of the major problems is the indiscriminate and uncontrolled use of these compounds, only in 1992 the world production of pesticides was estimated at 10 mill. Of ton. (López-Geta et al., 1992); of these more than 80% were used in Europe and the United States. Until the middle of the last century, about 40 compounds of botanical or inorganic type were used, among them, lead arsenate, copper aceto-arsenate and a mixture of copper sulfate and lime known as Caldo Bordelés (Albert, 2005). However, at present there is a lack of knowledge of the quantity and types of pesticides (active ingredients) applied in the fields; As well as the scarce control of wastes that are constantly exposed to environmental factors and are sometimes reused again.

In spite of the large number of agrochemicals that are constantly used, little is known about their toxicity in organisms, including humans, as well as the global environmental impact.

In this regard, soils that are the source of food globally, are vulnerable to the processes of degradation, desertification and their effect on the ecosystems they sustain. Among the risks are the loss of soil fertility, from the damage to humus and nutrients that make them productive, such as phosphorus, nitrogen, potassium and others (Orozco-Abundis, 2006). On the other hand, the inorganic particles that integrate the soil allow the accumulation and dispersion of pesticides, not only in the agricultural fields but also in the aquatic environments and organisms, which will depend on the persistence and degradation of the compounds (SEMARNAT, 2005). In this regard, FAO (2003) indicates that it is necessary to make regulations on the use and application of these compounds, especially to have a register and carry out monitoring programs on environmental pollution, intoxication and monitoring of the residues generated by these Compounds.

Pesticides include a wide variety of chemical compounds. Some are persistent; others are not persistent. Persistent chemicals, such as DDT, dieldrin and toxaphene, do not break down rapidly and accumulate in the environment.

Non-persistent pesticides, such as malathion or parathion, are most commonly used in Florida, especially in the southern Dade area, where they are used in citrus and vegetable farms. In 1969-70, they applied to nearly 11,900 acres of citrus and 47,000 acres of vegetable farms in Dade County. Approximately one million pounds of persistent pesticides were used annually on urban and agricultural land in the 1960s in Dade County.

Both persistent and non-persistent pesticides are found in the air, water, plants, and animals of South Dade County, predominantly persistent as DDT. Concentrations are usually at trace levels or below detectable levels in water, but at higher levels in bottom sediment and biota. Concentrations above 1000 μ / kg have been found in birds and mammals. A concentration of more than 16,500 μ / kg was measured on a bald eagle.

The accumulation of persistent insecticides in some birds causes metabolic disorders in their reproductive processes. Increasing concentrations of DDT coincide with the death and decreasing numbers of some species, particularly hawks, eagles and pelicans. Although local use of DDT and other persistent insecticides is declining, accumulation may continue for years as a result of the long life of some pesticides (up to 20 years for the DDT family) and as a result of extensive use elsewhere the world and the final atmospheric transport to Dade County. This is of particular concern in Everglades National Park because birds are a major attraction

Pesticides accumulate in humans at levels thousands of times greater than those of the water they drink and the food they eat. The mean total human fat DDT concentrations of Caucasians older than 5 years in Dade County were 8.4 μg / kg. For comparison, the concentrations in Caucasians from other 22 states ranged from 3.98 to 13.23 μ / kg. States with warm climates had higher averages (9.21 μ / kg) than those with cold climates (4.85 μ / kg). The effects of such concentrations on human health are not known

Impact on the environment. Unfortunately, terrestrial and marine aquatic systems are the most threatened by the contribution of pollutants such as pesticides, fertilizers, heavy metals, pathogenic organisms and others, through the increase of anthropogenic activities in adjacent areas that alter the natural conditions of ecosystems, including humans. The importance of water bodies, active biological sites, lies in the biological diversity and biogeochemical processes that are performed.

Alternatives to avoid pollution in agricultural fields. Ecological agriculture. It is a system of sustainable agricultural production that is used in Europe and is conceived as a viable alternative to the traditional approach of agriculture, food security and environmental problems (Orozco-Abundis, 2006).Based on the environmental and health risks of chemical pesticides, it is necessary to develop technology for the development of new biodegradable formulas for pest and disease control in the region, which are favorable to agriculture and the environment.

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Greenhouse Gases

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Abstract

Infrared (IR) dynamic gasses, basically water vapor (H2O), carbon dioxide (CO2), and ozone (O3), normally exhibit in the Earth's climate, assimilate warm IR radiation produced by the Earth's surface and environment. The air is warmed by this instrument and, thus, transmits IR radiation, with a noteworthy part of this vitality acting to warm the surface and the lower climate. As an outcome the normal surface air temperature of the Earth is around 30° C higher than it would be without air ingestion and reradiating of IR vitality. This wonder is prominently known as the "nursery impact," and the IR dynamic gasses in charge of the impact are moreover alluded to as "nursery gasses." The fast increment in groupings of nursery gasses since the mechanical period started has offered ascend to worry over potential resultant atmosphere changes.

Outline

What we know about Greenhouse Gases? Why the greenhouse effect occurs?

What are the kinds of greenhouses gases?

What are the human activities that produce the greenhouses gases?

How the greenhouse gases impact in this country?

Case: South Beach Miami

Climate change in South Beach Miami

Temperature in South Beach Miami

Animals and plants affected in South Beach Miami

Reference

Citations

What we know about Greenhouse Gases? Why the greenhouse effect occurs?

The phenomenon is called the greenhouse effect by which certain gases, which are components of the planetary atmosphere, retain part of the energy that the soil emits because it has been heated by solar radiation. It affects all planetary bodies endowed with atmosphere. According to most of the scientific community, the greenhouse effect is being accentuated on Earth by the emission of certain gases, such as carbon dioxide and methane, due to human activity. This phenomenon prevents that the solar energy constantly received by the Earth returns immediately to the space, producing at world scale an effect similar to the one observed in a greenhouse.

The greenhouse effect originates because the energy that comes from the sun, coming from a body of very high temperature, is formed by waves of high frequencies that pass through the atmosphere with great ease. The energy sent to the outside, from the Earth, coming from a much colder body, is in the form of waves of lower frequencies, and is absorbed by the gases with greenhouse effect. This retention of energy makes the temperature higher, although it is necessary to understand well that, in the end, under normal conditions, the amount of energy that reaches the Earth is equal to that which it emits. If it were not so, the temperature of our planet would have been steadily increasing, which, fortunately, has not happened.

We could say, in a very simplified way, that the greenhouse effect is to cause the energy that reaches the Earth to be "returned" more slowly, so it is "held" longer along the surface and is thus maintained The elevation of temperature.

Cambio Climático Global. Accessed July 19, 2017.

What are the kinds of greenhouses gases?

Atmospheric gases of natural and anthropogenic origins that absorbs and emit radiation at certain wavelengths of the infrared radiation spectrum emitted by the Earth's surface, atmosphere, and clouds. This property causes the greenhouse effect. Water vapor (H2O), carbon dioxide (CO2), nitrous oxide (N2O), methane (CH4), and ozone (O3) are the main greenhouse gases in the Earth's atmosphere. In addition, a series of man-made greenhouse gases exist in the atmosphere, such as halocarbons and other chlorine and bromine.

Greenhouses Gases molecules have the ability to absorb and re-emit long-wave radiation (this is infrared radiation, which is eminently thermal) that comes from the sun and reflects the Earth's surface into space, controlling the flow of natural energy through the climate system. Climate must somehow adjust to increases in Greenhouses Gases concentrations, which leads to an increase in the infrared radiation that is absorbed by Greenhouses Gases in the lower atmosphere (the troposphere), in order to maintain the energy balance Of the same. This adjustment will generate a climate change that will be manifested in an increase in global temperature (referred to as global warming) that will lead to an increase in sea level, changes in precipitation regimes and in the frequency and intensity of extreme weather events (Such as storms, hurricanes, Child and Child phenomena), and a variety of impacts will be presented on different components, such as agriculture, water resources, ecosystems, human health, among others.

El Efecto Invernadero. Accessed July 19, 2017.

How the greenhouse gases impact in this country?

South Florida is among the most vulnerable places in the world for thegreenhouse gases.

Climate change is already affecting the US

Climate change is transforming the United States into a country increasingly affected by storms, air pollution and disease, according to a new federal science report.

The different damages caused by climate change "will surely become more and more noticeable throughout the country in this century and beyond," concluded the National Climate Assessment on Tuesday.

The report highlighted how warming and changing weather conditions change people's daily lives, even using the phrase "climate change" as one more way of referring to global warming.

"We are all part of a single Earth, a unique atmosphere," said Assistant Director of Research of the Science Division of the US space agency, Jack Kaye, during a ceremony in Washington to commemorate Earth Day 44 last 22 of April.

Climate change, the result of human action, focuses the concerns of environmental groups and governments, as revealed by the director of the NASA Earth Science Division, Michael Freilich.

"The climate is changing and it will have a profound impact on all of us," said Freilich, who reminded the children at Union Station of their "great responsibility" for the future of the planet.

"Humans are the only creatures that can change what we do based on predictions of how the world will be," said Freilich, who emphasized our ability to change our behavior to be "better guardians of the Earth."

Glacier melting and ocean water warming accelerated sea level rise, which rose at a rate of two millimeters per year between 1971 and 2010, and even more rapidly over the last decade.

US President Barack Obama presaged in a statement released Monday that "the consequences of climate change will worsen in the years to come."

"Let us accept our responsibility to future generations and meet the current challenge with the same energy, passion and sense of duty" that led to the creation of the first Earth Day in 1970, Obama said.

Our planet was formed about 4.5 billion years ago and is the densest and fifth largest of the eight that make up the Solar System.

"Climate Miami Beach - Florida - Climate - United States." Accessed July 19, 2017.

Case: South Beach Miami

Located between the 1st and 25th Streets of Miami Beach, South Beach is Miami's busiest and most famous beach. It is an extensive white sand beach and crystalline shallow and calm waters. South Beach is one of the most visited beaches because it is beautiful, but at the same time it is being contaminated and affected both by the neglect of the man not to preserve this beach wonder as the climatic changes that today presents the Earth. South beach has certainly warm temperatures, presents a large range of animals as marriage as terrestrial, like the flora. It is climate is mostly warm.

Climate in South Beach Miami

Miami has a subtropical climate, with moderate and pleasant temperatures throughout the year. The city of Miami has an average of 3,000 hours of sunshine per year, making it one of the sunniest cities in the United States.

The summers in Miami are hot, very humid and quite rainy. The months in which there is a greater number of precipitations are those that go from May to September, dates that also coincide with those of greater risk of hurricanes, while in winters are usually fairly dry and not too cold, with a mean minimum temperature of 13ºC and maximum temperatures of up to 25ºC in the month of December.

Temperature in South Beach Miami

The surface water temperature close to shore at South Beach (Miami) can vary by several degrees compared with these open water averages. This occurs especially after heavy rains, near a South Beach (Miami) sea temperatures in the range 29 to 30 ° C (84 to 86 ° F) on the 10th of August and are at their coldest on the 10th of February in the range 22 to 24 ° C (72 to 75 ° F). Year round warm sea temperatures at South Beach (Miami) climb to their highest in early to mid August. Even then a rash vest and board shorts should be fine for surfing at any time of year. South Beach (Miami) are temperatures are always warm but dip to their coldest in early to mid February.

Weather2Travel.com. "South Beach Climate: Monthly Weather Averages, Florida." Weather2Travel.com. Accessed July 19, 2017.

Animals and Plants affected in South Beach Miami

Florida's vegetation is varied; there are up to 7 floral zones.

- Flatwoods: open forests with an abundance of flowers (up to 60 species of orchid).
- Scrublands: mostly small sand pines.
- Savannas: American lotus, water hyacinth and water lettuce.
- Grassy swamps: The Everglades
- Salt marshes: Mangroves
- Hardwood forest or Hammock: Trees growing on wet soil and in marshlands.
- Pinelands

Florida's fauna has also some native species, specific to Florida. Some of these species are endangered and, therefore, protected.

- Alligators and crocodiles
- Manatees
- The Killer whale, also called the Orca
- The Bottlenose dolphin
- Jellyfish

"Animals and Plants in Miami." Miami Beach Vaction Rentals. Accessed July 19, 2017.

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Mercury in the Everglades

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Abstract:

Water quality conditions in the Everglades are affected by mercury , the accumulation of mercury in aquatic ecosystems is of global concern due to health effects associated with eating fish with elevated Hg levels. Mercury contamination has been recognized as a critical health issue for humans and wildlife that consume fish from the Everglades. The state of Florida has advisories that restrict consumption of some species of fish

Methylmercury is also produced naturally from inorganic mercury in the aquatic environment by bacteria in sediments under conditions devoid of dissolved oxygen. Once produced, is readily taken up but eliminated by fish . This results in a phenomenon referred to as bioaccumulation. The ratio of the methylmercury concentration in a fish to the concentration in the surrounding water is its bioaccumulation factor (BAF). Fish will bioaccumulate higher concentrations of methylmercury, this results in a phenomenon referred to as biomagnification

Keywords: Everglades, mercury, contamination, bioaccumulation, biomagnification.

Introduction

The element mercury (Hg) is naturally present in the earth's crust. Pure elemental mercury, which is a silver colored liquid metal at room temperature, is obtained by smelting its most abundant ore, mercuric sulfide or cinnabar. Pre-industrial human uses of mercury were surprisingly significant, with the ancient Romans reported to have used more than two tons per year. Modern human uses of mercury include gold mining, chlor-alkali production, batteries, turf and seed treatments, contact explosives, silent and pressure switches, thermometers and manometers, fluorescent lights, house paints, and fillings for dental cavities

Mercury is a liquid metal at ambient temperatures and pressures. It forms salts in two ionic states. Mercury salts are much more common in the environment, and if soluble in water are bioavailable and considered toxic. Mercury also forms organometallic compounds, many of which have industrial and agricultural uses.

The toxicity of mercury salts and elemental mercury to humans has been known since the dawn of history. Toxicity to humans increases with the form of mercury in the order inorganic mercury salts, elemental mercury vapor, and methylmercury salts. Inorganic mercury and methylmercury are also highly toxic to wildlife species

The Everglades appears to be especially susceptible to a methylmercury problema because have the highest average concentrations of mercury of any area in Florida.

The Everglades

The Florida Everglades is among the largest freshwater wetlands in the world. It covers a region about 60 Km wide by 160 Km long and extends south of Lake Okeechobee to Florida Bay*).*

The Everglades ecosystem has been greatly altered during the last century to provide for urban and agricultural development, which has severely impacted this ecosystem.

In 1948, the Central and Southern Florida Flood Control Project was created by federal legislation in response to periods of drought in the 1930s and 1940s, and severe flooding with loss of human life in the 1920s and 1940s. This project is one ofthe most intensive public water management systems constructed that effectively provides flood control and water supply to facilitate urban and agricultural growth, as intended. The canal system quickly drains water from developed areas and the wetlands that remain.

The result is that some areas are too wet while other areas are too dry. Historically, most water slowly flowed across or soaked into the region's vast wetlands. Today, over one-half ofthe region's wetlands have been irreversibly drained. South Florida's population of about six million continues to grow and compete for the land and water of the Everglades ecosystem. One-fourth of the historic Everglades are now in agricultural production: sugar cane and vegetables are grown on the peat soils of drained sawgrass marshes. An extensive system of canals, levees, and water control structures have modified the Everglades water conditions and it is thought to provide a conduit for pollutant transport from urban and agricultural áreas

Mercury Problem

In the last two decades there has been an increased awareness of mercury contamination of game fish and wildlife in the Florida Everglades. About one million acres of the Everglades system reportedly contained large mouth bass with mercury concentrations above 2 mg/Kg double the FDA limit for human consumption. In addition, mercury accumulation may reduce the breeding success of fish-eating birds and an endangered Florida panther was found dead with a high liver methylmercury concentration of 110 ppm. In response, fish consumption advisories have been issued for almost the entire Everglades system.

To understand the biogeochemical cycling of mercury in the Everglades ecosystem, it is necessary to understand the processes and factors influencing the flux of mercury through this system. The mercuric ion is the predominant species in the Everglades aquatic environment. However, sulfate-reducing bacteria can transform inorganic mercury into methylmercury. Various hypotheses have been formulated to account for the apparent susceptibility of the Everglades to mercury impacts , including a high rate of net methylation of mercury and a high bioavailable fraction of methylmercury.

The sources, distribution, transport, transformations and pathways of mercury through the Everglades are not well understood. Among the possible mercury sources are natural mineral and peat deposits, atmospheric deposition, local emission sources such as medical and municipal waste incinerators, regional air emissions sources such as power plants, and local water sources such as agricultural runoff.

Sources of Mercury

Mercury in the natural environment originates in the soils and sediments deposited with the formation of the earth's crust and the early atmosphere. A significant source of atmospheric mercury is the natural evasion of elemental mercury from the surface of soil and water. Deposition from the atmosphere back to the earth's surface completes this cycle and ensures a continuous supply of newly available inorganic mercury for biogeochemical transformation, including formation of elemental mercury and methylmercury.

In addition to its natural background sources, atmospheric mercury is generated by a variety of human activities, including combustion of fossil fuel and waste, mining and smelting of mineral ores, and the use and disposal of mercury itself . Mercury may be removed from the air and deposited on water, soil, or plant surfaces in wet deposition (rain or snow) or dry deposition (particle settling and gas adsorption to the solid or liquid surface). Although the relative proportions may change depending on the source, mercury exists in the atmosphere in three forms, which differ greatly in their air chemistry and in the physical properties that determine their rates of removal from air by wet and dry deposition processes.

Cycling of Mercury

Mercury is found in aquatic ecosystems in three forms. In descending order of occurrence they are inorganic mercury, Hg, methylmercury, and elemental mercury. Once present in an aquatic environment, inorganic mercury can be converted to methylmercury by microbially mediated processes in the water but more often in the sediment.

Methylmercury is absorbed across the gut from food items . The most significant route of loss of methylmercury from fish is believed to be across the gill membrane. As

a consequence, methylmercury is only slowly excreted by fish too.

Because the methylmercury depuration rates decrease and bioaccumulation factors increase with increasing size in fish and age in fish and also the average fish size increases with each trophic level , large, top predator fish will bioaccumulate methylmercury up to several million times the concentration in the water column, as is the case for several species of top predator fish at some locations in the Everglades. A number of environmental factors are believed to influence methylmercury bioaccumulation in fish in aquatic ecosystems. Methylmercury bioaccumulation tends to be higher in fish in waters with high temperature

The increase in methylmercury production first manifests itself as an increase in the methylmercury concentrations in water and the one-celled plants and animals that form the base of the food chain. This increase then propagates up the food chain with biomagnification at each link, peaking in top predator fish.

Human Health Effects from Everglades Mercury

Based upon current knowledge of mercury toxicity, there are no direct effects to human beings from drinking or contact with waters containing the levels of inorganic mercury and methylmercury that are found in the Everglades. The only quantitatively significant pathway for methylmercury to exert its toxic effects on humans by consumption of predators high in the food chain, which have bioaccumulated high levels of mercury. If humans, particularly pregnant women, were to eat sport fish from the Everglades, they would be at risk from methylmercury toxicity. Signs at some water access points warn of these effects. Literature prepared by the Game and Fresh Water

Fish Commission for distribution with fishing licenses also contains these warnings.

No documented adverse human health impacts from environmental methylmercury exposures are known in South Florida. Studies of people eating fish caught in South Florida carried out by the University of Miami and the Centers for Disease Control found that mercury body burdens were proportional to fish consumed, but not sufficiently elevated to cause toxicity. However, these studies had limited representation of subsistence fishermen.

Solving the Everglades Mercury Problem

The solution to the Everglades mercury problem has several steps. The first step is to learn what level of mercury in fish is safe for both humans and wildlife. The second step is to learn what human actions are causing or contributing to the Everglades mercury problem. Potential causes include present day atmospheric deposition, mercury in stormwater runoff and reentry into the ecosystem of mercury that was previously buried in Everglades peat soil. Potential contributing factors include changes in water quantity and quality that might liberate buried mercury for recycling in the Everglades or facilitate its accumulation in fish and wildlife. The third step is to understand how the Everglades processes inorganic mercury from atmospheric deposition, runoff, and peat soil into methylmercury, the most toxic form of mercury in the aquatic environment. The fourth step is to understand how to relate the quantities of inorganic mercury added to the Everglades ecosystem each year to the concentration of methylmercury in fish. This is to be done with mathematical models that represent all of the key processes governing methylmercury production and bioaccumulation. The fifth step is to determine the best way to reduce the levels of methylmercury in fish to safe levels by managing mercury

sources and water quantity and quality using the model. Potential candidates for management are emissions from local air pollution sources, chemical constituents in stormwater runoff from the Everglades Agricultural Area, and water depth and flow.

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Pollution in Everglades

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Abstract

The Everglade is common called “The river of grass” is most important river for Florida, The Everglades cover about 1,500,000 acres where habit wonderful species of animals and plants, the Everglades also supply fresh water for almost all Florida and it’s a land suitable for farming, the benefits that we receives of this area are a lot, but unfortunately the humans has contaminated this sources of water for many years. The pollution consequences affect all type of being livening in Florida, animals, plants include population Floridian. The Everglades is not only a principal source of water but also is a perfect land for agriculture, since the war world end the farmers began growing vegetable and sugar cane. The Everglades is a home for many species of animals animal and plants that make a wonderful place, but the pollution other factor produced by man to cause problem in this beautiful place.

Key Word: Pollution, Everglades, water, animals, plants.

Everglades

The everglades, common called the “river of grass”it is a home for many fauna and plants. The Everglades cover a huge part of Florida, from to Lake Okeechobee to the Florida Bay. It´s approximate a distance of more than 240 miles and it’s this lake that provided, unfortunately many birds, fish, reptiles, amphibians, mammals, and plants have disappeared due the canals and levees of the lakes have been increased, flooding other parts with more water than they need.

Pollution in Everglades

The Everglades is polluted all days by humans, Even thought the Everglades have too much water for ministry all Floridathe water conditions worsened. The Kissimmee River is the main source of fresh water for Lake Okeechobee and the southern wetland. This problem is affecting all of the species and living beings that habit there and the distribution to parts of Florida.The construction around to Everglade throw its waste and contaminate the habit, this sharply reduce the flow of water into the Everglades with disastrous results for plants and wildlife, the fertilizer used in farming and lawns throw chemicals that damage the soil and water. The Agricultural and storm water runoff has degraded water quality and brought about the species living there grow in low phosphorus conditions.

Causes of pollution in Everglades

In 1900 the massive development in south Florida caused damage in Everglades, because the cities surrounding the Everglade use huge amounts of the water supply. The increase population around Everglades is polluting fresh water for Florida,the agriculture has also contributed to pollution in Everglades: Sugar plantation and vegetable farms consume much water and harmful chemicals used in agriculture run off into the water supply, the plantation of other not native plants produce problems in the area, when such seeds take root and develop into plants, they can over power and replace native Everglades species.

Consequences of pollution in Everglades

The human intervention now threatens the existence in Everglades, the live in the Everglades is affecting by pollution; different species of animals can be found nowhere else in the world is suffers because its habit is damaging by pollution and the food chain is breakout, this it entails kill innocents animals and problem with balance in food chain.The use skin alligator for profit almost disappeared from State. High phosphorus causes loss of the natural communities of algae that are defining characteristics of the Everglades, loss of water dissolved oxygen that fish need, changes in the native plant communities that result in a loss of the open water areas where wading birds feed.

Importance to Everglades

The Everglades is the principal source of water to all people living in Florida: The everglades supply fresh water all Florida, the Everglades help to represent the culture, economy and natural heritage for Florida, also have a wonderful marshes, freshwater ponds, prairies and forested uplands and here live in many animals such as: squirrels, raccoons, armadillos, opossums, deer, turtles, rabbits, otters, snakes, rats, mice, alligator, and gophers thrive inland. Florida panthers live primarily in the Everglades. Ordinary birds, such as blue jays, vultures, crows, woodpeckers, robins, owls, geese, cardinals, and mockingbirds, dot the state. But the Everglades have rare birds too, like long-billed roseate spoonbill and the anhinga.

Saving Everglades

In actuality existed some groups in pro to save Everglades;since 1991 the U.S The Army Corps Engineers cooperated to reconnect some parts fragmented by building of artificial barriers and drainage canals in Everglades, also They continue working to restoring to the Kissimmee to its original course. The state of Florida has also collaborated with the mission for saving the Everglades: The state of Florida has created a mechanism to clean the

lakes around Okeechobee: they constructed new marshes that will store water and filter out toxic chemicals before releasing the additional water into the wetlands to the south.

*Who can we do for help the Everglades?*Turn off the water while you brush your teeth , water your lawn in the early morning, late evening or at night, take quick showers instead of baths, fix leaks around you house, apply three “R”: Recycle, Reuse, Reduce.

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Overview of PT-QSRR models for predicting yield of reaction

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Introduction

In organic chemistry the prediction of yield of reaction Yld(%) is very important. In almost all cases, organic chemists infer qualitatively the yield of a query reaction $Yld(\%)_{new}$ taking into consideration the experimental results for a previous reaction of reference $Yld(\%)_{ref}$. The PT-QSRR models are a quantitative expression of this idea because they applied Perturbation Theory (PT) to seek Quantitative Structure-Reactivity Relationship models. PT-QSRR predict the yield of some reactions comparing quantitatively the molecular properties of components such as catalyst, substrate, product, and nucleophile as well as controlled variables such as time, temperature and catalyst loading of both the new reaction and the reaction of reference. Other authors have previously developed a PT-QSPR approach, which combines perturbation theory (PT) and QSRR ideas, to correlate and predict different outputs (properties) in complex molecular systems (metabolic reactions) nanoparticles, and so forth (1). The method has also been extended to predict the enantioselectivity and/or yield of intramolecular carbolithiation and Heck–Heck cascade reactions (2). In some cases, the developed PT-QSRR models use trace operators, like spectral moments, or eigenvalues of chemical structure matrices, like bond adjacency matrix, as the inputs.

Methods

The molecular descriptors of type k, structural variables, $V_k(Mi)$ are calculated for each molecule Mi in both reactions. Next, the deviations $\Delta V_k(Mi) = V_k(Mi)_{new} - V_k(Mi)_{ref}$, can be used to quantify the structural perturbations or structural changes in the new molecules with respect to the query ones. In the same form deviation operators canc be used to measure perturbations on variables $V_k(cj)$ depending on the experimental conditions cj, $\Delta V_k(cj) = V_k(cj)_{new} - V_k(cj)_{ref}$.

Model

$$Yld(\%)new = Yld(\%)ref + \sum ak \cdot \Delta\mathrm{Vk(Mi)} + \sum ak \cdot \Delta\mathrm{Vk(Mi)} + e0$$

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Editorial: EHUDW01, First EHU-DELFIN Program Workshop, Bilbao, Jul, 2017

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The UPVEHUDW01: First UPV-EHU DELFIN Program Workshop, Bilbao, Jul, 2017 is a scientific and educational workshop organized by professors of the University of the Basque Country (UPV/EHU) and IKERBASQUE, Basque Foundation for Sciences. The workshops is associated to the MOL2NET International Conference Series on Multidisciplinary Sciences, MDPI Sciforum, Basel, Switzerland. The workshop aim is to promote the scientific, educational, and cultural interchange of professors and students of UPV/EHU with students of the international education network DELFIN, Mexico. The DELFIN program was created in 1995 with the aim of strengthening the collaborative culture between the Higher Education Institutions and Research Centers that are members of the Program, through the mobility of professors-researchers, students and the dissemination of scientific and technological products. In particular, the workshop aims to strengthen the development of research and national graduate. Specifically, student mobility is promoted through academic research stays, within the framework of the Summer of Scientific and Technological Research of the Pacific. This mobility program strengthens the vocation of young people for science and technology and influences their decision to join postgraduate programs at home and abroad.

UPVEHUDW01: La primera jornada UPF-EHU DELFIN, Bilbao, Jul, 2017, es una jornada científico y educativa organizada por profesores de la Universidad del País Vasco / Euskal Herriko Unibertsitatea UPV/EHU y/o profesores de IKERBASQUE, Fundación Vasca para las Ciencias. El objetivo del taller es promover el intercambio científico, educativo y cultural de profesores y estudiantes de la UPV / EHU con los estudiantes de la red internacional de educación DELFIN, México. El programa DELFIN se creó en 1995 con el objetivo fortalecer la cultura de colaboración entre las Instituciones de Educación Superior y Centros de Investigación integrantes del Programa, a través de la movilidad de profesores-investigadores, estudiantes y de la divulgación de productos científicos y tecnológicos. En lo particular para fortalecer el desarrollo de la investigación y el posgrado nacional. Específicamente, se promueve la movilidad estudiantil mediante estancias académicas de investigación, en el marco del **Verano de la Investigación Científica y**

Tecnológica del Pacífico. Este programa de movilidad, fortalece la vocación de los jóvenes por la ciencia y la tecnología e influye en su decisión por integrarse a programas de posgrado en el país y el extranjero.

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Biotechnology in plants genomics: a legal and bioethics overview.

Anisley Negrín Ruiz [1], Lázaro Pino Rivero [2]

1

2

ABSTRACT
Concepts like bio-security and bioethics have been put into the test whit the rapid advance of the Biotechnology. Specifically the plant's genome manipulation is worthy to be reconsidered by the ethical and juridical point of views. Plants varieties and products obtained by these new biotechnological methods are important achievements but also can be a risk for the human health and the environment. On the other hand, a monopoly of commercial exploitation for the holder of the patent and other exclusives titles like Certificates of Vegetable Obtaining can leave out the fair access to the technological progresses. In this work we make a valuation about those aspects of Biotechnology related with the genome of the plants and their juridical protection.

Key words: Biotechnology, Genetic Engineer, Plants genome, Organisms genetically modified, Vegetable variety, obtainer, Bio-security, Bioethics.

1. PLANTS GENETIC MODIFICATION: THE RISK-BENEFIT DICHOTOMIES

The modern Biotechnology innovations regarding plants genome are presented as an alternative for solves some of climatic changes troubles. Because of the difficulties to predict long term effects of innovation in agricultural ecosystems such point of view is discussible, and the supporters of the "biotechnical agriculture" number it is comparable to their detractors.

One reason why many people are worry about biotech agriculture is the assumption, in some circles, that almost any problem of production or plague control in agriculture can be solved through genetic manipulation. From this perspective, working with the right genes will eliminate any problem, or at least make management it much easier. This idea is based on a dangerous lack of knowledge about what happens in agricultural ecosystems and is one of the reasons why Biotechnology worries so many people (1).

On the other hand, Intellectual Property recognizes rights equivalent to a patent to the obtainer's of new varieties of plants that could have come along the path of genetic manipulation. The Certificate of Plant Variety is thus established as a monopoly of exploitation rights. Thus the questions are: if the genetic modification of plants, applied essentially to agriculture, is a real alternative, why to recognize the exclusives rights of the breeder that prevents society from free consumption? And if -on the contrary- it constitutes a danger, why to protect genetic manipulation on plants?

Certainly the application of Genetic Engineering techniques in the agricultural environment has generated a debate about the advantages and risks of genetically modified plants, both for human health and for the environment. Advantage example is the obtaining more resistant to diseases and pests crops; in this way, it can avoid the use of insecticides that produce environmental problems, while preventing viruses, fungi and insects from becoming more resistant every day. It is also advantageous to achieve crops that are more resistant to adverse soil and climate factors such as heat, frost, drought, salinity or acidity. So is the improvement of the nutritional quality and the appearance of the fruits, providing them with a balanced nutritional content and a better taste and texture.

The application of genetic engineering in animals and plants for the production of drugs, industrial chemical compounds, fuels, plastics, medical products and other materials could also be understood as an advantage (8). As well as the so-called phyto-remediation, or application of certain plants for the regeneration of contaminated soils" (8).

Nonetheless the genetic modification of plants also involves risks, both for the environment and human health. Among the former, it is necessary to emphasize the uncontrollable dispersion of the offspring of the transgenic plant and the genetic contamination of genetically modified plants to others. Concern is centered mainly on the resistance gene being transferred to the herbicide, creating also resistant weeds. Another important risk to take into account lies with the extend the resistance of genetically modified plants to external agents that they want to control, such as weeds, insects, viruses and fungi. What we would be talking about is a natural inversion of the technique.

Genetic Engineering allows selecting the qualities that are desired in a plant and from there to create an unlimited number of plants whose genomes are identical to each other. The cultivation of these plants will lead, in the opinion of some authors, to the genetic uniformity of the crops, with the increasing deterioration of the biological diversity and vulnerability to diseases, pests or adverse factors of the soil and the climate that would suppose.

With regard to human health, the risks of the use of Genetic Engineering have been valued in the food sector. In this sense, two possibilities are contemplated, organisms that can be used as food and have undergone Genetic Engineering, and organisms that contain an ingredient derived from a genetically modified organism or that have taken place using enzymes or other similar products in their elaboration (9-17). The concern has focused especially about the allergy cause of these foods. In this case, the consumer would be harmed if the composition of the food is not properly reported on the labels. There is talk, then, of an allergic effect produced by the toxicity of some of these foods. Another issue worth noting in terms of the risks to human health is communication to the resident bacteria in humans of antibiotic resistance. The concern is that resistance to bacteria from the human body, making us invulnerable to certain antibiotics.

2. SOME PRINCIPLES APPLICABLE TO PLANTS GENETIC MODIFICATION

The search for an appropriate balance between the potential risks and benefits of genetically modified organisms, in order to avoid any harmful effects on human health or the environment, calls for the application of certain principles relating to the conservation and sustainable development. Such is the case of the "precautionary principle" and the "development principle".

As has been stated in the Biosafety Protocol of Cartagena, the concept of precaution recognizes that the determination of the level of acceptable risk rests in scientists, expressly stating that "lack of scientific knowledge or scientific consensus will not necessarily be understood as indicators of such level of risk, risk or existence of acceptable risk (9-17). This principle should therefore be kept in mind when scientific information is not sufficient, inconclusive or uncertain, and when there are indications of possible effects on the environment and plant, animal or human health which may be potentially dangerous and incompatible with the chosen protection level (18-22).

The United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992, established this principle at firth time, and the signatory countries assumed the duty to apply it when there could be a danger of a serious or irreversible damage for the environment. However, it was in the Bio-security Cartagena Protocol, signed in January 2000, where the true role of this principle was confirmed in the field of modern biotechnology. This Protocol, whose main objective is to ensure that the movement of genetically modified organisms from one country to another is carried out safely for the environment and human health, incorporates this principle in Articles 10.6

and 11.8, leaving the importing party with the decision about whether the conditions of importation, as well as prohibit importation, request additional information or delay such decision. Mostly when appreciates the lack of scientific knowledge about the effects of genetic modification on a living organism on human health or the environment, and to avoid or reduce these adverse effects (9-17).
However, in many countries and contexts other principles are considered relevant, which are increasingly accepted in law and are part of Biotechnology and Intellectual Property policies. Among them, we can find the principle of "sustainable development". Most Latin American developing countries claim that it is not possible to apply the precautionary principle as an unwavering rule, but must be analyzed in conjunction with other options as education, information, Recycling, polluting production, rights management and adaptation management (18-22).

3. BIO-SECURITY, ETHICS AND BIOETHICS IN PLANTS GENOMICS.

With the development of Biotechnology, there is a need to create norms and mechanisms capable of preventing and controlling the impact and negative effects of the research, production, release and introduction of new species and genetically modified products, which may undermine the integrity of the environmental, technological, socio-economic and cultural aspects, also on food safety and the quality of life of human beings.
In these sense, biosecurity is associated with concepts such as "risk", "benefit", "effectiveness", "diffusion or dispersion"; As well as the "environmental effect of transgenic organisms". And for the analysis of the risks of products derived from modern biotechnology, we must take into account ethical values and alternative forms in technological development that can lead to the same result.
Bioethics can now be defined as the "analysis of ethical issues arising in Biology and Medicine, especially those produced by human activity in society through Biotechnology" (30); Being also known as the ethics of biosecurity. An ethical behavior in Biosafety must revolve according to the economic sector when the biotechnological advances are applied.

4. CONCLUSIONS

Resultants products of genetic modification in plants can arise benefits as same as risks. The balance is necessary; as well as the setting in practice of political socio-economic not ruled by the mercantilist vision of many of the holders of Intellectual Property rights on this products, but for the willingness of governments to make the benefits of modern biotechnology reach everyone. The combination of the precautionary and the sustainable development principles will ensure the necessary balance. The consumer of such products should be offered the option of choosing between those genetically modified or those of an organic nature, unmodified. Intellectual Property offers the possibility of *sui generis* protection for genetically modified plants, different from patent protection and the rights of the holder of plant varieties. Therefore it is left to the will of the States to establish policies of intellectual property more rigid or more flexible, as well as to define in the hands of who will be such Intellectual Property rights.

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Climate Change and the National Park Everglades

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Abstract

The National Park Everglades is already damaged by the warming climate. The sea level is rising and has already brought several changes to the landscape. In the future these changes will be worse.The Everglades as we call them, find their origins 3200 years ago, when the rhythm of rising seas dropped significantly from 9 inches to 1.5 inches per century. This dropped of the tide rise in the sea level allowed an urbanization of mud, shells and sand at the Florida's Southern Coast. This kind of ridge acted as a low dam and stopped ocean water from crossing over it. This natural dam hinder rainfall and overflow from Lake Okeechobee forming a freshwater environment, the Everglades. A large portion of this exceptional landscape is now protected as Everglades National Park.

Why we should conserve and take care of the Everglades.

Everglades National park is one of the largest parks (1.5 million acres) in the country. It has an extraordinary amount of significant resources inside its limits, among them we have: the largest stand of sawgrass prairie in North America, the largest protected mangrove forest in the northern hemisphere, the vast estuary of Florida Bay, and cultural resources chronicling approximately 10,000 years of human experience. In addition, Everglades National Park is the only subtropical wilderness area in North America where, by federal law, people must make no impact on the land and ecosystem. However, the influence of man is increasingly being felt on every acre of the Everglades in the form of human-caused climate change.We must protect the natural and historical livings beings and objects within the park.This way present and future generations would enjoy of this fascinated place.

The Climate Change

During the last century, experts have noticed that the global temperature has raised significantly. The researchers are 99% sure that the higher global temperature is caused by human activities that increase greenhouse gases in the atmosphere:

- Water vapor (H_2O)
- Carbon dioxide (CO_2)
- Methane (CH_4) rn
- Nitrous oxide (N_2O)
- Ozone (O_3)
- Chlorofluorocarbons (CFCs)
- Hydrofluorocarbons (incl. HCFCs and HFCs)

The problem is that greenhouse gases are destined to cause more warming of the global climate with greater proportions than what was experienced in the 20th century. Warmer temperatures influence other aspects of the climate system as precipitation that many living things depend upon. In fact, there are many species' normal life-history patterns that have been changed because of the global warming. For instance, winter ranges of bird species have shifted northwards in over 50 parks, small mammals' habitats have shifted upslope in Yosemite, and conifer tree mortality has risen in four parks.

Because of the warmer temperatures, researchers have observed an increase in the global sea level, and of course it has a transcendental impact for South Florida. Normally, the sea level measurementswere alike in the south Florida Region, until 1840s, which was one of the longest records in United States.The average rate was 5 inches per century for the period from 1846 to 1992 according to the monitoring station in Key West. This estimated was consequent with the 4¾ inches per century from the Intergovernmental Panel on Climate Change First Assessment Report in 1990. This fast rate of rise caused by the climate change is affecting the Everglades in many ways, for instance, don't let the animals and plants to adapt to their environment.

How the Climate Change is damaging the Everglades.

The surroundings of south Florida and the Everglades is distinctive because of its low altitude and subtropical climate. At all the coast, freshwater from the north encounter the continuous changes of the tides that feed several different ecosystems, as well as the buttonwood forests. These coastal ecosystems are home to many rare and scarce plants such as tropical orchids and herbs, some of which are found only in south Florida. Unluckily, these species' home is in risk because the habitat is varying, in part, due to sea level rise,generating the salinization of groundwater and the soils above.

It is uncertainwhether these species can bear the increased salinity in consequential of the sea level rise due to climate change.

Experts have checked the water levels in the whole park, even the numerous inland, freshwaters habitats. The water level in these zones fluctuates with variations in rainfall, freshwater flow and ocean tides. In the last 50 years, researchers have notice a growth in the water level at some, inland freshwater areas in the park, this is consequent with the growth detected in regional sea level. This is very dangerous because we don't know what type repercussions will bring to freshwater environments, however this means that the sea level rise would get far inland.

How the Climate Change is affecting the Cape Sable in the Everglades.

The Cape Sable is an extensive coastal area located at the southwestern of Florida. Once, was characterized by large interior freshwater marsh and connected freshwater lakes. This area has one of the major changes in landscape in the Everglades. At the beginning of the 1900s, the population started to use this area for agriculture and began extracting freshwater out to the ocean thus the ground could dry. Now, the canalsbuilt by the settlers, due to the impacts of hurricanes and the use of water farther north, have converted the coastal system of Cape Sable, and most of this change has been intensified by climate change.

Experts have studied the landscape to analyze the sea level in South Florida. The study demonstrate that the sea level rise was slow through the past 3200 years. However, current equipment has documented an increase rate of rise over the past century, which had evident effects on Cape Sable. The canals are today a conduct of salty water and sediments aimed to inland, mainly for the period of high tides or with the support of strong wind and surge from tropical storms. These

last years, the interior freshwater marsh has practically vanished and the closest lakes have almost been filled with marine sediments. These changes in the Cape Sable have repercussions for the mangrove trees that are situated at the edge waters. Due to the growth of the sea level and the several flooding, the trees are moving inland where the habitat is more appropriate for them. In addition, throughout the coast high tides and storm surge have contributes to removethe sediments from their roots and have facilitated the erosion in the Cape Sable.

How the climate change is affecting the Saline Glades in the Everglades.

The saline glades is anextensive linear area scarcely vegetated marsh, most of which is inside Everglades National Park. This zone receives limited freshwater flow and it is out of range of the Tides, this characteristic is not good for the development of the majority of inland and coastal plant species. There are just some plant species that can survive there: stunted red mangroves, sawgrass, and spike rush.

In the last 50 years the vegetation of red mangroves has extended its reach inland (upon 1km in some zones) and has moved other freshwater species. The red mangrove can grow in the inland due to the trade of freshand saline water in the marsh helped by roads, canals, and sea level rise. This progressively more salty environment makes it easier for saline species to develop, and diminishes the whole area of freshwater marsh.

How to strive against the Climate Change.

A way to checked if our environment is varying is to identify how it was in the past. Researchers persistently measure these changes through the time. These interpretations are made by indicators as: coral health, nesting of wading birds, vegetation communities, fish abundance and diversity, hydrology and water quality, threatened and endangered species, and cultural sites.

As well we have to adapt our landscape to the inclemency of the climate change. For instance, through 20th century, many canals were made to drain the freshwater marsh of Cape Sable.

During the last century, these canals in conjunction with the sea level rise made an evident change to the former freshwater environment. To break the incursion of salt water to Cape Sable, The National Park Everglades has plugged some of the canals, the last one was executed in 2011 with the expectations to intensifythe resilience against sea level rise as a result of climate change.

The Comprehensive Everglades Restoration Plan (CERP) is a multibillion dollar project approved by Congress in 2000. Since then, the work has increasingly been observed as south Florida's outstandingplan against climate change.

Will take over forty years to carry out by the U.S. Army Corps of Engineers and the South Florida Water Management District. CERP hopes to increase freshwater storage, improve water quality, and restore the natural water flow through the greater Everglades ecosystem. If it is achieved, these struggles will keep out subterranean aquifers from salt water invasion, postponed the effects of sea level rise along the coast, and get valuable time for wildlife to deal with their fluctuating environment.

Researchers are working in two renovation plans to recover flow in the everglades. One is the C-111 canal pump stations to the east of the park. A pump will aim water from the C-111 canal to a confinement area that will aid to avoid the leak of water from Taylor Slough and the eastern portion of the park. The other one is the L-29 canal dam and one-mile bridge on Tamiami Trail. Rise a one-milepart of Tamiami Trail, this way water can passeasily into the park without harming the road.

How contribute against Climate Change.

Strategies:

At the Ernest Coe Visitor Center, the Park uses solar power to light the parking lot when dark. Operating lights on solar energy helps decrease the contribution of greenhouse gases from the use of fossil fuels.As well, at the Flamingo campgrounds, the Park uses solar powered heaters to provide hot water for showers. Hot water was not available until the solar heaters were installed in 2010

Inaddition, in 2012, Shark Valley Tram Tours replaced its fleet of petroleum diesel trams with biodiesel trams. Biodiesel fuel is made from vegetable oil, animal fats, or recycled restaurant grease and must meet certain standards to be used. Using biodiesel fuel significantly reduces the emission of greenhouse gases and harmful pollutants, bringing the Park closer to achieving its goal to make operations more sustainable. A finally, replaced most of the park's vehicles with hybrid cars -Replaced old air conditioning systems with higher energy efficiency systems -Adopted an energy conservation strategy for all park facilities

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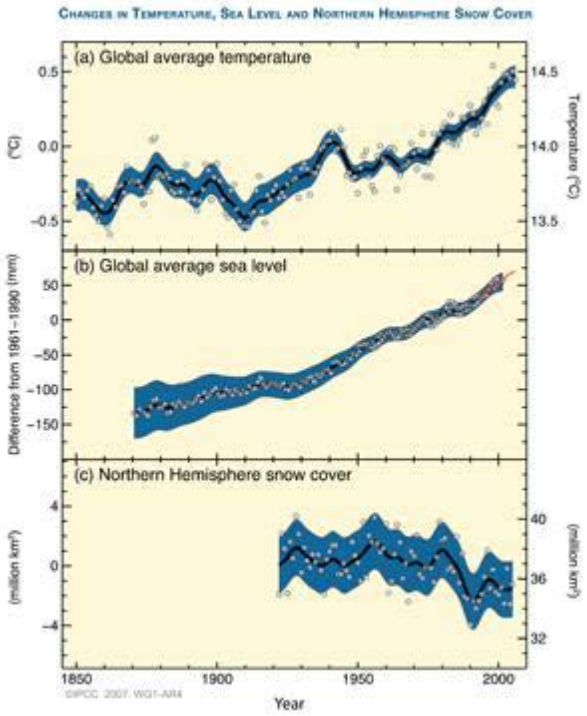
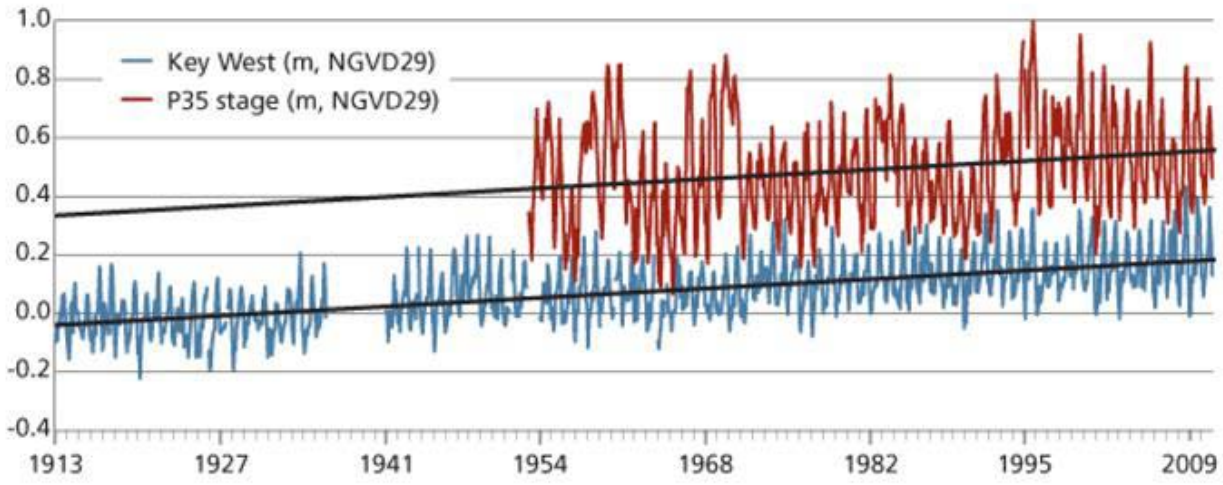
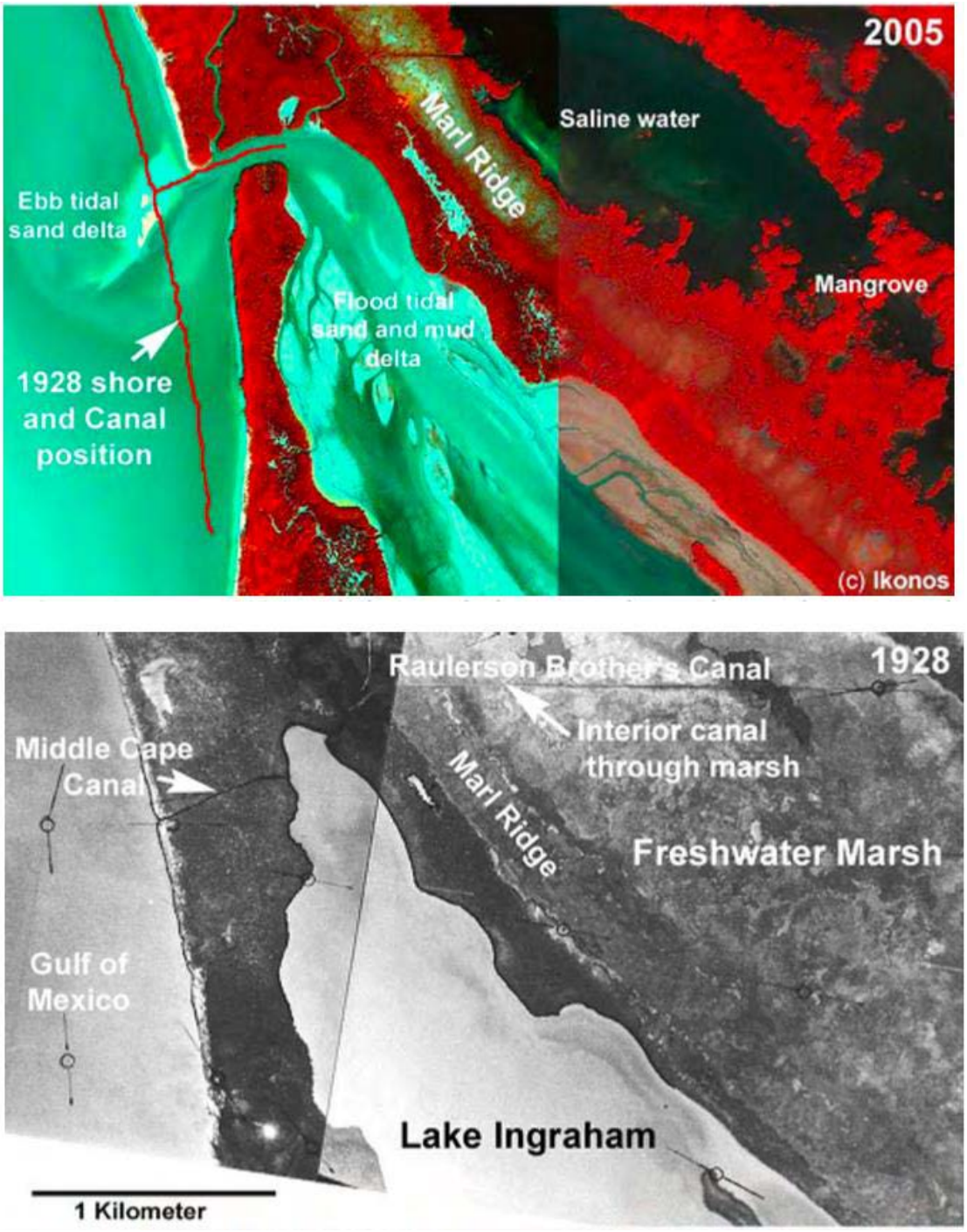
Graphics



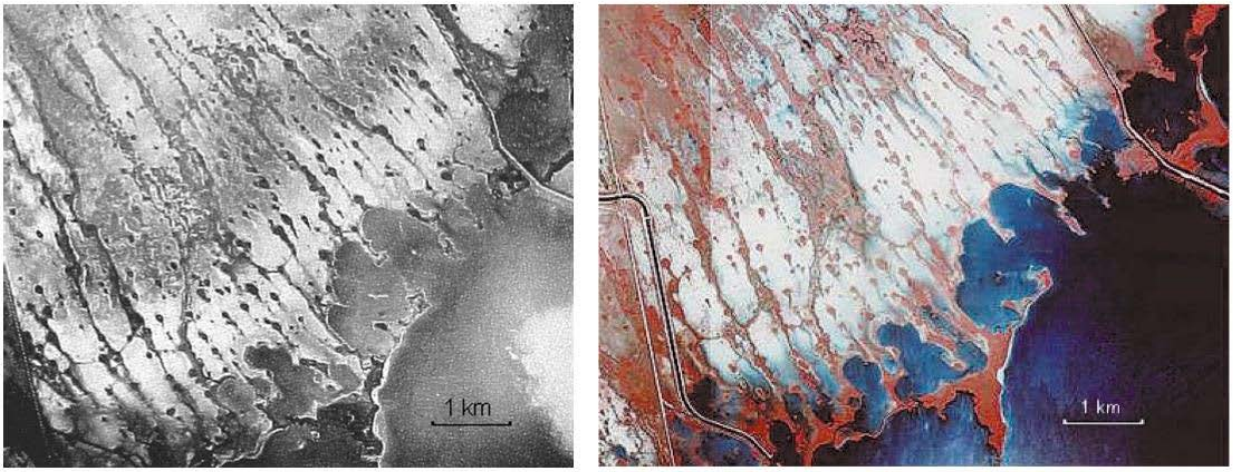
a) Shows observations of global average surface temperature; (b) shows global average sea level from tide gauges, (the red line is from satellite data); (c) shows Northern Hemisphere snow cover for March and April. The differences are calculated from the 1961-1990 averages.



Graph shows that the rate of marsh water-level rise in the park is like the rate of sea level rise observed in Key West. The black line is the trend line for the time series.



These images show the expansion of Middle Cape Canal, the retreat of the shoreline, and the conversion of freshwater marsh to saline water and mangroves from 1928 to 2005.



1940 **1994**

The red line in the photographs show the interior boundary of the saline glades has moved inland from 1940 to 1991.

A view south from the plug at East Cape Canal looking towards Florida Bay.

Note the difference between the silted tidal water to the south of the plug,

and the clearer freshwater behind it.

The C-111 canal pump stations became operational in early 2013.

Greenhouse Effect in Miami, Florida

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Abstract

Greenhouse gases in the Earth's atmosphere absorb the infrared radiation from the sun and release it. Much of the released heat reaches the earth, along with the heat of the sun that has already penetrated the atmosphere. Now both solar heat and radiated heat are absorbed by the earth and released. Some are reabsorbed by greenhouse gases to perpetuate the cycle. The problem is that the more of these gases exist, the more heat is prevented from escaping into space and, therefore, the earth is heated. This increase in heat is called the greenhouse effect.

*Keywords:*Methane, Carbon dioxide, pollution, greenhouse, temperature, levels.

Greenhouse Effect in Miami, Florida

According to the US National Oceanic and Atmospheric Administration (NOAA), the greenhouse effect is an increase in average earth temperature that occurs because certain gases absorb the infrared heat that normally radiates into space. Now, since carbon dioxide absorbs this heat, the more carbon dioxide there is in the atmosphere, the hotter the air will be. If the air gets too hot, the balance of life will be interrupted. Which will cause plant and animal species to die and the food chain to be altered. And this would cause many serious problems around the planet.

Atmospheric concentrations of both the natural and man-made gases have been rising over the last few centuries due to the industrial revolution. As the global population has increased and our reliance on fossil fuels (such as coal, oil and natural gas) has been firmly solidified, so emissions of these gases have risen. While gases such as carbon dioxide occur naturally in the atmosphere, through our interference with the carbon cycle (through burning forest lands), we artificially move carbon from solid storage to its gaseous state, thereby increasing atmospheric concentrations.

Climate Change

Climate Change versus global warming the term global warming has evolved over the past decade to the term climate change because we have realized that the greenhouse effect does not merely lead to warmer temperatures. The term global warming represents the long-term rise in the average global temperature and can be misleading because the actual weather effects associated with global warming can be highly variable, depending on the region. As a result, we now use the term climate change, which more accurately reflects that our concerns are much broader than global temperature increases.

Climate change is defined as a function of increased average temperature over time while the actual weather-related effects of a changing climate can impact variability and

extremes – including potential for warmer and cooler temperatures, wetter and dryer conditions, and changes in the intensity, frequency and patterns of storms. Global temperature increases affect many forces, including global weather patterns, ocean conditions and sea levels. Warming will not be the same for every region; long-term changes in average temperatures will have different impacts in different locations.

Greenhouse Effect in Miami

Florida is considered one of the most vulnerable areas to climate change, with southeast Florida especially susceptible to impacts such as rising sea levels. Miami-Dade County has been at the forefront of these issues for many years. The Board of County Commissioners and administrative departments have been implementing policies and initiatives to address climate change, environmental protection and other important sustainability issues, including energy efficiency and water conservation. Through early monitoring of greenhouse gas (GHG) emissions and analysis of climate change data.

One of the most significant challenges facing the southeast Florida region and the world is the threat of climate change although the planet has experienced natural cycles in atmospheric concentrations of carbon dioxide and temperature for more than 600,000 years, there is now an unprecedented rate of greenhouse gas (GHG) build up in the earth's atmosphere due to human activities. As a result, more and more of the sun's heat energy is trapped. Worldwide, changes are occurring to many interconnected forces that determine precipitation, temperature, severe weather patterns, sea level, ocean currents and acidification.

Current science is projecting that the southeastern United States could experience a general increase in average temperatures anywhere from 4.5o F to 9o F in the coming century depending on the Intergovernmental Panel on Climate Change greenhouse gas emissions scenario utilized for the projections (Intergovernmental Panel on Climate Change (IPCC), "Special Report" Appendix 1). This temperature change will likely manifest itself as an

increase in the number of days over 90o F, with the greatest temperature increases expected during the summer months.

Rising sea levels:It is important to note that relative sea-level trends vary across the world. Local sea level or the local mean sea-level trend is used for various planning needs associated with climate change adaptation planning.

The National Oceanic and Atmospheric Administration (NOAA) has three meters located in the southeastern region of Florida that measure the average sea level trend; Miami Beach, Vaca Key and Key West. According to NOAA, the average increase in sea level trend in the Key West tide indicator is about 2.24 millimeters per year from 1913 to 2006, which is roughly equivalent to a change of 0.73 feet in 100 years. A recent NOAA study indicates that additional tide gauges in the southeastern Florida area would allow more accurate regional integration of sea level rise and tidal fluctuations. These additional data can be useful as Miami-Dade County develops flood maps to assess vulnerable areas and infrastructure. The bottom line, however, is simple: sea level is increasing.

Temperature Increase in Florida.During the first seven months of 2010, southeastern Florida experienced firsthand the types of extreme temperatures that can occur as a result of climate change. According to the National Weather Service, the period from December 2009 to February 2010 was the coldest of three months in nearly four decades, with average daily temperatures of two to three degrees below normal throughout the region. Miami Beach experienced its second coldest winter, with an average temperature of 5.6 degrees below normal, and broke its coolest January-February mark of setting in 1958. United States Department of Commerce NOAA, "Hottest summer" n. In contrast, summer 2010 experienced higher than normal temperatures. These unusually high temperatures began in May and continued through the summer. In fact, the period from June to August was the hot summer of the history of the four major climate sites in South Florida such as Miami, Ft.

Lauderdale, Palm Beach and Naples. According to the United States Department of Commerce. NOAA

Rain Ends. Changes in precipitation patterns are also projected to affect the climate of Miami-Dade County. These changes may increase the likelihood of flooding and drought, which would have different but damaging impacts on the quality and supply of County water. Since the early 1900s, South Florida's spring, summer, and fall precipitation have fallen by almost 10 percent, as established by the United States Department of Commerce, NOAA, in 2009. However, local data indicate that there has been an increase in heavy downpours in the region, and a 2009 report from the Oceans and Coastal Council of Florida indicates that this trend may increase and be combined with longer droughts.

On the other hand, the six-month period from November 2008 to April 2009 was ranked as the second driest period recorded in most of South Florida. In May, this extremely dry season was quickly reversed to a very humid rainy season, with rainfall above normal that lasted until mid-July. In fact, a summer storm on June 5, 2009 threw a total of 9.3 inches of rainfall in South Beach, most of it in less than three hours, overwhelming the storm water drainage system by gravity. This caused severe flooding in areas of South Beach, Miami Beach and downtown Miami, leaving some areas with up to three feet of standing water.

Coastal erosion and shallow coastal flooding. Beaches and sand dunes are an important component of the region's coastline and are very dynamic, constantly changing due to natural erosion and the movement of sand by wind, currents and wave action. They can provide a first line of defense against the storm surge, and are significant assets that make the region an attractive tourist destination. Various climatic events leading to higher winds, tides and waves cause additional erosion, which can be severe. That is the case, which in Miami Dade County have addressed this issue since 1975 with their acclaimed Miami-Dade Beach Erosion Control Project. It is a highly recognized program as a national model and has won

numerous national and state awards. It is important to note that although the specific amount varies from year to year and from one project to another, Miami-Dade County invests approximately $ 6 million annually in beach restoration. On the other hand, communities adjacent to the ocean and intracoastal waterways regularly experience high tide floods, which occasionally inundate coastal communities through storm water drainage systems and low sea walls, to damage in infrastructure and goods.

Tide levels during June 2009 were six inches to two feet above normal, according to experts, the moon cycle contributed to this event, which is not necessarily unusual. But nevertheless, the geographic extension of this event along all the east coast made that this event of high tide was anomalous.

Storms and extreme Damage. There is a lot of scientific research on the causes, trends and complex factors that affect the development of tropical storms and hurricanes. As the atmosphere warms, sea surface temperatures and wind shear will also increase, these factors can have opposite effects in tropical storms. Also, the role of sea temperatures is complicated, as the temperature rises, the general frequency of storms may decrease, but the intensity of the strongest storms may increase.

However, account must also be taken of the problems they produce from climate change and its effects on these storms, and future trends and impacts become extremely abnormal. While what is clear is that our community will continue to experience these events, and they can have devastating impacts.

As is well known, the region is no stranger to hurricanes, as demonstrated by Hurricane Andrew in 1992 and the busy 2005 hurricane season where we live the power of Katrina and Wilma.

Our source of electricity. Florida Power and Light (FPL) is Miami-Dade County's main electricity provider, with two FPL power plants within the boundaries of Miami-Dade

County. Turkey Point, is located in Biscayne Bay, which holds two nuclear power units and is also an oil and gas facility. The 2,337.5 megawatt (MW) plant has the capacity to meet the annual electricity needs of more than 450,000 households. Cutler Ridge, an oil and gas plant, has a considerably smaller capacity of 236.5 MW (FPL).

Homestead Electric, a municipal-owned company, also serves Miami-Dade County, but supplies less than one percent of its electricity. In 2008, 219.6 million megawatts (MWh) were consumed in the state of Florida, approximately 27.3 million of which were used by Miami-Dade County households, businesses and government operations. Thus Miami-Dade's electricity consumption accounted for approximately 12.5 percent of Florida's total consumption, while its residents accounted for approximately 13.5 percent of the state's population.

Strategy: Expanding the industries of alternative fuels (biodiesel/biodiesel based on waste) or renewable energy is the most accessible and cost-effective way to reduce water and energy consumption. The use of renewable energy sources that can provide global sustainability benefits to our community should be encouraged: sustainable biodiesel, including waste-based biodiesel and renewable energy. The use of these nontraditional energy sources can be accelerated by the deployment of technology and the construction of local infrastructure, as the State of Florida does not have a renewable portfolio standard, and the community does not aggressively seek distributed solar or energy efficiency facilities, the initiatives in this strategy address the need to stimulate the renewable energy market.

New fuels should be analyzed to ensure they are of sustainable origin and have net environmental benefits. To estimate the impacts of renewable and alternative fuels on GHG emissions, the entire fuel life cycle, including fossil fuel extraction or raw material growth, fuel production, distribution and combustion, should be evaluated. No emission reductions

were calculated for this strategy; however, it is clear that by shifting the use of fossil fuels, large reductions of GHG emissions can be achieved.

An effective measure, it could be to replace conventional vehicles with their hybrid counterparts that are 25 percent more fuel efficient, we will have reduced emissions by 25 percent per year.As well as deploying more trains and buses, even though this action would increase overall fuel usage, implementing more means of transportation would help reduce miles traveled by personal vehicles.

Another way to reduce the effects of climate change is to use energy and water more efficiently and more conservatively, which would allow for the reduction of greenhouse gas emissions.

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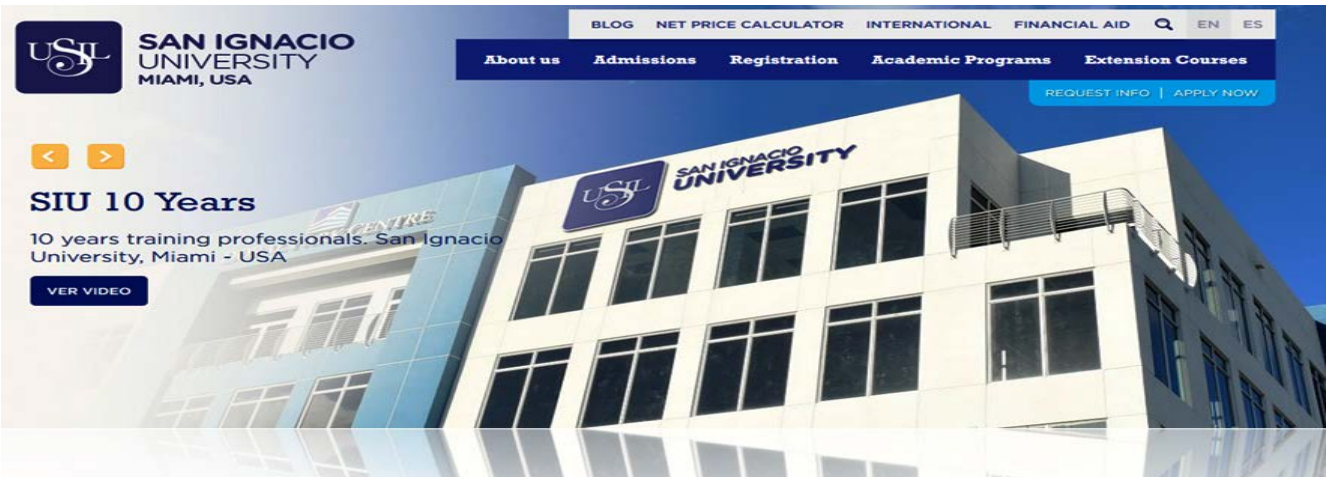

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SIUSCI-01: San Ignacio University Sciences Workshop, Miami, USA, 2017

Ph.D. Lázaro Pino Rivero

Adjunct Faculty at San Ignacio University Miami, USA, Chemistry Professor at West Coast University, Miami Campus and Chemistry Professor at Miami Dade College, Miami, FL, USA

Dear colleagues worldwide,

We are glad to invite you to participate in the workshop SIUSci-01. SIUSci-01 workshop series will be held at San Ignacio University (SIU), FL, Miami, USA. San Ignacio University is an innovative educational institution dedicated to the creation of the leaders of tomorrow accredited by the Accrediting Council for Independent Colleges and Schools (ACICS). The present workshop focus on topics of multidisciplinary sciences relevant to the interests of SIU students and professors. SIUSCI-01 is also devoted to strength the collaborations and networking between SIU professors and students with other students and researchers in FL education system and worldwide.

In this sense, SIUSci-01 is a workshop associated to and hosted online by the MOL2NET Conference Series on Multidisciplinary Sciences, MDPI Sciforum, Basel Switzerland. This means that all communications are going to be published online at Sciforum platform. All presentations will be peer reviewed and a DOI number will be assigned. MOL2NET conference of Sciforum is one of the platforms internationally recognized for scientific exchange. This annual edition is full of diversity in topics, approaches, and integration of disciplines, representing one of the common paradigms of modern science, interdisciplinary teamwork, and networking. I hope you will enjoy the program and the presentations.

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USE OF NATIVE MICROORGANISMS OF THE RHIZOPHORE OF THE AMAZON TO ACCELERATE THE PROCESS OF DECOMPOSITION OF ORGANIC AGRICULTURAL RESIDUES

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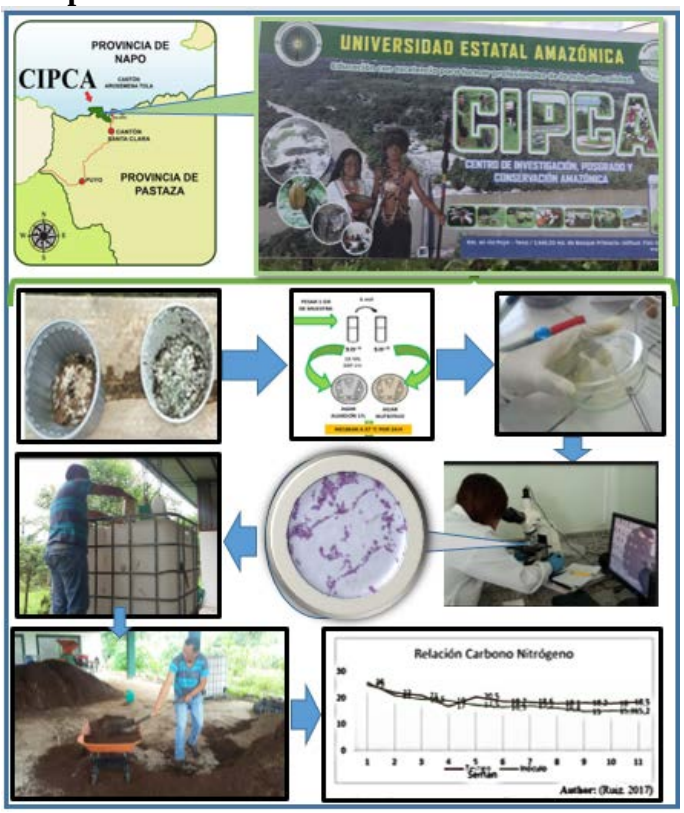
Graphical Abstract



Abstract

The objective of this research was to accelerate the composting process of solid waste generated in the agroindustry of the Amazon by inoculating composting beds with a solution of isolated native microorganisms on the surface of the CIPCA forests. The microorganisms identified in this work were 2 fungi *Aspergillus fumigatus*, *Penicillum* sp. And the bacteria *Bacillus subtillis* and *Pseudomos fluorences*. A biomass of microorganisms with a concentration of 1 X 10^7 CFU*mL^{-1} per isolated microorganism was applied and sprayed 4 L*m^{-1} 3 of substrate to compost. The following variables were evaluated: temperature, humidity, pH, C/N texture and physical structure, organic matter, electrical conductivity and cation exchange capacity. The results indicated that the beds inoculated with the microbial solution reached the physical, chemical and biological characteristics of a mature compost with the difference of five weeks before the control bed. The response in these characteristics indicated that the inoculum solution significantly accelerates the composting process.

Key words: *Amazon, Pseudomos fluorences, agricultural residues, microorganisms.*

Introduction

The man with an industrial and / or agricultural economic activity generates a large amount of waste that often causes important environmental problems due to its inadequate storage and treatment, Moreno C et al., (2017). The total decomposition of organic molecules into carbon dioxide, inert inorganic waste or minerals is incorporated back into the structure of the soil to be assimilated by microorganisms and plants. The final product of this process, called compost, contains nutrients such as nitrogen, potassium phosphorus and a concentration of microorganisms that promotes plant growth. However, this process can be very slow and expensive, so it is necessary to look for some ways to accelerate the process without deteriorating the quality of the product and to increase the final benefits such as obtaining an organic substrate of good quality at the end of the treatment. (Soto G et al., 2002).

Currently, the natural composting process is one of the efficient methods in the transformation of such waste; this activity allows obtaining a usable by-product for agriculture. One of these forms is based on the acceleration of the process using native microorganisms to the soil where they naturally degrade organic matter, applying the bioaugmentation technique. This transformation consists of several processes that have a variable duration, conditioned by climatic factors, quality of the waste, its size, disposal in the composting bed, aeration, humidity and biological population. The production of accelerated compost from agro-industrial waste will contribute to the conservation and recovery of the ecosystem reported by Faure Vargas-García, et al., (2007).

It was possible to obtain a compost with the adequate levels of the physico-chemical parameters with the reduction of the time of composting, using native microorganisms to achieve a rapid acceleration of the process.

The objective of this research was to accelerate the waste composting process by inoculating native microorganisms in order to reduce the time of composting and obtain a high quality compost that fulfills the parameters required by the agro quality for its registration.

Materials and Methods

In the experimental research phase, a comparative experimental study was made using a field test for 150 days. The test was carried out in two stages, the first one consisted of the isolation and identification of the microorganisms, and it was processed in the Microbiology Lab of the Amazon State University, (UEA) located km $2^{1/2}$ via Napo (side step) Puyo Pastaza. The second stage of the research was carried out in the organic fertilizer production plant of the Center for Research and Postgraduate Studies and Conservation of the Amazon Biodiversity CIPCA, located at Km 44 of the Puyo Napo road of the essay preparing the composting piles. They were processed under cover conditions by the adverse climatic conditions of the amazon region. The composting beds were placed on a cement platform covered with geo membrane, surrounded by gutters to collect the leachates from the process of decomposition. The piles were made with the dimensions of 4 x 1 x 1, giving a capacity of $4m^3$ equal to 640 kg of organic material. Prior to its formation, all the material was subjected to grinding in a hammer mill with fine sieve to obtain particles between 1 to 3 cm^3 size, it was indicated to accelerate the colonization of microorganisms and aeration for their first decomposition process, reported by De Carlo *et al.* (2001).

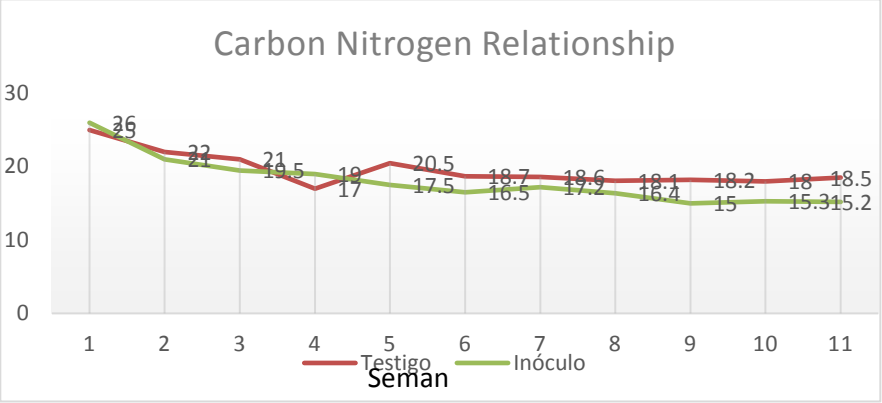
Mechanized turning was performed weekly to guarantee and control the process. Isolation, identification and characterization of the sample microorganisms, collected from the CIPCA rhizosphere, was made with dilutions of the samples 1 x 10^{-4}. It was dissolved in a physiological solution, due to the presence of low concentration microorganisms; it was isolated in plates with soybean tryptophene Sabaoureaud culture medium, being incubated at 28 ° C for 5 to 7 days, specifically for fungi and for bacteria at 37 ° C per 48 hours. Each of the different isolated strains was sown on nutritive agar, enriched with 2% starch and using lugol as an indicator. The amylolytic characteristic was quantified to the diameter of the halo of degradation around each colony, recorded in the research of Andreoni, V. *et al* (2004.).

The fungal strains were identified, using morphological identification keys and registering typical characteristics of each strain. The amylolytic and cellulitic capacity of 20 bacterial strains was evaluated, 10 of them were selected from 10 strains of fungi, from which two most active strains were selected, added the two groups were obtained a total of 12 strains. Subsequently, the soybean tryptamine agar culture medium was prepared, where the strains were seeded. Bacterial colonies were collected and suspended in a physiological solution that was adjusted to the 0.5 standard of the Mc Farland`s scale from the surface of the culture medium.

On the other hand, fungi grown in Lactrimel medium is collected by flooding the petrick boxes with the help of the spore-counting chamber, a concentration inoculum of 1 x 10^{7} in physiological saline was prepared. The inoculation of the composting beds was made with the help of the "Compost SYSTEM" Turning Machine and its incorporation of the spray system was inoculated the microbial content, 4 liters of solution per m^3 of organic matter with a concentration of 1 x 10^{7} CFU * ml^{-1} by strain. The application was made only once in the mesophyte stage of the composting process.

Results and Discussion

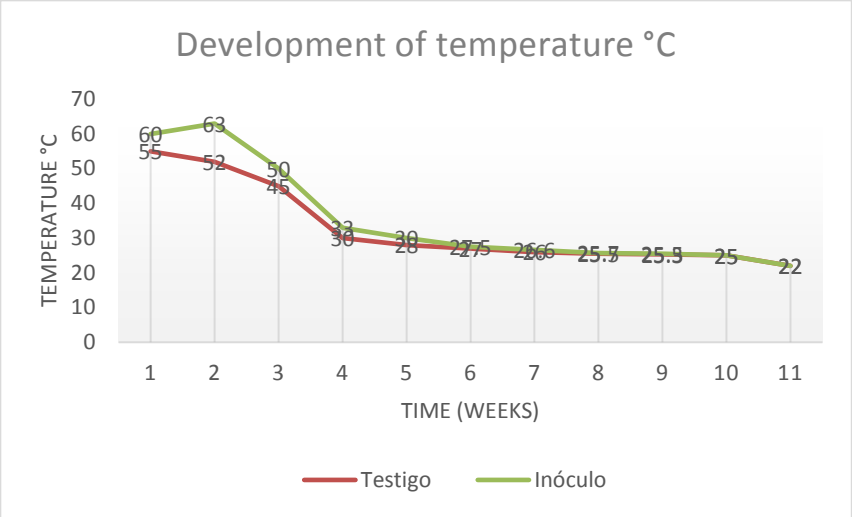
It was isolated and identified native microorganisms of rhizosphere from the CIPCA involved in the biodegradation process such as: *Aspergillus fumigatus, Bacillus subtillis* and *Pseudomos fluorences*. It was the inoculum used in a concentration of $1x10^{7}$ and inoculated 4 liters per m^3 of organic matter for the concentration research, recommended by other research reports in the research of Farfán VF *et al.*, (2002).



Author: (Ruiz. 2017)

Figure 1. *Evolution of the C / N ratio during the composting process of agro industrial solid waste.*

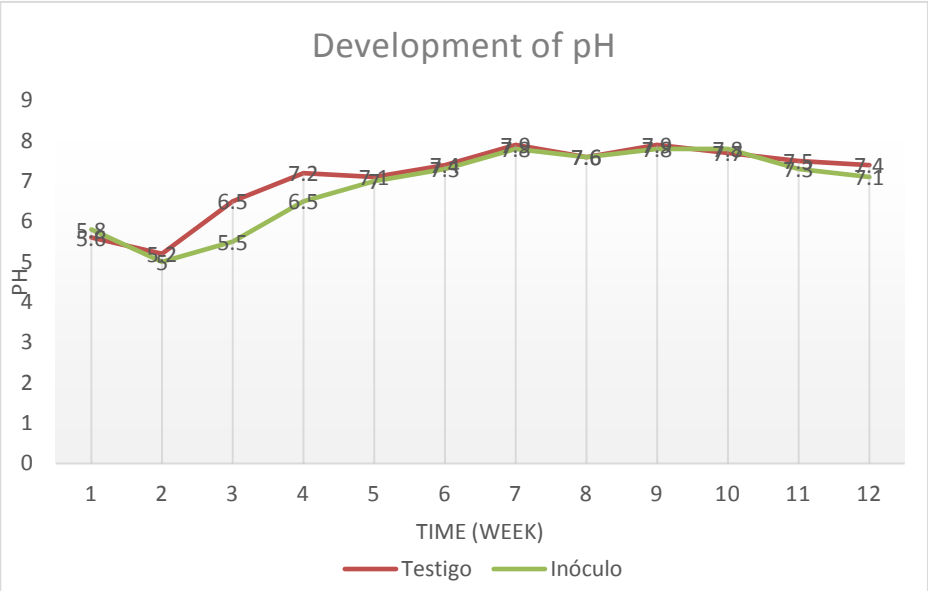
As indicated in ***fig. 1***, the result of the process was a compost with good oxidizable organic carbon content and therefore it is a good source of organic matter, as demonstrated by the C / N ratio in pile 1 with 15%, in the stack 2 with 14.0% and stack 4 with 11.5%. When comparing these results, with those of the reference (<20%), obtain similar results indicated by Soto G *et al.*, (2002).



Author: (Ruiz. 2017)

Figure 2. *Evolution of the temperature in the agro-industrial waste composting process.*

The ***Fig. 2***. Temperature reached a value of 65 ° C in all piles in its mesophyte stage, reaching a stability of 40 ° C at the end of its process, which allows demonstrating the active metabolic processes that ensure stability and maturity in a shorter time, a recorded indicator were reported by similar data in the research of Araujo *et al.*, (2005).



Author: (Ruiz. 2017)

Figure 3. *Evolution of pH in the composting process of agro industrial solid waste*

The humidity of the compost was recorded in its final stage of maturation of each of the tests, obtained a humidity below 40%. Final humidity was recorded in weeks 19- and 20 coinciding with data reported by Blandion CG et al., (1999). During the first 4 weeks of the process, the pH in the inoculum batteries

were kept below that of the control batteries (***Fig. 3***), it was reached at the end of a record in the battery 1, 7 pH, battery 2, 7.1 pH and battery 4, 6.5pH. This was originated by the aeration obtained in the turning that increased the aerobic degradation of organic matter, recorded too in the research of Jiménez and García et al., (1989).

The amount of organic matter (OM), being solid waste, was based on a high value of 87% at the end of the process, a value of 52% was obtained in the witnessed piles and 63% in the inoculated ones. The electrical conductivity (C.E) values reported in the batteries, both inoculated and control, varied in the five months between 0.57 and 3.80 dScm-1. These values are related to the concentrations of Ca ++, K + and Na + and they did not contribute in our research an indicator of maturity for the study. Considering the values obtained from the cation exchange capacity (CIC), the values were 86.6 cmol, pile 1, 85.5 cmol, pile 2, and 83 cmol, pile 4 (+) kg-1, compost, versus 57.6 cmol (+). kg-1 of the control pile data, recorded by Moreno C et al., (2007) parameter, that would be beneficial since the compost obtained from the inoculated beds, would have greater nutrient retention capacity and a great retention capacity of phytotoxic substances as well as higher buffer capacity, indicated in this mode the degree of maturity. The product obtained at the end of the process presented characteristics such as the smell of earth, texture and structure uniform dark color, neutral pH, where it does not present characteristics of the initial organic matter reducing its initial volume by 35%.

Conclusions

The main objective was to isolate the strains of native microorganisms from the rhizophor to accelerate the composting process of solid organic waste. The microorganisms selected were bacterial strains Bacillus subtilis and Psudomonas fluorencens and the fungal strains Aspergillus fumigatus. According to the indicators and characteristics of maturity stability of the composting piles inoculated with the solution of these native microorganisms, reaching their stability and maturity in less time compared to the control without inoculating. The final compost presented the quality standards established by the production of fermented organic fertilizers in Ecuador. Controlled and registered for use by Agroquality. These selected microorganisms provide a safety in the natural decomposition process of organic matter in a free or controlled manner, minimizing its negative impact on nature and human as a direct manipulator.

Acknowledgments

To all who made this research possible.

Author Contributions

All authors have the same contribution**.**

Conflicts of Interest

There is no conflict of interest of the authors.

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Osteosarcoma gene prioritization through combined bioinformatics analysis.

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Abstract: This is the abstract section in English (mandatory). One paragraph only (Maximum 300 words approx.).

Osteosarcoma (OS) is a rare genetic disease that represents 20% of all types of malignant and benign neoplasms of the bone, and 2% of pediatric cancers. Therefore, our aim in this study is to generate a consensus gene list associated with the pathogenicity of OS by using several theoretical approaches that let to propose new drivers associated to this sarcoma, and also possible biomarkers. Firstly, we evaluated the consensus between 9 prioritization strategies to early determine pathogenic genes related to OS. From these genes, we performed a communality analysis in the protein-protein interaction network further enrichment analysis. The consensus prioritized gene list consisted of 1295 genes. Our results revealed that consensus strategy proposes genes related to control in the cell cycle that describe the etiology of cancer in general, and prioritizes not only suppressors already described for OS such as RB1 and TP53, but also postulates new candidates that would help to describe its pathogenesis.

Keywords: Consensus analysis; Gene periodization; Osteosarcoma, Communality analysis; Pathogenesis; Early recognition.

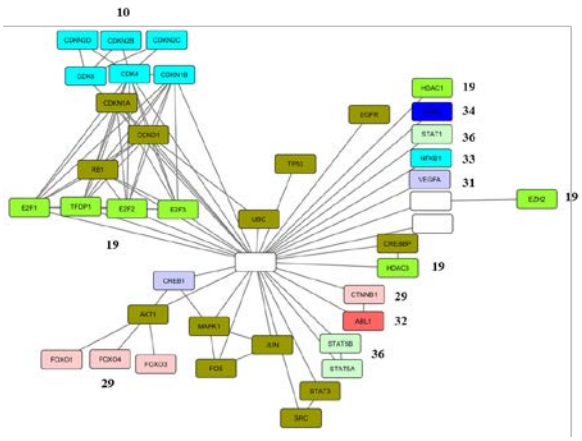

Figure 1. Network analysis from gene communities prioritized for OS.

Protein interaction of prioritized OS genes base on STRING database. Each community is represented by specific colors: light blue, comm. 10; green, 19; light green, 36; blue, 34; pink, 29; red, 32; purple. Genes present in two or more communities are olive-green colored.

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Antiproliferative activity of *Psidium guajava* essential oil: a preliminary study

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Graphical Abstract

Psidium guajava OE - Antiproliferative activity

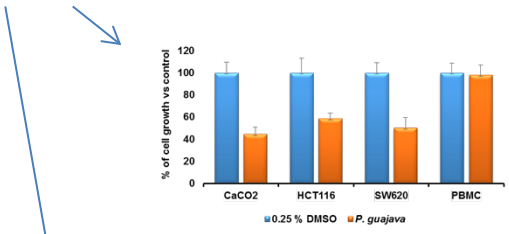

Figure 2

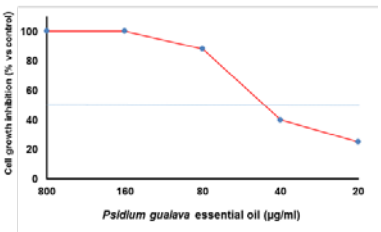

Figure 1

Abstract.

Psidium guajava essential oil (EO) from Ecuador was screened for its antiproliferative activity against three human colon carcinoma cell lines: HCT116 a human colon adenocrcinoma grade II, sensitive to 5-fluorouracil and oxaliplatin; CaCO2, a primary adenocarcinoma grade II; SW620, a highly resistant cell line deriving from a lymph-node mestastasis. Peripheral Blood Mononucleated Cells (PBMC) from a healthy donor buffy coat was utilized as healthy control cells. Cells were treated with increasing concentrations of P. guajava EO (20-800 µg/ml) dissolved in 0.25 DMSO and added to growth medium. 24 or 48 hours after cells were detached with trypsin and number of viable cells was determined by Tripan blue exclusion and/or or calcein-AM method, thus providing percentage of viable cells and rate of proliferation as well. Dose dependent antiproliferative effects were observed, with a percentage of growth inhibition ranging from 40 to 80 % (20-80 µg/ml). 48h treatment did not increased effectiveness of the EO probably because of a short half-life of the oil. Interestingly treating PBMC with 50µg/ml no anti-proliferative effects were observed, suggesting that this concentration, corresponding to IC 50 of this OE, did not lead evaluable toxicity to healthy cells such as PBMC. Further studies regarding chemical characterization and combination with commonly utilized anticancer drugs may be done as next step.

Key words: *Psidium guajava*, essential oil, antiproliferative activity

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Introduction

Psidium guajava is an important medicinal plant very well-known in several tropical countries, where extracts and metabolites of this plant are used in traditional medicine for the treatment of several diseases such as diabetes mellitus, diarrhea, dysentery, cardiovascular disorders and cancer. Many authors have reported traditional uses, folk medicine, phytochemistry and several studies demonstrating biological activities of *P. guajava* extracts [1-6]. Furthermore some papers suggest the anticancer potential of *P. guajava* leaf extracts by acting as inhibitors of tumor cells proliferation and motility and also acting as proapoptotic agents [7-10]. Differently from extracts, anti-proliferative effects of *P. guajava* essential oil (EO) is poorly characterized [11-12]. In this preliminary study, we investigated the antiproliferative activity of the *P. guajava* essential oil against human colon carcinoma cell lines.

Materials and Methods

Plant material

Leaves of *P. guajava* were collected in the Amazonian region of Pastaza (Ecuador) and species authentication were certified by Dr. David Neill, voucher specimens were deposited at the Herbarium ECUAMZ of the Amazonian State University (UEA) in Ecuador (voucher specimen: Asanza 4814).
The essential oil was obtained by hydrodistillation in a stainless steel distiller equipped with a Clevenger apparatus. Essential oil was obtained performing three distinct distillations and essential oil (moisture-free) yield was 0.14%. The oil was dried over anhydrous sodium sulphate and stored in sealed amber vials at 4°C.

Cells

PBMC: Peripheral Blood Mononucleated Cells (PBMC) were isolated from healthy donor's buffy coats by gradient centrifugation. Colon cancer cell lines: CaCO2, a well differentiated grade II primary adenocarcinoma sensitive to 5-fluorouracil and oxaliplatin, HCT116 a malignant grade II colon adenocarcinoma and SW620, a highly resistant cell line derived from of a lymph-node mestastasis of grade III-IV colon adenocarcinoma patient. PMBC and cell lines were grown in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

Antiproliferative activity:

Cells were counted and plated in 24 multiwell plates at the same initial density. 24 after cell seeding, growth medium was replaced by fresh medium containing different concentrations of the oil dissolved in 0.25 % DMSO. 24 or 48 hours following *P. guajava* oil treatment (800 - 20 μg/ml), cells were trypsinized, centrifugated, and resuspended in standard medium, and the anti-proliferative effect was quantified by performing a cell count with a hemocytometer. The cell viability was reported as a percentage of living cells compared to untreated control cells, by using the formula: Percentage of Living cells = Nb of treated cells/Nb of control cells × 100. Untreated control was considered as 100% living cells. Appropriate control groups with diluents only (0.25 % DMSO) and blank control were included.
Cell viability was determined using the trypan blue (0.2% TB solution) exclusion test. The percentage of dead cells is calculated as (number of stained cells/number of total cells) × 100.
P. guajava EO effects on viability or cytotoxicity were also evaluated by utilizing Calcein-AM/PI Double Stain Kit (Molecular Probes) according to the manufacturer protocol.

Results and Discussion

As preliminary experiments, we setup a dose response curve to identify the range of oil concertation as optimal dose for the antitumor experiments. To address this issue we utilized HCT116 cells treated for 24 hours with different concentrations of *P. guajava* EO. Results shown in **Figure 1**, allowed us to select the concentration of 50 µg/ml, roughly corresponding to the IC50 of the oil for these cells. Antiproliferative effects of *P. guajava* oil were then evaluated treating HCT116, SW480 and CaCO2 three different colon carcinoma cell lines for 24 or 48 hours with 50 µg/ml *P. guajava* oil in 0.25 % DMSO. As control 0.25 % DMSO was utilized, since no differences were observed between 0.25-1% DMSO medium and DMSO free RPMI. The results shown in **Figure 2** clearly suggest the cell growth inhibitory effects of the oil in all cell lines tested that ranged from 50 to 60 %. 48h treatment did not increase the inhibitory effects in cell growth, probably because of a short half-life of the oil in the experimental conditions utilized (not shown). Furthermore no significant differences in cell viability between control and EO treated cells were observed, allowing us to hypothesize that cell growth inhibition, comes from antiproliferative, rather than cytotoxic effects. In these experiments, we assessed EO effects on Peripheral Blood Mononucleated Cells (PBMC) deriving from a healthy donor, to preliminarily assay potential toxic effects of oil; as shown in figure 2 *P. guajava* oil did not give any inhibitory or cytotoxic effects

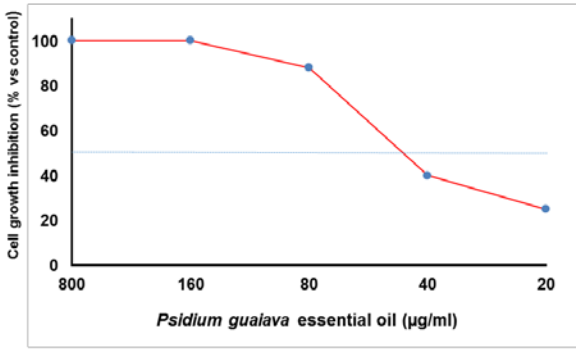

Figure 1

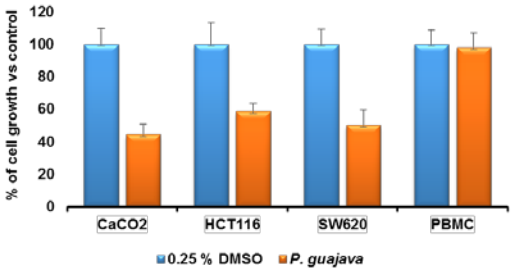

Figure 2

Conclusions

In this manuscript, we describe for the first time anti-proliferative effects of *P. guajava* EO against colon cancer cells that represent one of the most aggressive human cancer. Although there is two papers about cytotoxic effects of this oil reviewed in [13], no data were available about its toxicity. Here we show the potential antitumor effects of *P. guajava* EO in an interval of concentration that is in the same range of several cytotoxic drugs already utilized for this type of tumors. Our observation about cytotoxicity, allowed us to hypothesize that this EO induces a cell growth arrest more than cytotoxicity, since did not verify significant differences in cell mortality. Interestingly we also observed that PBMC were unaffected by OE treatment, probably because of their state of resting, non-proliferating cells, suggesting that at the concentration utilized there are no toxic effects on healthy cells such as blood cells.

From these encouraging results, we have planned to investigate the efficacy of these oils in combination with commonly utilized anticancer drugs. Next step will be the in vivo analysis of efficacy and toxicity, ad for the most promising oils the analysis of mechanism(s) of action in order to identify the target pathways.

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SMANN: Screening Model of Artificial Neural Networks for Brain Connectome

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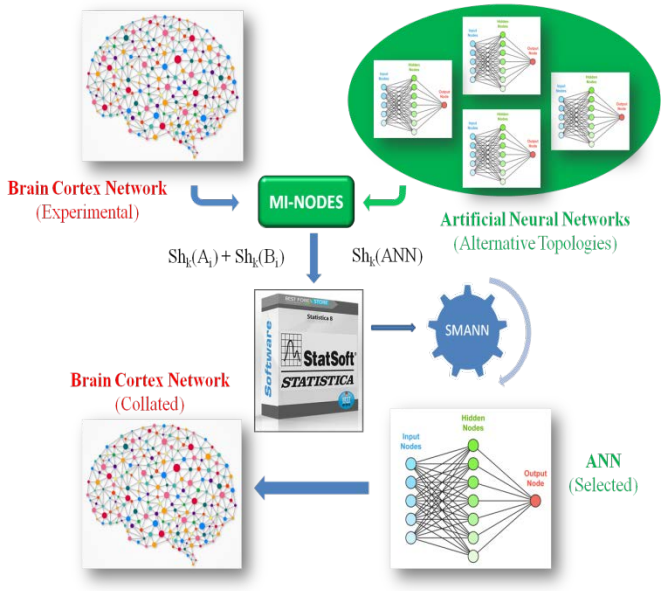

$$^{q}S(L_{ij}) = -70.198 \cdot Sh_2(A_i) - 77.605 \cdot Sh_2(E_j) + 0.297 \cdot Sh_2(N$$

SMANNs workflow used in this work

Abstract. W can represent the brain as a Brain Connectome Network (BCN) formed by n_i brain cortex regions (groups of neurons) interacting with others (L_{ij} = 1) or not (L_{ij} = 0) by means of electrophysiological co-activation (synapses). The large number of links to be study and their complex connectivity patterns made difficult to select the appropriate topology in order to predict them with Artificial Neural Networks (ANNs) algorithms. In this work, we developed a new Screening Model for ANN (SMANN) algorithm to solve this problem. In so doing, we can quantify topological (connectivity) information of both the complex networks under study and a set of ANNs trained using Shannon measures. We developed one SMANN model with >85% of accuracy for 52690 outputs of 10 different ANNs and 52690 pairs in the BCN.

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CNT Mitoprotective activity in mitochondrial swelling

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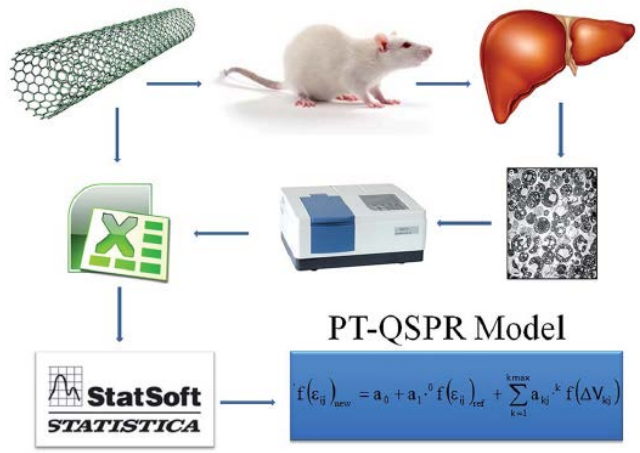

Abstract. We used different experimental protocols to determine the mitoprotective activity (%P) of different carbon nanotubes (CNT) against mitochondrial swelling. The experimental conditions were combinations of the following factors: different mitochondrial swelling assays using the MPT-inductor (Ca2+, Fe3+, H2O2) combined or not with a second MPT-inductor and swelling control assays using MPT-inhibitor (CsA, RR, EGTA), exposure time (0–600 s), and CNT concentrations (0–5 mg ml1). Other factors changed were the CNT structural parameters CNT type (SW, SW + DW, MW), CNT functionalization type (H, OH, COOH). We also changed different physico-chemical properties of CNT properties like molecular weight/functionalization ratio (minW/maxW) or maximal and minimal diameter (Dmin/Dmax).

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In silico study of the natural compounds inhibiting angiotensin converting enzyme II

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Abstract: Hypertension is a health problem of high prevalence worldwide. Because it is an important cardiovascular risk factor, the development of new drugs that are more effective and with fewer side effects is extremely important. Recent studies have shown that several natural compounds have good antihypertensive activity by inhibiting the angiotensin II converting enzyme (ACE), which makes them good candidates the prototype for the development of new drugs. Based on this perspective, this work proposes to evaluate the solubility (partition coefficient and water solubility) of the natural compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligandstroside, through the software ALOGPS 2.1, and observe their interaction with the ACE, through molecular docking, with the software Autodock 4.2, aiming to corroborate the experimental data widely described in the literature. it was observed that all the compounds involved in the study had adequate partition coefficient and water solubility to interact with aqueous (biological fluids) and liposoluble (plasma membrane) surfaces. It was also observed, through the molecular docking study, that all the compounds interacted attractively with the active site of the enzyme, forming intermolecular interactions with the amino acids of the site and with the zinc ion, which is of extreme importance for the enzyme to convert angiotensin I in angiotensin II. Among the compounds involved in the study, epicatechin 3-O-gallate showed the most stable interaction with the active site, with energy at -8.02 kcal / mol. The theoretical results developed in this work allowed a better view, at a molecular level, of the interactions between several natural compounds with the active site of ACE. It can be observed that the polar groups of the compounds are of extreme importance for the interaction of the zinc ion and for its biological activities.

Keywords: Hypertension, molecular docking, molecular modeling, natural compound

1. Introduction:

Hypertension is one of the main health problems in the world, besides being considered a serious risk factor for cardiovascular diseases and one of the causes of the reduction of the quality and life expectancy of individuals [1].

Currently in the pharmaceutical market there is a wide range of antihypertensives with varied mechanisms of action. Among these, a class that has gained prominence are the angiotensin converting enzyme (ACE) inhibitors [2]. Despite the large amount of active compounds on the market, it is still necessary to search for new substances, which are more effective and have fewer adverse effects.

Recent research has shown that a number of natural compounds have antihypertensive activity by inhibiting ACE [3], which makes them good prototype candidates for the synthesis of new antihypertensives. Although the amount of naturally occurring drugs is declining while the advance in molecular synthesis increases, there is still a lot to be analyzed in molecules already isolated [4], because from these structures, new substances can be synthesized.

In the development of a drug, it is necessary to take into account the pharmacokinetic characteristics of the substance, besides pharmacodynamics, because, if the substance does not arrive at the appropriate place of action, the activity is compromised [5]. Among the several methods used for the development of new drugs, molecular modeling has been gaining strength over time, since it has tools that contribute satisfactorily with corroboration of experimental studies to evaluate the pharmacokinetic and pharmacodynamic aspects of the compounds.

Based on this context, this work aims to evaluate, through a molecular modeling study, the solubility of natural compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligandstroside, as well as to verify the interaction of these compounds with ACE by molecular docking in order to corroborate with experimental studies.

2. Materials and Methods:

2.1 Molecular Docking Study

The molecular docking was run using AutoDock 4.0 software [6]. The crystallographic structure of the human testicular angiotensin converting enzyme (ACE) was obtained from the Protein Data Bank database [PDB ID: 1UZE] [7]. This enzyme was elucidated by X - ray crystallography, with a resolution of 1.82 Å. A set of five natural molecules, found in plants, was chosen for the study, according to reports in the literature on inhibition of angiotensin converting enzyme. The molecules were: oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligstroside. Ligands were obtained through the Pubchem database. The AutoDock Tools module was used to prepare and analyze the computational simulations. Gasteiger loads and polar hydrogens required for power calculations were added considering the target structure, with the water molecules removed. Gasteiger charges were also assigned to the ligands, with non-polar hydrogens being suppressed. AutoDock requires pre-calculated three-dimensional maps arranged in a box composed of a three-dimensional grid of points in a region defined in the macromolecule. The AutoGrid 4.0 program was used to generate the maps for the ligands. The box was positioned in the catalytic region of the enzyme with dimensions in the X-, Y- and Z- axis were, respectively, 66 Å 68 Å 74 Å with spacing of 0.375 Å. The Lamarckian Genetic algorithm (GA-LS) [8] was chosen to search for the best conformations with 100 runs for each ligand (genetic algorithm with local search). During the search process, the enzyme was held rigid, while the ligands were kept flexible. The initial population was defined as 150 and the search process occurred through random initial conformations. The maximum value of energy ratings chosen was 25,000,000. The maximum number of generations was 27,000. The number of elitism chosen was 1. Gene and crossover mutation rates were respectively defined as 0.02 and 0.80. At the end of the calculations, 100 different poses were obtained and grouped into

different clusters, defined by energy proximity and RMS values (Root Mean Square deviation), according to the AutoDock default. The validation of the methodology used was done through the redocking technique.

2.2 Solubility study

The software ALOPS 2.1 [9], which is based on machine learning calculations by neural networks, was used for the partition coefficient (Log P) and water solubility (Log S) calculations of the compounds. The ALOGPS was built on the Associated Neural Network (ASNN). The system implemented in ALOGPS for log P calculations was developed with 12908 molecules from the PHYSPROP database, using 75 E-state indices. Sixty-four neural networks were enabled using 50% of molecules chosen by coincidence from the whole set. The accuracy of the prediction log P presents an RMS value of 0.35 and mean standard error S = 0.26 [10-11]. For the calculation of water solubility, ALOGPS was developed using 1291 molecules. The accuracy of the log S prediction presents RMS = 0.49 and mean standard error S = 0.38 [12].

3. Results and discussion:

3.1 Solubility study

The present study investigated the solubility of the compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligstroside in the middle of the phase of aqueous and lipophilic equilibrium, since it is known the great importance that the solubility of the molecules has in relation to their pharmacological activity , this fact is due to the need of the compound to cross the lipophilic barrier and to interact with biological fluids [13].

The partition coefficient (log P) and water solubility (log S) obtained for the chemical compounds target of this study can be visualized in **Table 1**.

Table 1. Results of log P and log S of substances calculated by ALOPS 2.1

Compound	Log S (calc)	Log P (calc)
Oleroupein	-2.86	0.63
Guanosine	-1,26	-1.61
Epicatechin 3-O-gallate	-3.80	2.38
Mirtilin	-2.75	0.58
Ligrostroside	-2.55	0.77

Analyzing the values calculated by ALOPS 2.1 software (**Table 1**) we can observe that the epicatechin 3-O-gallate molecule has the lowest log S (less water soluble), while guanosine presented the highest value, representing greater solubility in water. It is observed that log P with the highest value was that of epicantequina3-O-gallate, in agreement with the values of log S.

The calculations show that all molecules proposed in this work have sufficient solubility to cross hydrophobic barriers and interact with aqueous fluids, since compounds with log S values between -1 and -5 present satisfactory hydrophilicity for aqueous solubility and lipophilicity to interact with hydrophobic surfaces [14]. Molecules having log S values

above -1 are very polar and have difficulty transposing into hydrophobic surfaces [14].

3.2 Molecular Docking

Table 2 shows the results obtained through the docking study between the compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligstroside with the angiotensin-converting enzyme (ACE).

Table 2. Value of the molecular docking energy of the compounds against the ACE

Compound	Docking Free Energy (kcal/mol)	Vdw, Hydrogen Bond and Solubility Interaction Energy (kcal/mol)	Eletrostatic Energy (kcal/mol)	Torsional Energy (kcal/mol)
Oleroupein	-6,16	-10,76	-0,47	5,07
Guanosine	-6,77	-8,39	-0,17	1.79
Epicatechin 3-O-gallate	-8,02	-10,69	-0,61	3.28
Mirtilin	-5,37	-8,29	-0,96	3,88
Ligrostroside	-6,56	-10,95	-0,38	4,77

Table 2 shows that all compounds involved in this study interacted with the angiotensin converting enzyme (ACE) in an attractive manner, and the compound 3-O-gallate epicatechin obtained lower interaction energy, being shown to be more stable in complex with the site of the macromolecule. It can also be observed that the ligand and oleroupein compounds obtained van der waals interaction, hydrogen bonding and solvation energies as satisfactory as the epicatechin 3-O-gallate, but with higher torsional energies, directly affecting the free energy of the docking .

Figure 1 shows the more stable conformation of the compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin, ligstroside at the active site of the angiotensin converting enzyme (ACE). It can be observed that in addition to interacting with the amino acids of the active site, all compounds studied interact ion-dipole with the zinc ion (Zn ++). This fact is of great importance because the enzyme requires this component for the conversion of angiotensin I to angiotensin II, that is, with the occupation of the active site and interaction with Zn ++, the enzyme is unable to convert substrate into the final product.

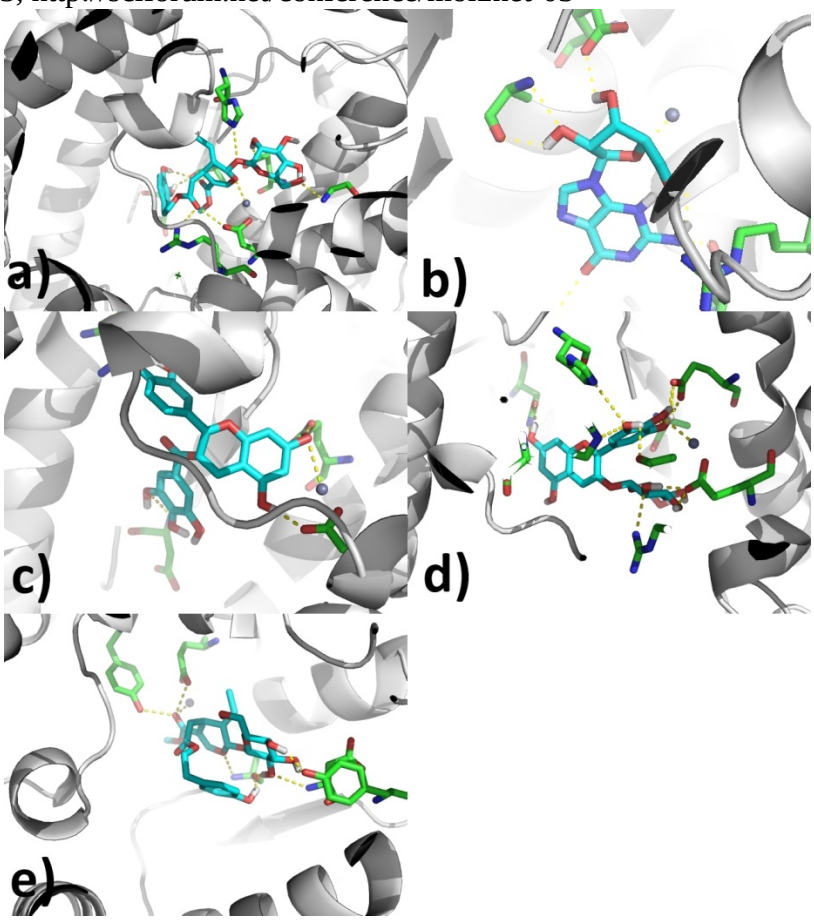

Figure 1: more stable conformations of the complex compounds/enzyme. a) Oleroupin; b) Guanosine; c) Epicatechin 3-O-gallate; d) Mirtilin; e) Ligstroside

Table 3. Description of the hydrogen bonds formed between ligands and active site of the enzyme

Compound	Number of H-bonds (n)	Aceptor H-bond	Donor – L-H	Distance H-bond (Å)
Oleroupein	2	ALA 356	LIG – O	1.45
		LIG – H	GLU 384	1.89
Guanosine	5	LIG – H	ALA 356	2.23
		LIG – H	GLU 411	2.15
		LIG – H	GLU 411	1.34
		ARG 522	LIG – O	2.10
		ALA 356	LIG – O	1.65
Epicatechin 3-O-gallate	5	LIG - H	ASN 70	1.62
		LIG - H	GLU 411	1.55
		LIG - H	ASN 70	1.67
		LIG - H	GLU 384	2.3
		ASP 358 - H	LIG – O	1.89
Mirtilin	4	LIG – H	ASN 70	1.34
		LIG – H	GLU 411	1.89
		LIG – H	GLU 384	2.10
		HIS 513	LIG - O	1.67
Ligrostroside	7	ASP 358	LIG – O	1.78
		SER 355	LIG – O	1.99
		HIS 513	LIG – O	1.76
		LIG – H	TYR 523	2.4
		LIG – H	ASN 70	1.32
		LIG – H	SER 516	1.56
		LIG – H	ASN 70	2.15

In **Table 3** the main characteristics of the hydrogen bonds formed between the binding compounds and the active site amino acids of the enzyme can be observed.

Ligands interacted through hydrogen bonds with similar active site amino acids, such as ASN 70, ASP 358, ALA 356, and others. This shows the importance of the polar groups of these compounds for the interaction with the active site of the enzyme, because in addition to the large number of hydrogen bonds, all interact with the zinc ion, as already mentioned above.

4. Conclusion

The computational study carried out in this work allowed a better view, at a molecular level, regarding the interaction of compounds oleroupein, guanosine, epicatechin 3-O-gallate, mirtilin and ligandstroside with the enzyme, showing that the compounds can be used as a prototype for synthesis of new ACE inhibitors.

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Quality indicators in *Ochroma pyramidale* seeds from three sites in the Ecuadorian Amazon for reforestation in degraded areas

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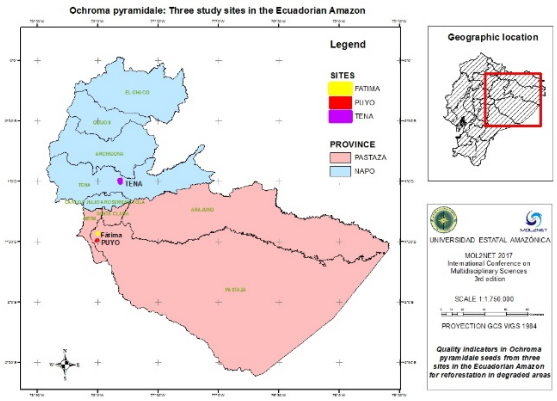
Graphical Abstract



Sites	P	GE	GC	UV	VG
San Juan	98.76	7,36	92.00	90.86	4.08
Fátima	98.03	2.80	70.02	68.64	3.10
Puyo	98.03	2.70	69.50	68.13	3.08

Abstract

Ochroma pyramidale, is a species recognized for its economic and ecological importance, widely used in forest plantation programs. The aim of this work was to evaluate seed quality indicators of *Ochroma pyramidale* in three sites in the Amazon region of Ecuador for reforestation purpose in degraded areas. The results indicated that the seeds of the species in the three study sites are of good quality, expressed through the germinative capacity, germination energy, useful value and germinative vigor, although in San Juan they presented higher values as a reflection of their vitality and exuberant nature.

Introduction

Ochroma pyramidale (Balsa), is a forest species belonging to family Malvaceae, recognized for its high demand in the international market, wide distribution, diversity of uses and very fast growth that produces low density wood, the lowest of the commercial wood in the world (Betancourt, 1968; Espinosa, 2007).

The species is cultivated naturally and for reforestation purposes, especially in the sub-tropical forest of Ecuador, where it is one of the most exploited timber forest resources; for this reason, it is one

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of the most economically important forest sectors with a high level of development, from its reforestation to its subsequent transformation, making it one of the highest quality timber in the world (González et al., 2010). It has also been used to enrich agricultural and livestock depleted soils, as well as to rehabilitate degraded areas as a result of frequent burning (Levy and Duncan, 2004).

Ecuador has more than 20 thousand hectares of plantations between natural forests and reforestation of Balsa, whose production is very profitable due to the turn of use of only 4-6 years, according to the quality of the site (Obregón, 2005). These plantations are an excellent option for the investor in the short term and require the increasing availability of a high quality reproductive material to meet the needs of the population.

In many forest programs, inappropriate management practices and use of low quality seed are evident, limiting the success of establishing forest plantations.

The aim of this work was to evaluate seed quality indicators of *Ochroma pyramidale* in three sites in the Amazon region of Ecuador for reforestation in degraded areas.

Materials and Methods

Selection of seeds

Fifty fresh seeds harvested in February 2017 were collected from three sites (San Juan, Fatima and Puyo), corresponding to Pastaza and Napo provinces, in the eastern region of Ecuador.

Germination process

The germination process was recorded by daily physical counting of seed germinated by bags for 46 days. The sowing was carried out in the experimental nursery of the Municipality of Pastaza, located at the entrance of the "Paseo Turístico" (Tourist Route) of Puyo. Polyethylene bags and natural soil mixtures were used with sawdust as a substrate.

Parameters of seed quality

Five indicators were calculated to evaluate seed quality at each site:

- Purity (%)
- Germinative energy (u)
- Germinative capacity (%)
- Useful value (%)
- Germinative Vigor (u)

Results and discussion

Percentage of accumulated germination

Figure 1 shows the percentage of germination accumulated in the three study sites in its germination phase representing the emergence of the radicle. In all three sites a linear behavior with determination coefficients higher than 98% was presented, which indicated a good fit. It is notable that the harvested seeds of the San Juan site presented a higher percentage of accumulated germination and the germination onset was inferior to the rest of the sites.

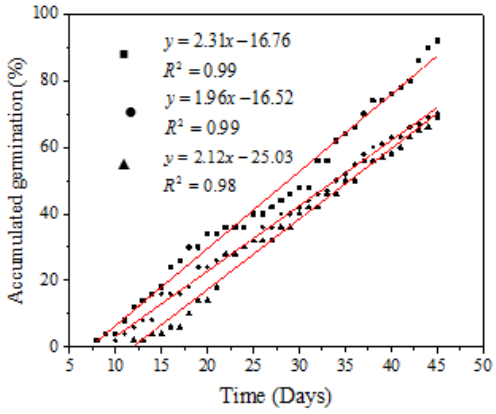

Figure 1. Percentage of accumulated germination of Ochroma pyramidale seeds at three sites.(■) San Juan, (●) Fátima, (▲) Puyo

Table 1 shows a similar behavior in the seed quality indicators. It is representative that at the San Juan site presented greater germinative energy, germinative capacity, useful value and

germinative vigor, as a reflection of a seed of superior quality, which could be an expression of its vitality and exuberant nature, but all the sites, according to the parameters obtained, may be appropriate to obtain good quality reproductive material for the success of the establishment of forest plantations under the dissimilar ecological conditions of the Amazon. As reported by González et al., (2010) the species reached a germinative capacity in all three sites in adequate ranges. These results will increase the genetic variability and the germplasm of species of high ecological and economic importance, which will provide a good quality material to foment sources of exploitation that contribute to eliminate logging of the Amazon jungle.

Table 1. Quality indicators in *Ochroma pyramidale* seeds

Sites	P	GE	GC	UV	VG
San Juan	98.76	7,36	92.00	90.86	4.08
Fátima	98.03	2.80	70.02	68.64	3.10
Puyo	98.03	2.70	69.50	68.13	3.08

Legend: Purity (P), Germinative energy (GE), Germinative capacity (GC), Useful value (UV), Vigor germinative (VG).

It is very important to homogenize the seeds in restoration and conservation plans of tree species in order to maintain genetic variability since the diversity of genes determines their response capacity in their establishment (Barbour *et al*., 2009; Ruíz *et al*., 2011; Ramírez *et al*., 2012).

Conclusions

It was demonstrated that the seeds of *Ochroma pyramidale* species of the three study sites (San Juan, Fatima and Puyo) were a high quality material that will increase the genetic variability for their use in reforestation plans in degraded areas of the Amazon region.

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[b] *<Insert affiliation here>*

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Alignment-Free Model for Prediction of B-cell Epitopes

Saúl G. Martínez-Arzate[§], [*] *Esvieta Tenorio-Borroto*[§], *Alberto Barbabosa Pliego*[§], *Héctor M. Díaz-Albiter*[#,Δ], *and Juan C. Vázquez-Chagoyán* [§,*]

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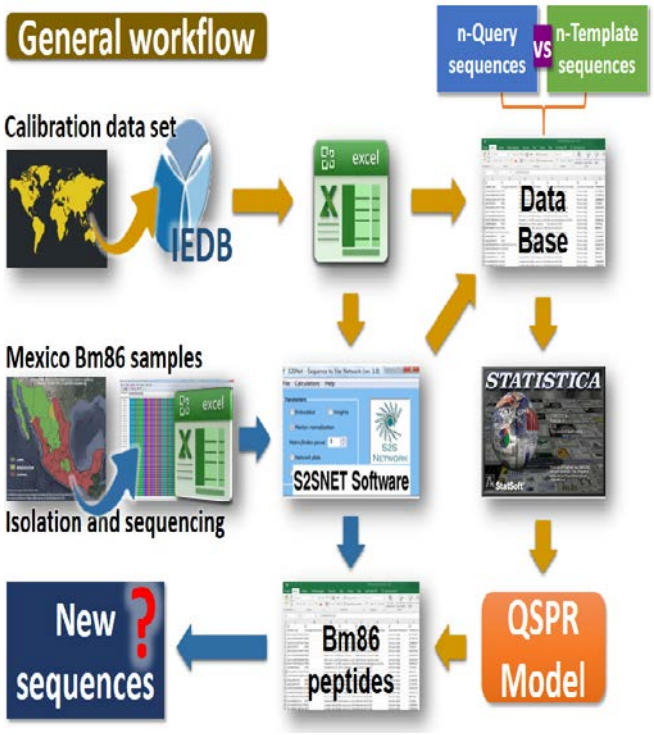
Graphical Abstract



Abstract. In this work, we developed a general Perturbation Theory model for prediction of B-cell epitopes in vaccine design. The method predicts the epitope activity $\varepsilon_q(c_{qj})$ of one query peptide (q-peptide) in a set of experimental query conditions (c_{qj}). The model proposed here is able to classify 1,048,190 pairs of query and reference peptide sequences reported on IEDB database with perturbations in sequence or assay conditions. The model has accuracy, sensitivity, and specificity between 71% and 80% for training and external validation series. The model may become a useful tool for epitope selection towards vaccine design. The theoretic-experimental results on Bm86 protein may help on the future design of a new vaccine based on this protein. **Ref:** J Proteome Res. 2017 Sep 18. doi: 10.1021/acs.jproteome.7b00477**.**

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The water relations of *Inga multinervis* for efficient water use in forest systems

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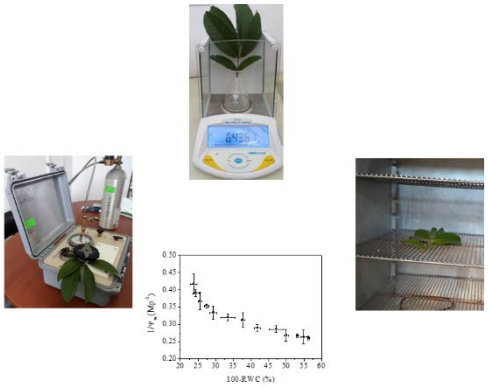
Graphical Abstract



Abstract

Inga multinervis, a little-known species, is being used in agroforestry systems for nitrogen fixation and soil improvement. The aim of this research was to characterize the water relations of the species *I. multinervis*from pressure–volume measurements. The results indicated that the species has the capacity for osmotic and elastic adjustment, given to the low solute potentials and elasticity of the cell walls, thus its use is recommended in degraded forest systems with low water levels in the soil.

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Introduction

Inga multinervis is a species of legume in the Fabaceae family, which grows only in Ecuador. Its natural habitats are subtropical or tropical moist lowland forests and subtropical or tropical moist montane forests (Neill and Pitman, 2004). This little-known species is being used in agroforestry systems for nitrogen fixation and soil improvement. Consequently, from the physiological point of view it's important to increase the knowledge of this speciesfor future forest management, which will allow its use in mitigating environmental impacts.

Pressure–volume (P–V) curves are frequently used to analyze water relation properties of woody plants in response to transpiration-induced tissue water loss. Generally, P–V-derived parameters reflect the environmental conditions of growth.

Reforestation of degraded land requires the use of selected species which shouldprovide sustainable long-term ecological services. Eco-physiological properties of trees are commonly considered when their capacity for growth and stress tolerance are evaluated (Kozlowski and Pallardy, 1997; Larcher, 2003).

The aim of this research was to characterize the water relations of the species *I. multinervis* forefficient use of water in forest systems.

Materials and Methods

Study site and plant materials

The study was carried out at Universidad EstatalAmazónica, located in the Province of Pastaza, Ecuador. Sampling was carried out in the proximity to the university. Plant material included the tree specie*I. multinervis.*

P–V curve analysis

Measurements for P–V analyses were performed using a pressure chamber (Model 1000, PMS instruments Corvallis, OR) following the method described in previous studies (Tyree and Hammel 1972; Kubiske and Abrams 1990).

Statistical analysis

Statistical analyses were performed using analysis of variance.

Results and discussion

P–V parameters

Figure 1 shows typical Höfler diagrams obtained from P–V curves for *I. multinervis*. These diagrams represent dynamic changes of water potential (Ψ_w), osmotic potential (Ψ_p), pressure potential (Ψ_p) andbulk elastic modulus (ε)in relation to relative symplastic water content (SWC).

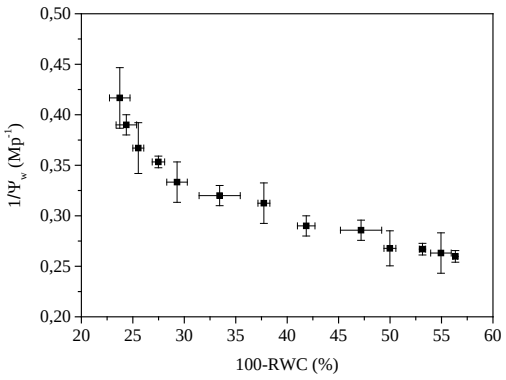

Figure 1. Plots of relative water content (RWC) against water potential (Ψw).

Table 1 shows the water relation parameters derived from P–V curves for *I. multinervis.*

Table 1. Water parameters from *I. multinervis*

Osmotic potential (MPa)	-2.41
Osmotic potential at water saturation with full Turgor (MPa)	-3.62
Bulk elastic modulus (MPa)	7.81
Relative water content at turgor loss point (%)	73.31

I. multinervis showed higher osmotic potential and osmotic potential at water saturation with full turgor than other species, such as *Robiniapseudoacacia, Quercusliaotungensis, Syringaoblata, Acer stenolobum, Armeniacasibirica, Pyrusbetulaefolia, Caraganamicrophylla, Rosa hugonis*according to reported by Yan *et al.*,(2013). These authors reported for these species bulk elastic modulus and relative water content at turgor loss point above those shown by *I. multinervis*.Bulk elastic modulusis one of the key leaf physiological traits of plantdrought tolerance estimated from the relationship betweenthe leaf–water potential and leaf–water volume, alsoknown as the pressure–volume curve.ε is mechanistically related to other P–V parameters that includeosmotic potential at turgor loss point, osmoticpotential at full turgor, and relative water content at turgor loss point. These parameters have alsobeen correlated with various aspects of drought tolerance(Lenz *et al*.2006; Bartlett *et al*.2012; Touchette*et al*., 2014). For instance, a more negativeosmotic potential at turgor loss pointextends therange of leaf–water potential at which the leaf remainsturgid and maintains stomatal and hydraulic conductance,photosynthetic gas exchange, and plant growth, which isespecially important when drought occurs during thegrowing season (Lenz *et al*., 2006; Bartlett *et al*., 2012).

These results indicate that the species presents high water absorption capacity of the soil, so it is recommended to use it for low water consumption and consequently less impact on the soil and the environment.

Conclusions

The determination of the water parameters from the P-V curves allows the characterization of the water relations of forest species. *I. multinervis* presented low values in the osmotic potential at water saturation with full turgor and in the water potential at turgor loss point, as well as low bulk elastic modulus, indicating that it is a suitable species for forest systems in low water content soils. The species is recommended to mitigate the environmental impacts associated with drought degraded soils.

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Relationship between the fed substrates and the physical chemical parameters of an anaerobic biodigester in Ecuadorian Amazon Region.

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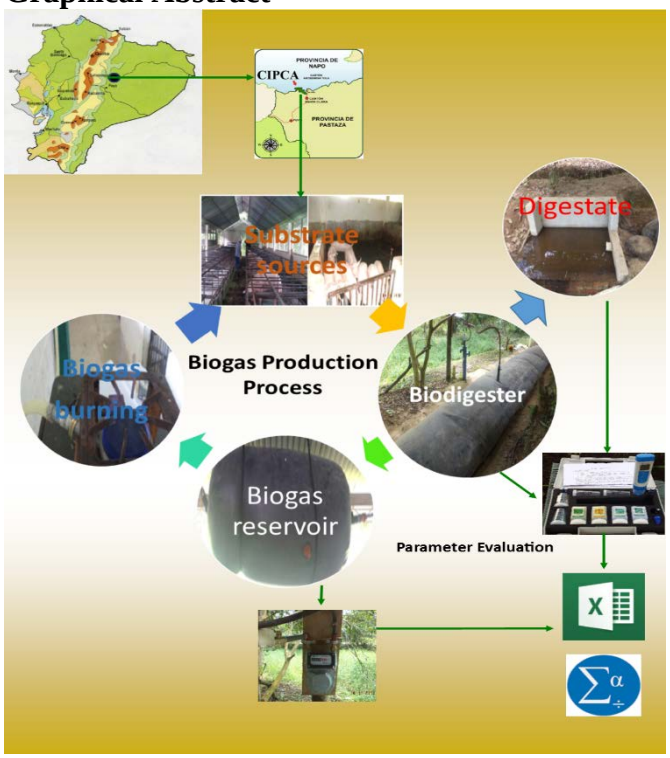
Graphical Abstract



Abstract.

At present the search for renewable energy sources and raw materials is the attention of many countries that seek to modify their energy and productive matrix. Anaerobic digestion of different substrates is one of the most studied processes. Not only with the aim of generating a renewable energy source but also by the use of waste generated in industry, agriculture and our homes. The operational parameters of this process directly influence the quality of the biogas and digestate that is produced. In addition to influencing the performance of the process. Amazon State University's (UEA) has a bioreactor that is fed mainly with animal manure so that the variability of the raw material quality can influence the product obtained quality. This work aims to relate the influence of the different

	substrates fed on the physical chemical parameter variability (pH, salinity, conductivity) that affect the digestion process. Keywords: Anaerobic digestion, animal manure, operational parameters

Acknowledgments

The authors acknowledge the Catalonia branch of "Ingeniería Sin Fronteras" and the project "Fortalecimiento de la cooperación universitaria/municipal en la implementación de tecnologías apropiadas para el tratamiento y aprovechamiento de residuos orgánicos". They also PhD Jaime Marti and Ing. Janeth Sanchez for cooperation.

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Trends in Medicinal Chemistry

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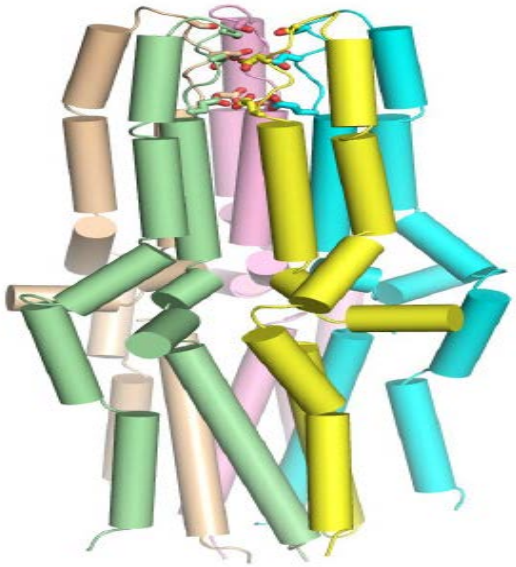
Graphical Abstract *(mandatory)*



Abstract. The ultimate goal of medicinal chemistry is to find most effective ways to treat various diseases and extend human beings' life as long as possible. In fact, Medicinal chemistry is currently undergoing an unprecedented revolution. Accompanied with such a revolution is the emergence of many new concepts, terminologies, approaches, and techniques. In a recent review, Chou discuss these processes from several different aspects. Ref: Current Topics in Medicinal Chemistry, 2017, 17, 2337-2358. See: *http://www.eurekaselect.com/151622/article*

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Leave-Species-Out Procedure in Multi-target QSAR models

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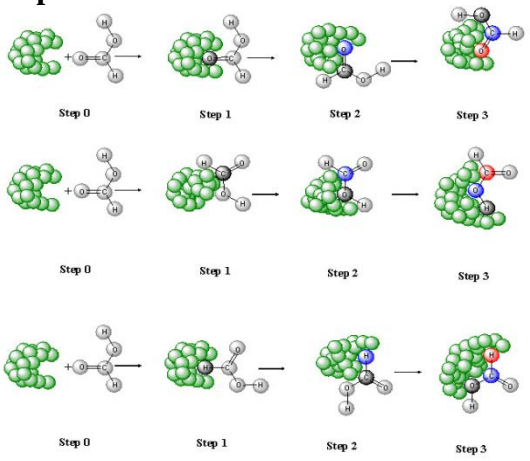
Biomedical Sciences Department, Health Sciences Division,
University of Quintana Roo, UQROO, 77039, Mexico.

Graphical Abstract



Abstract.

In this paper we generalized QSAR models to predict the biological activity of antifungal drugs against 87 fungi species. The data was processed by Linear Discriminant Analysis (LDA) classifying drugs as active or non-active. The model correctly classifies 338 out of 368 active compounds (91.85%) and 89 out of 123 non-active compounds (72.36%). Overall training predictability was 86.97% (427 out of 491 compounds). Validation of the model was carried out by means of Leave-Species-Out (LSO) procedure. After elimination step-by-step of all drugs tested against one specific species we record the percentage of good classification of leave-out compounds (LSO-predictability). Ref: Bioorg Med Chem. 2006 Sep 1;14(17):5973-80.

Ref: Ref: Bioorg Med Chem. 2006 Sep 1;14(17):5973-80.
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Intelligent consensus predictor: Towards more precise predictions for external set compounds

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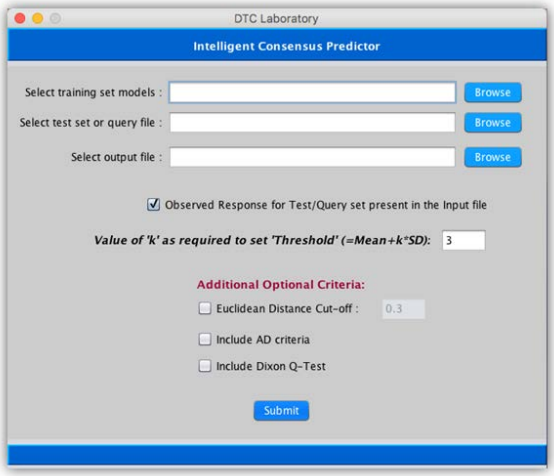
Graphical Abstract



Abstract

Quantitative structure-activity relationship (QSAR) modeling has travelled a long journey in drug discovery process as well as in prediction of property and/or toxicity data of diverse chemicals in order to fill the data gaps. The goodness-of-fit and quality of a model and its prediction capability for untested compounds are assessed through diverse validation metrics. There is a constant endeavor among QSAR researchers to get better the quality of predictions for lowering the predicted residuals for external compounds. The objective of the present study has been to improve the prediction quality for external compounds with implication of "intelligent" consensus modeling approach. Three different forms of consensus models were developed for six different datasets to explore their prediction capability on query chemicals. The types are average of predictions from all qualifying individual models (CM1), weighted average predictions from all qualifying individual models (CM2), and best selection of predictions (compound-wise) from individual models (CM3). Among three consensus models, newer strategies like CM2 and CM3 are evolved as the "winners" considering prediction errors of query compounds for the studied six data sets irrespective of diverse responses, number of data points as well as dissimilar modeling algorithm. We have also developed a tool named "Intelligent Consensus Predictor" which is freely accessible via the web http://teqip.jdvu.ac.in/QSAR_Tools/ and http://dtclab.webs.com/software-tools. The details of this work have been presented in *Conferentia Chemometrica* http://cc2017.ttk.mta.hu/ in Hungary during September 3-6, 2017.

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QSAR of natural sesquiterpene lactones as inhibitors of Myb-dependent gene expression

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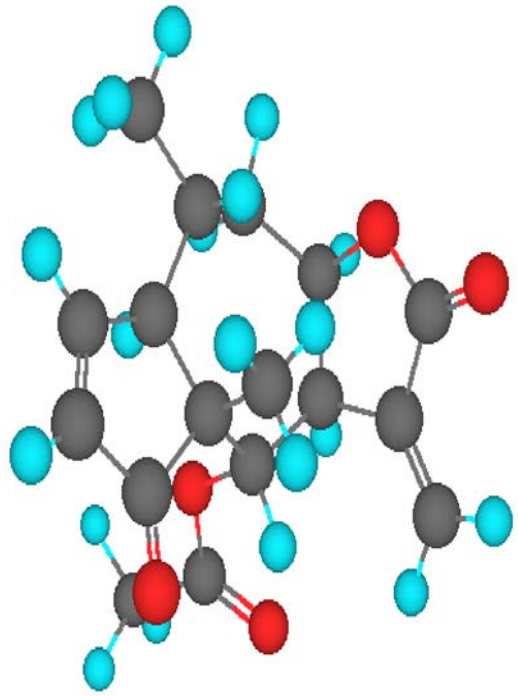
Graphical Abstract



Abstract. Protein c-Myb is a therapeutic target. Some sesquiterpene lactones suppress Myb-dependent gene expression, which results in a mechanism for their potential anti-cancer activity. Database ChEMBL is representative of lactones for physicochemical and physiochemical properties. We studied a dataset with 31 natural lactones are discussed in terms of quantitative structure–activity relationships, which objective is to predict inhibitors of Myb-induced gene expression. Several constitutional descriptors are related to structure–activity. Coefficients standard errors result acceptable in almost all equations. After cross-validation, linear equations for lactones, pseudoguaianolides and germacranolides are the most predictive. Most descriptors are constitutional variables.

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Mosquito-borne viruses: A computational search for antiviral drugs

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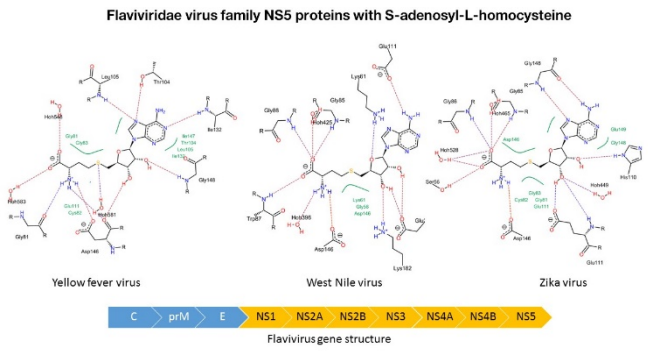
Graphical Abstract



Abstract.

Mosquito-borne viruses of Flaviviridae virus family are dangerous for human. To develop drugs and vaccines against Flaviviridae viruses, promising targets must be identified. The genomes and biochemistry of Yellow fever (YFV), West Nile (WNV), and Zika (ZIKV) viruses are similar. Therefore, the main aim of this project was to identify lead compounds which could simultaneously inhibit all three viruses targeting one or more viral proteins.

Introduction

Mosquito-borne viruses of Flaviviridae virus family are dangerous for human [1]. To develop efficient drugs and vaccines against Flaviviridae viruses, promising protein targets must be identified.

The genomes and biochemistry of Yellow fever (YFV), West Nile (WNV), and Zika (ZIKV) viruses are similar [2]. Therefore, the main aim of this project was to identify lead compounds which could simultaneously inhibit all three viruses targeting one or more viral proteins. Activation of non-structural proteins NS1, NS2A, NS3 and NS5 inside of mosquito-borne viruses is necessary for virial replication, as well as structural envelope E is responsible for entry of viral particles into the cell. Hence, the inhibition of at least one type of protein could neutralize the entire virus.

Materials and Methods

At the first step, RCSB Protein Data Bank was used to extracted data on sequences variations for target proteins [3]. Next, Basic Local Alignment Search Tool (BLAST) tool was applied to find similarity between studied proteins of YFV, WNV, and ZIKV [4].

A series of FDA approved drugs from Binding database (https://www.bindingdb.org) and DrugBank (http://www.drugbank.ca/drugs) were screened [5,6]. Selected proteins were prepared for molecular docking: native ligand and waters were removed, and polar hydrogens were added to the protein. The active sites of the enzymes were defined to include residues within 8.5 Å radius around inhibitor. Both crystallography-based and suggested allosteric sites were considered for docking. Final scores were used for database ranking. The best pose with the highest score was selected to analyze the interactions between ligand and protein. At the next step, hits were used as references for deeper screening of ZINK database [7]. Results were compared with literature data. Docking, scoring, and screening procedures were performed using BioSolveIt suite [8].

Results and Discussion

Ten target proteins were extracted from RCSB: structural envelope (E), non-structural hydrolase and transferase for YFV, non-structural hydrolase and methyltransferase for WNV and non-structural methyltransferase, helicase, protease RNA-dependent polymerase and structural envelope for ZIKV. These proteins contained bonded ligands, so locations of these ligands were used as a reference for initial search of possible binding pockets. In addition to it, allosteric sites were analyzed.

While genomes of YFV, WNV, and ZIKV are quite similar, the qualitative analysis based on BLAST revealed, that the best binding sites for promising hits were located in different places for same types of proteins. In some cases, these differences are drastic. For instance, similarities between non-structural NS1 proteins were: ~ 55 % for ZIKA and DENV, ~50 % for ZIKA and YFV, ~ 55 % for ZIKA and WNV, ~ 45 % for YFV and DENV, ~ 45 % for YFV and WNV, and ~ 50 % for DNV and WNV.

Molecular docking was performed for more than 6000 drug-like compounds. Free energies of binding varied from −35 kJ/mol to -6 kJ/mol for hits. A series of compounds were identified as inhibitors for proteins of certain type. For example, quinacrine and its derivatives acted in the same way against all nonstructural NS3 proteins. Other examples are nanchangmycin and lovastatin, that interacted with allosteric sites of NS2A and NS3 proteins. Specific poses were identified and analyzed. For instance, in the case of Zika, selected drugs mainly bonded to Glu234, Gln396, and Glu 231 inside of NS3 protein.

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[4] Basic Local Alignment Search Tool, https://blast.ncbi.nlm.nih.gov/Blast.cgi

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The role of excipients in neglected tropical diseases

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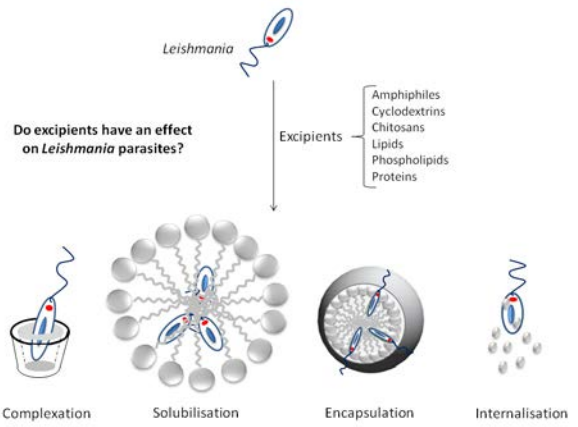
[±] First co-authorship shared

Keywords: Excipients, leishmaniasis, amphiphile, surfactant, lipids, Eudragit, Labrasol, medium chain fatty acids, lauric acid, sodium deoxycholate

Graphical Abstract



Abstract.
Leishmaniasis is a neglected tropical disease responsible for the ninth largest disease burden in the world. Excipients are necessary for ensuring the stability and bioavailability of currently available antileishmaniasis drugs. In a recent work, we have evaluated the *in vitro* activity of 30 commercially available excipients against different *Leishmania* spp., their cytotoxicity and potential use for inclusion in novel formulations.

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QSAR with ETA indices: Insecticidal activity of plant derived compounds against zika virus vector *Aedes aegypti*

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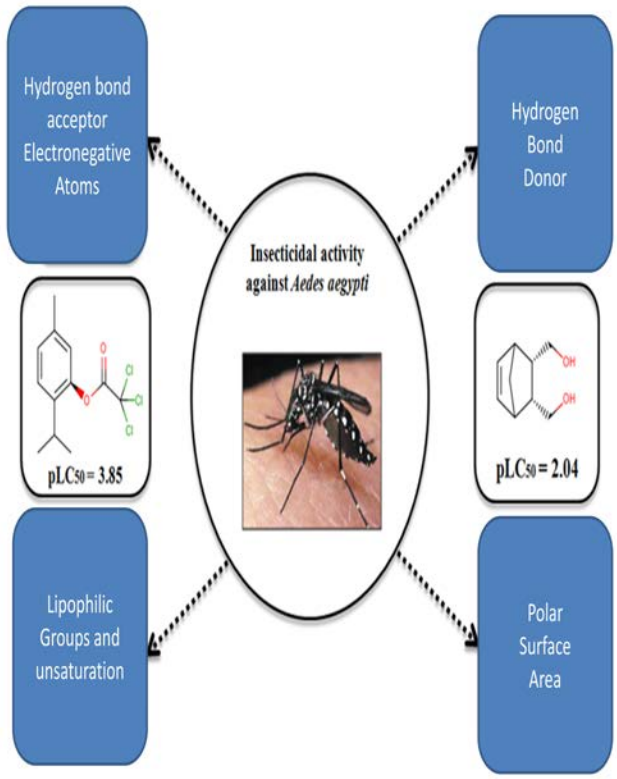
Graphical Abstract



Abstract

Dengue, zika and chikungunya have severe public health concerns in several countries. Human modification of the natural environment continues to create habitats in which mosquitoes, vectors of a wide variety of human and animal pathogens, thrive which can bring about enormous negative impact on public health if not controlled properly. Quantitative Structure–Activity Relationship (QSAR) modeling was applied in this work with the aim to explore features contributing to promising larvicidal and insecticidal property against the vector *Aedes aegypti* (Diptera:Culicidae). A dataset of 62 plant derived compounds obtained from the previous literatures was used in this present study where Genetic Algorithm (GA) was used for model development employing Double Cross Validation (DCV) tool. Simple topological descriptors like Extended Topochemical Atom (ETA) indices developed by the present authors' group were used for model development. A number of models were generated by the GA method and the descriptors obtained were pooled for Best Subset Selection method (BSS). Further, the best model obtained from BSS was used for Partial Least Square (PLS) regression to

obtain the final model. The model was validated extensively using different validation metrics to check the robustness and predictivity of the model for regulatory acceptance and enhancing confidence in QSAR predictions. Based on the insights obtained from the PLS model, we can conclude that presence of hydrogen bond acceptor atoms, presence of multiple bonds as well as sufficient lipophilicity and limited polar surface area play crucial roles in regulating the activity of the compounds.

Keywords: QSAR, Zika, Insecticidal activity, Double cross-validation

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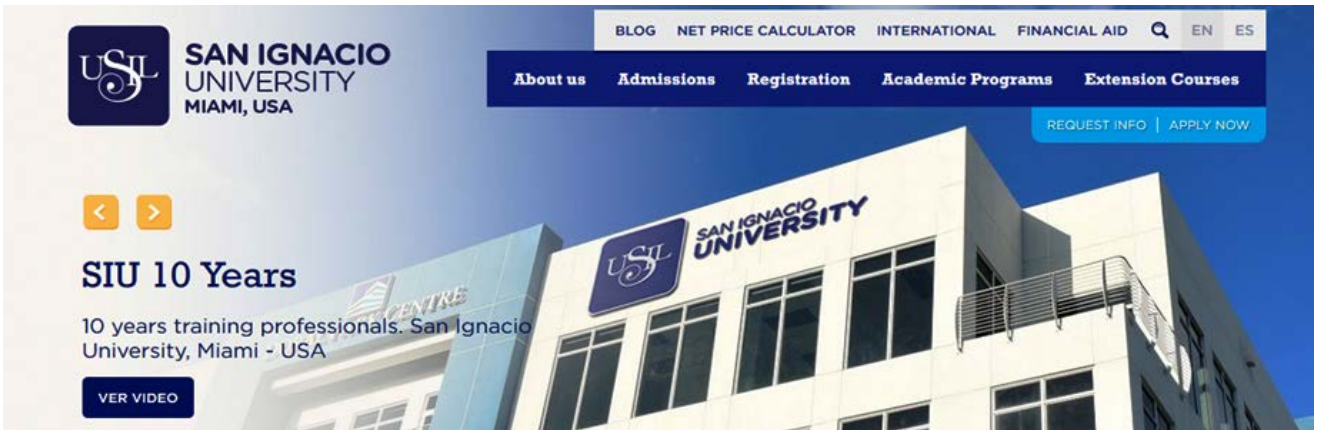

MOL2NET 2017

Flavonoid interaction with chitosan: planning active packing with antioxidant and antimicrobial activity

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Abstract: Active packaging is a packaging system that has incorporated additives and that interact directly with the food in order to prolong its quality and its useful life. Due to the bioindecomposability and toxicity of synthetic polymers and additives, the search for natural substances, which present more suitable characteristics for the production of active packages, such as chitosan, which is a naturally occurring polymer and flavonoids, increase, because they have low toxicity and activities antioxidant and antimicrobial. The purpose of this study to perform the interaction of flavonoids quercetin, rutin, quercitrin and artemetin with chitosan by molecular docking, aiming at the planning of new biodegradable and non-toxic active films. The molecular docking study was performed using Autodock 4.0. The three-dimensional structure of the chitosan was obtained through the PolySac3DB bank, while the flavonoid structures were acquired through PubChem. The results showed that the flavonoids quercetin, quercitrin and artemetin interacted attractively with chitosan. Quercetin was the flavonoid that interacted more stable, with an energy expenditure of -3.61 kcal / mol. The rutin was the only flavonoid, among those involved in the study, that did not interact attractively with chitosan, as its binding energy was 0.49 kcal / mol. It is observed that the interaction of rutin with chitosan is impaired due to its high level of torsion. It was observed that the flavonoids targets of this study, with the exception of rutin, interacted attractively with chitosan, suggesting that they are good candidates for additives for the production of active films.

Keywords: chitosan, flavonoid, molecular docking, active packaging

Introduction:

Active packaging is a packaging system that has built-in additives that will interact directly with the packaged food in order to prolong its quality and shelf life [1-2]. The packaging must support the microbiological and sensory competence of the food, in a way that contributes to the preservation of the quality of the packaged product, from its biological activities [3]. Recent studies have presented promising results regarding the use of flavonoids as additives in active packaging [4].

Flavonoids are compounds found in fruits and vegetables, responsible for the vibrant colors that attract pollinating insects and filter the ultraviolet rays of the sun. Flavonoids attracted interest from the scientific community, due to its diverse biological activities, such as antimicrobial, anti-inflammatory, antithrombotic and antioxidant activity, among others [5-6]. Flavonoids are compounds that belong to a certain class of natural compounds currently classified as micronutrients [7].

Chitosan is a naturally occurring polymer derived from the deacetylation process of chitin, and besides being considered the second most abundant polysaccharide in nature, it also has numerous technological and biological characteristics, finding applications in a variety of fields, , in the development of active films due to their favorable characteristics, such as biodegradability, biocompatibility, gel formation and bioactivity [8-10].

Molecular modeling techniques have been widely used in development studies of new active materials [11]. Molecular docking, specifically, can be used to predict the interaction of ligands with polymers [12]. Molecular docking is a fundamental tool to seek a better adjustment orientation of a ligand in a protein, in advance, that is, method of finding the best fit of two molecules [13-14].

Based on the characteristics of chitosan and the biological properties of flavonoids, the present study sought to investigate in silico, by molecular docking, the interaction of flavonoids quercetin, quercitrin, artemetin and rutin with the polymer, aiming at a better understanding of the mechanistic behavior of compounds in interaction with chitosan (flavonoid-polymer), in order to corroborate with experimental data widely described in the literature.

Materials and Methods:

The molecular docking study was performed through Autodock 4.0 [15]. The three-dimensional structure of chitosan with 12-mers (**Figure 1**) was obtained through the PolySac3DB bank, while the flavonoid structures (**Figure 2**) were acquired through the PubChem molecule bank. For orientation of the ligands, a grid was positioned around the entire molecule with dimensions of 58 Å on the X- axis, 126 Å on the Y-axis and 56 Å on the Z- axis. For the searches the Lamarckian Genetic Algorithm [16-18] was used in 100 runs.bThe initial population was defined as 150 and the search process occurred through random initial conformations. The maximum value of energy assessments chosen was 25,000,000, while the maximum number of generations was maintained at 27,000, as well as the number of elitism was maintained at 1. The rates of genetic mutation and crossover were respectively 0.02 and 0, 80. After completing the calculations, 100 different conformations were obtained and grouped into different clusters, defined by energy proximity and RMS values, according to the *AutoDock* default [15].

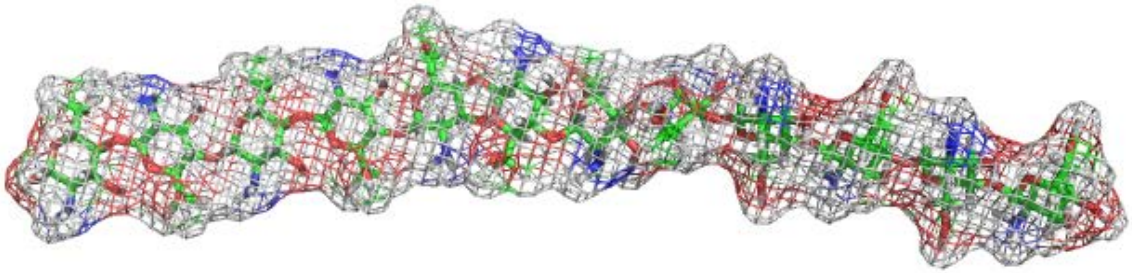

Figure 1. Three-dimensional molecular structure of chitosan with 12 mers

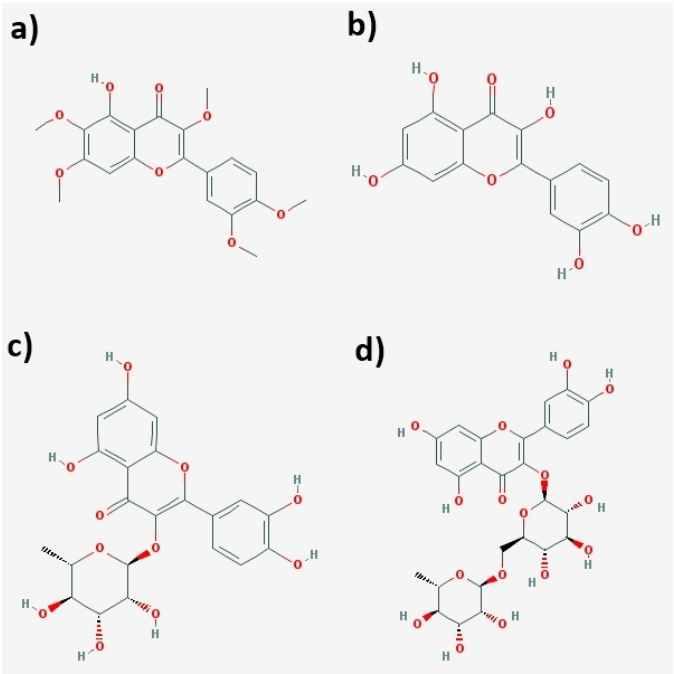

Figure 2. Two-dimensional structure of the flavonoids involved in the study. a) artemetin; b) quercetin; c) quercitrin; and d) rutin

Results and discussion:

The results showed that the flavonoids quercetin, quercitrin and artemetin interacted attractively with chitosan, as shown in **Figure 3** and **Table 1**. Quercetin was the flavonoid that interacted more stable with an energy expenditure of -3, 61 kcal / mol. The rutin was the only flavonoid, among those involved in the study, that did not interact attractively with chitosan, as its binding energy was 0.49 kcal / mol. It is observed that the interaction of rutin with chitosan is impaired due to its high torsion level (**Table 1**).

In addition to the van der waals interactions formed between the flavonoid ring groups and the chitosan ring groups, several hydrogen bonds are formed between the polar groups of flavonoids with polar groups of chitosan.

The present study is similar to other experimental studies developed, where flavonoid quercetin was used as an additive and incorporated into chitosan efficiently [19-21].

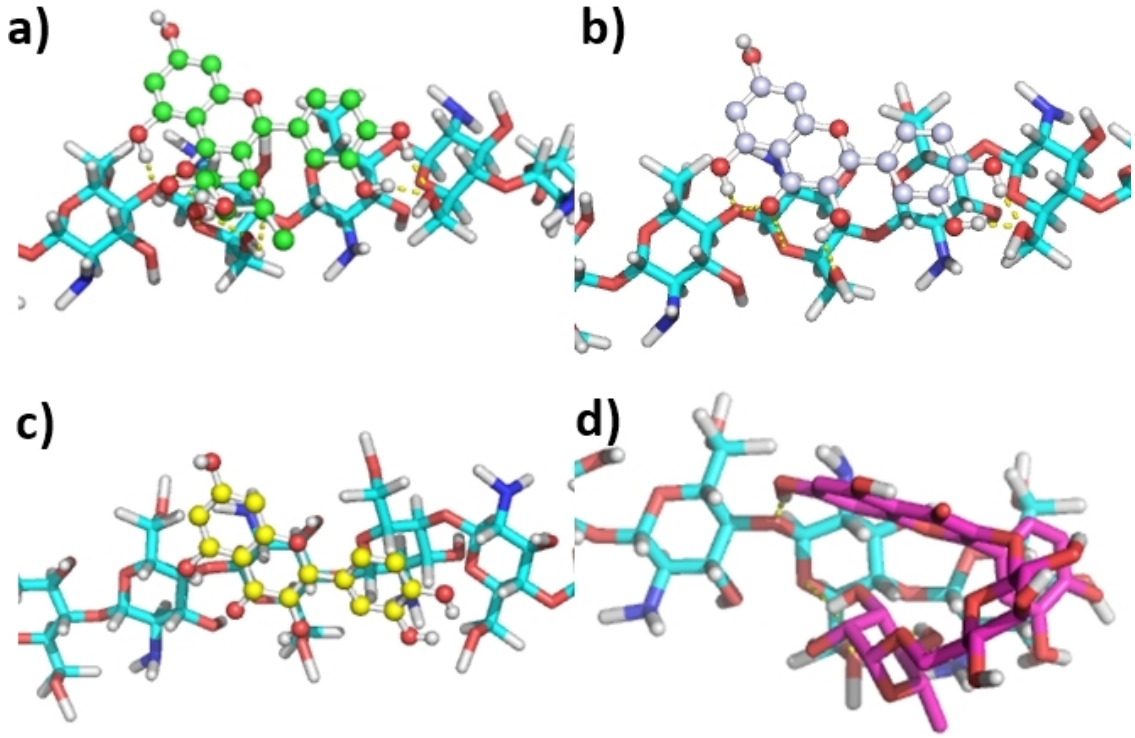

Figure 3. Interaction of ligands with chitosan. a) quercitrin; b) artemetin; c) Quercetin; d) Rutin

Table 1. Values obtained by molecular docking

Complex	Free energy docking (kcal/mol)	Electrostatic interaction energy (kcal/mol)	Van der Waals interaction and hydrogen bonding energy (kcal/mol)	Torsional Energy (kcal/mol)
chitosan + Quercetin	-3.61	-0.34	-5.05	1.79
Chitosan + Quercitrin	-2.25	-0.31	-4.92	2.98
Chitosan + Rutin	0.49	-0.42	-3.86	4.77
Chitosan + Artemetin	-2.52	-0.34	-4.27	2.09

Conclusion

It was observed that the flavonoids targets of this study, with the exception of rutin, interacted attractively with chitosan, suggesting that they are good candidates for additives for the production of active films.

Conflicts of Interest:

The authors declare no conflict of interest

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An Overview on Cyclopentenyl Fatty Acids

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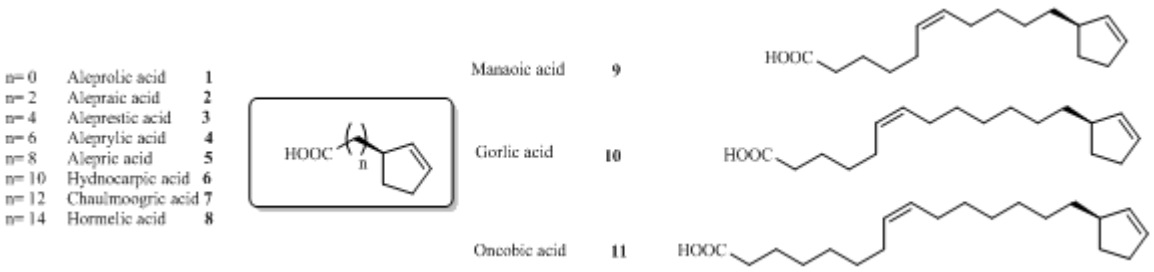
Graphical Abstract



Abstract. This review discusses the substantial cyclopentenyl fatty acid class of naturally occurring lipids. These compounds are historically important and have recently been shown to exhibit remarkable biological activity relevant to producing new antibiotic agents. Information about the history of cyclopentenyl fatty acids, their use in traditional and modern medicine, as well as biological activity, and methods for their synthesis are given.

***Keywords*:** *Cyclopentenyl fatty acid, chaulmoogra oil, tuberculosis, leprosy, hydnocarpic acid, chaulmoogric acid.*

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Almahli H. Cyclopentenyl Fatty Acids: History, Biological Activity and Synthesis. Curr Top Med Chem. 2017 Aug 21
https://www.ncbi.nlm.nih.gov/pubmed/28828992

MOL2NET, International Conference Series on Multidisciplinary Sciences
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Squalene-based Nanosystems for Controlled Drug Release

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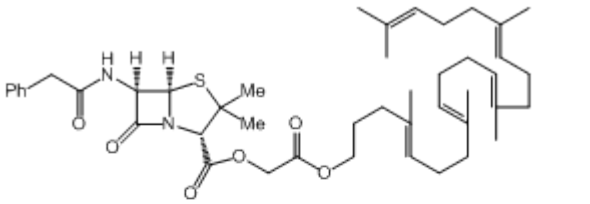
Graphical Abstract



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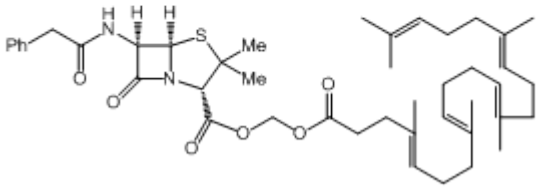

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Abstract: This article reviews the innovative and original concept the “squalenoylation”, a technology allowing the formulation of a wide range of drug molecules (both hydrophilic and lipophilic) as nanoparticles. The "squalenoylation" approach is based on the covalent linkage between the squalene, a natural and biocompatible lipid belonging to the terpenoid family, and a drug, in order to increase its pharmacological efficacy. Fundamentally, the dynamically folded conformation of squalene triggers the resulting squalene-drug bioconjugates to self-assemble as nanoparticles of 100–300 nm. In general, these nanoparticles showed long blood circulation times after intravenous administration and improved pharmacological activity with reduced side effects and toxicity. This flexible and generic technique opens exciting perspectives in the drug delivery field.

Keywords: Squalenoylation, Prodrug, Nanoassemblies, Drug loading, Oncology, Intracellular infections, Neurological disorders

References

Ref: Curr Top Med Chem 2017, https://www.ncbi.nlm.nih.gov/pubmed/28730957

Enhancement of quantum efficiency of hybrid photoelecrochemical cell: Effect of funtionalized carbon nanotube with Cu doped ZnO nanocomplex

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The great energy challenge facing by mankind is due to the depletion of fossil fuels at an alarming rate with exponential increase in population and growing demand in their modern lifestyle. To overcome this situation, utiliszation of omnipresent and abundant solar energy has become the most promising one. Current research work is concentrated on developing new and novel systems to harvest solar energy with greater efficiency. The emergence of nanomaterials has opened up many innovative ways and new initiatives are taken for fabrication of hybrid solar cell. ZnO nanoparticles are considered to be one of the most important material owing to several unique features and wide range of technologically important applications such as solar cell. The optical and electrical properties of ZnO are significantly improved by Cu doping. For further development of optical properties, researches on hybrid materials of ZnO and carbon nanotubes (CNTs) have received extensive attention.

We have studied the effect of addition of Cu doped ZnO nanoparicle (CZNP) and functionalized carbon nanotubes complex (CZNP/FMWCNT) with PSF dye for photovoltage generation in hybrid PEC cell. The CZNP-FMWCNT complexes were synthesized via wet chemical method. XRD, FESEM, EDAX and Raman spectroscopy confirmed the formation of well crystalline complex system with a certain concentration of FMWCNT.

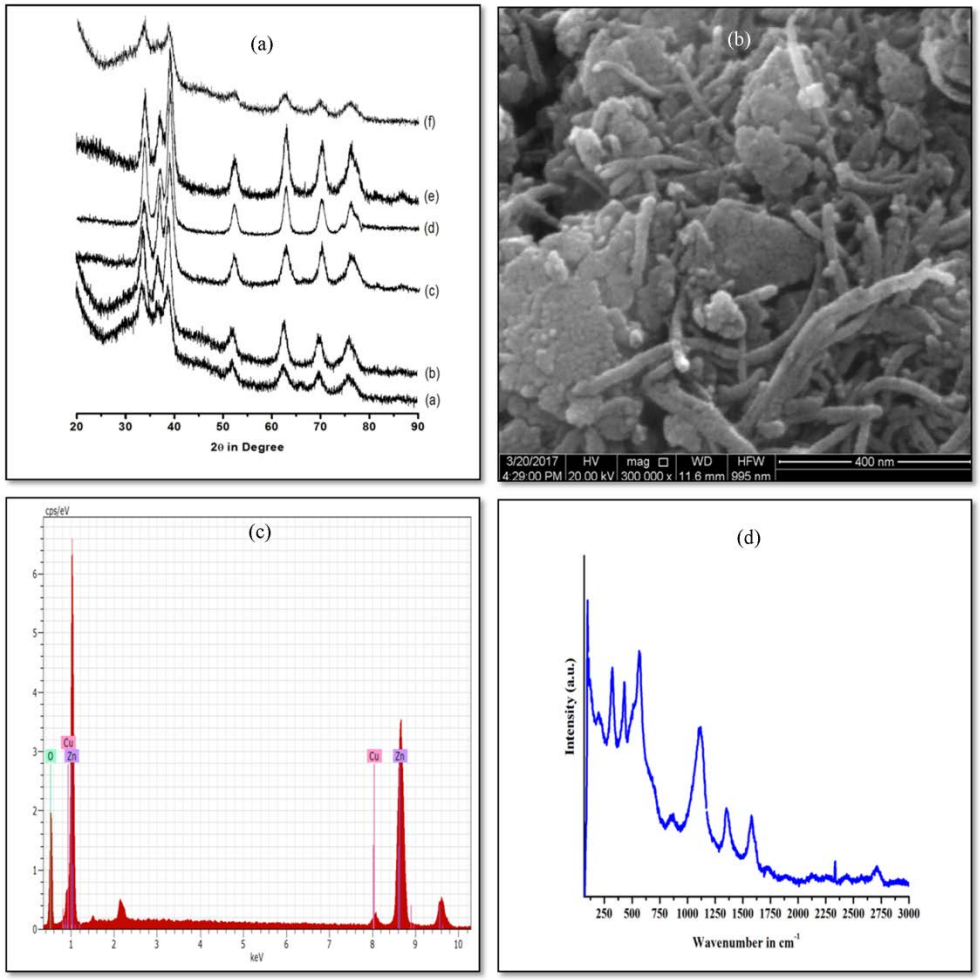

Figure 1. Different characterization of CZNP/FMWCNT complexes (a) XRD, (b) FESEM, (c) EDAX & (d) Raman spectroscopy

Then PSF dye was added to this complex and used in a hybrid photoelectrochemical (PEC) cell for photovoltage generation. The maximum voltage generation was of the order of 712.6 mV and storage duration was ~55 hrs. The energy conversion efficiency (η%) was 3.34%. UV-Vis spectra of the system with unchanged peak position of dye ascertained adsorption of dye molecules on the surface of CZNP/FMWCNT.

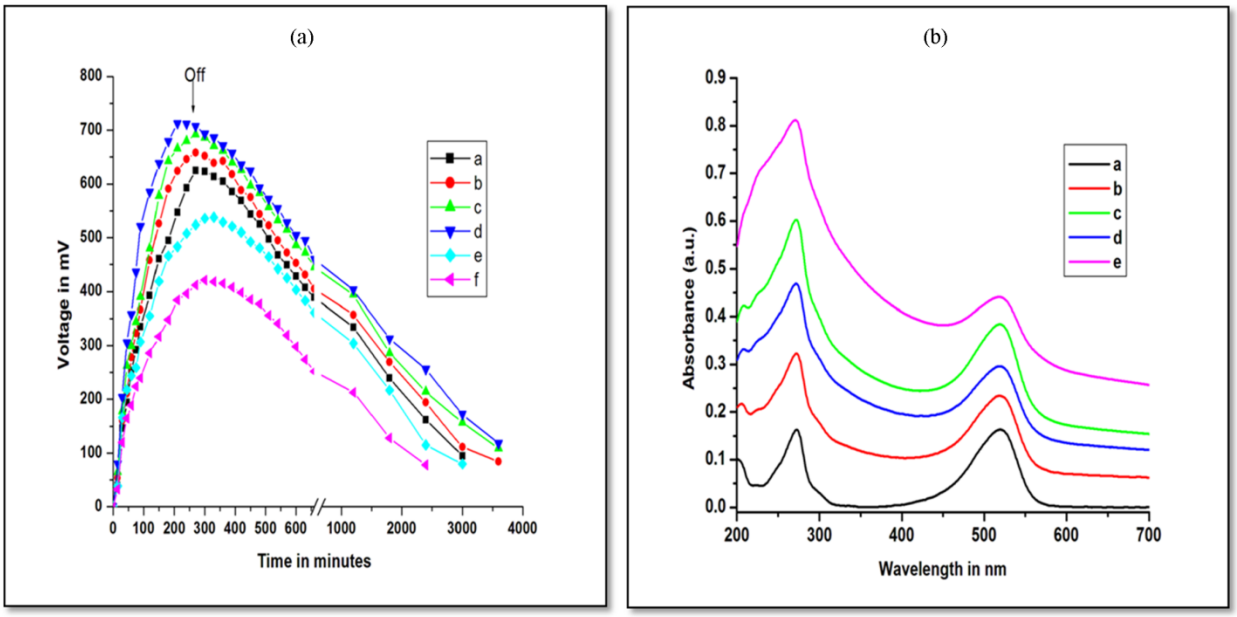

Figure 2 (A) Photovoltage generation with CZNP/FMWCNT complexes with increasing concentration of FMWCNTs (a to f) and (B) UV-VIS spectroscopy.

ZnO and CNT, an important subset of nanomaterials, received exceptional attention for their unique structures and pertinent physical and chemical properties. Photovoltage generation and light conversion efficiency got significantly enhanced than only PSF solution and/or PSF adsorbed CZNP system, due to incorporation of FMWCNTs in PSF-CZNP/FMWCNT system. This might be attributed to the fact the CNTs have a large storage capacity and their conductivity is almost similar to metals, so MWCNTs played an important role in photogenerated charge transfer Formation of new energy level by Cu 3d level between conduction and valance band of ZnO helped in easy and higher photodissociated charge transport. All these helped in efficient charge collection reducing the electrolyte interfacial resistance and hence the recombination probability which resulted in enhanced photovoltage, good storage capacity and improved photo energy conversion efficiency.

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Affectation of *thrips palmi* in the quality and production of the naranjilla in the Ecuadorian Amazon.

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Abstract:

Many amazon producers used to apply various agrochemicals on crops of naranjilla (*Solanum quitoense* Lam.) with the objective of controlling different pests, and also increasing the size of the fruit. This kind of crop has a very high acceptance among the producers of Pastaza province. The low level of identification of *Trips Palmi* leads us to believe that the symptomatology and damages are associated with the phytotoxicity of the plant caused by the various pesticides, masking the high incidence of this pest and generating a decline in yields. This has an impact on economy of producers and for this reason, it was necessary to carry out this research in order to determine the impact of *T. Palmi* on crops of naranjilla. This experiment took place in two different productive farms located in the community of San Cristoball, canton of Santa Clara in Pastaza province. A sample of 35 plants was taken to identify the pest first. It was indispensable to know the specific location for which quantity and quality variables were proposed. It was demonstrated that, in the quantity variable (plant height), there were highly significant differences, in comparison with the average values between farms at all times (days: 1, 8, 16 and 24). It became apparent that there is a 100% impact of *Thrips Palmi* in both farms. This pest is located on the upper side of young leaves causing chlorotic and rosulate yellowing in upper, middle and lower leaves as well.

Keywords: affectation, naranjilla cultivation, *Solanum quitoense* Lam.

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1. Introduction

In 2008, the world production of tropical fruits was estimated at 87.2 million tons, so much so, in the last decade the average growth rates of fruit production reached 5%. Latin America contributes to the world production of fruits with 21% (Muñoz, 2010).

Among the species of fruit trees, the naranjilla (*Solanum quitoense* Lam.) Is a crop of great importance for Ecuador, since it is a fruit tree with important markets, in turn of interest for international markets, the naranjilla has been cataloged as an essential factor in the peasant family economy due to the fact that it constitutes one of the main sources of income for 19,000 families in Ecuador (Muñoz, 2010) This Solanaceae is a short cycle crop, due to its constant production generates weekly income, main factor that stabilizes the family economy.

The cultivation of this fruit has been affected by high populations of the *Thrips palmi* insect that causes economic damages in the crop, since they produce lesions that do not allow the correct development of the plants and the quality of the fruit to be harvested (Murguido *et al*., 2002).

Due to the above, in most of the countries where the fight against this insect has been launched, it has been difficult to eradicate it, due to the little or no quality of the applications of the chemical products, the ability of the insect to adapt to conditions unfavorable as it is the case of prolonged droughts, added to this, the reduced number of natural enemies that this has (Vázquez, 2003).

The Farmers in their effort to stop the development of this pest have used different insecticides, which in many cases failed to meet their expectation of eradicating the insect from their crops, caused by the lack of information on their effectiveness in controlling the plague. For these reasons, it has been suggested that *T. palmi* has a natural resistance to insecticides, which hurts and makes it a difficult pest to control and eradicate (Murguido *et al*., 2002).

2. Materials and Methods

For the experiment, two agro-productive farms were chosen from Santa Clara - Pastaza Province, each one was named as replicas 1 and 2 respectively, these were divided into three levels. Medium and low, for the sampling of data, 35 Plants for each level during days 1, 8, 16 and 24 per plant were evaluated a high branch, a middle branch and a low branch, based on an exploratory investigation (Bermeo, 2011), since *Thrips palmi* is a pest with a wide range of hosts, especially plants of the Solanaceae family, an experimental design with dependent and independent variables was applied in order to obtain the relations of the incidence of *T. palmi* in the culture of the naranjilla each farm, the determination of the presence of the plague was made by direct observation to the plant with appropriate magnifying glasses, and the data were saved in database and then developed with the formuls

The incidence was calculated using the following formula:

$$\% \text{ of incidence: } \frac{\text{\# affected plants}}{\text{\# total plants}} \times 100$$

Author: Barea, G. 2008

The severity was determined based on four levels of attack; each level corresponds to a percentage of damage of the foliar area attacked from the fourth leaf (counting from the apex of the branch) selected.

The severity was calculated using the following formula:

$$\% \text{ of Severity: } \frac{\text{Affected area}}{\text{Total area sheet}} \times 100$$

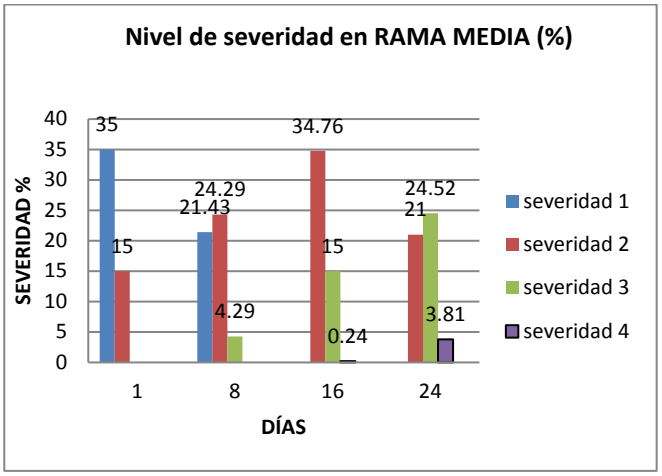
Author: Reis, 1994

In the end the presence of the characteristic symptoms caused by *T. palmi* in the organs of the plant was confirmed.

3. Results and Discussion

The research allowed to verify the existence and direct incidence of *T. palmi* with the lowering of the quality of the fruit and mechanical damage leaves and new shoots in the agroproductive farms under study.

Highly significant differences with respect to the values obtained from height of the plants on days 1, 8, 16 and 24 in relation to the comparison of farms.



Author: (Chicaiza, 2017)

Fig. 1. *Treatment To levels of attack severity Trips palmi in the high branch.*

Treatment A Regarding the location where sampling was considered, (Suris and Plana 2001) indicate that the ideal place is the middle stratum of the plants since the individuals (larvae) have better living conditions because they are less exposed to the action of natural enemies and solar radiation, in addition to being at this level leaves, which due to their age, could be more appropriate to guarantee food to this state. The sequential sampling plan (1, 8, 16, and 24) with fixed levels that indicate the presence and affectations of *T. palmi.,* based on the results obtained in ***Fig. 1.,*** related to the spatial distribution of the pest in the plant, the representative state of

its populations and the selection of the sample unit, it is proposed to modify the sampling procedures indicated in the phytosanitary management program for the cultivation of naranjilla in the following aspects: The apical leaflet will be selected as the sample unit, which will be chosen in the middle stratum of the plants and the state to be quantified will be the larva. These elements will be common in any monitoring procedure.

4. Conclusions

Treatment A allows us to take into account the initial behavior of the pest, once it has confirmed its presence in silver and its location in the high branch.

The research proved that by applying the treatments on the farms in the established days, they indicate that the pest is present with greater rigor in the high and low branches, which was verified through the monitoring on days 1, 8, 16, 24 also indicated the direct impact of Trips palmi as a cause of damage to leaves and rolling of young leaves and the direct impact on the quality of the fruit, prior to this investigation the producer associated the symptoms of the pest with intoxications manifested by overdoses of agrochemicals not specialized and even to *trips palmi*

Acknowledgments

To all who made this research possible.

Author Contributions

All authors have the same contribution.

Conflicts of Interest

There is no conflict of interest of the authors.

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Physico-chemical and electrochemical properties of nanoparticulate NiO/C composites for high performance lithium and sodium ion battery anodes

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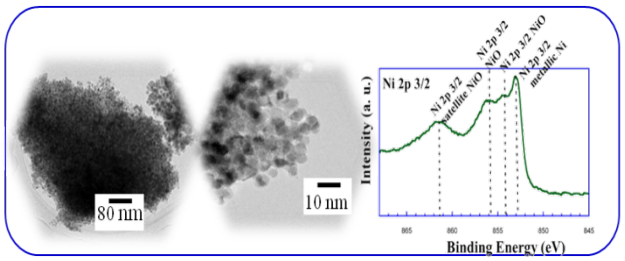
Graphical Abstract



Abstract.

Nanoparticulate NiO and NiO/C composites with different carbon proportions have been prepared for anode application in lithium and sodium ion batteries. Structural characterization demonstrated the presence of metallic Ni in the composites. Morphological study revealed that the NiO and Ni nanoparticles were well dispersed in the matrix of amorphous carbon. The electrochemical study showed that the lithium ion batteries (LIBs) containing composites with carbon have promising electrochemical performances delivering specific discharge capacities of 550 mAh/g after operating for 100 cycles at 1C. These excellent results could be explained by the homogeneity of particle size and structure as well as the uniform distribution of NiO/Ni nanoparticles in the *in situ* generated amorphous carbon matrix. On the other hand, the sodium ion battery (NIB) with the NiO/C composite revealed a poor cycling stability. Post-mortem analyses revealed that this fact could be ascribed to the absence of a stable SEI or passivation layer upon cycling.

Introduction

.As one of the most important and widely used rechargeable power sources, lithium ion batteries (LIBs) have been widely used in portable electronics, electric vehicles (EVs) and hybrid electric vehicles(HEVs)[1 4] .

Additionally, they are supposed to be one of the most promising candidates for next generation power sources. Besides of LIBs, recently, sodium ion batteries (NIBs) have received increased attention as an alternative to LIBs for stationary storage due to the abundance and low cost of Na. Actually, NIBs were initially studied when the development of LIBs began in the 1970s, but due to the fast advances in the development of LIBs, NIBs were unregarded[5]. Even if the fundamental principles of the NIBs and LIBs are almost the same, NIBs usually exhibit low specific capacities, short cycle lifes and poor rate capabilities due to increased radius and mass of Na (1.02Å, 22.99 g/mol) compared to that of Li (0.59Å, 6.94 g/mol) [6]. Additionally, sodium has a higher standard electrode potential compared to lithium (-2.71 V vs SHE as compared to -3.02 V vs SHE for lithium). Consequently, NIBs will often fall short in terms of energy[7]. Nevertheless, the weight of cyclable lithium and sodium is only a small part of the mass of the components of the electrode.

Nowadays, even if graphite is the most widely used anode material due to its low cost, high abundance, and outstanding electrochemical performance, this material exhibits a theoretical capacity of 372 mAh/g. Consequently, in order to fulfill the requirements as to large scale applications, higher energy density systems need to be developed. This purpose implies the necessity of denser and higher capacity anode materials are needed.

In this sense, 3d transition metal oxides (MO_x) are among one of the most promising next-generation anode materials under consideration due to their low cost, high theoretical capacities (500-1000 mAh/g) and easy fabrication [8,9].

NiO has been regarded as one of the most popular choices of metal oxides due to its high theoretical capacity (718 mAh/g), high corrosion resistance and low materials and processing costs[10]. However, further optimization of nickel oxides as anode materials is needed due to their poor capacity retention or rate capability owed to low electric conductivity and large volume change during the conversion reaction[11,12].

Even if transition metal oxides have been extensively studied in LIBs, only a few metal oxides have been studied for application in NIBs [13 , 14] . Among these studies, some previous reports have demonstrated the potential application of NiO in NIBs[15]. Meanwhile, other researchers have revealed the electrochemical inactivity of NiO with Na, while exhibiting outstanding performances in LIBs. In this regard, the reason why this is happening is not clearly understood yet[16]. As far as we are aware, very little research has been done in the field of NiO anodes for NIBs application up to now.

In this study, three different composites based on nanosized NiO and carbon, were successfully synthesized by the freeze-drying method. We report on the structural, morphologic, magnetic, spectroscopic and electrochemical characterization (vs Li and Na) of the synthesized samples, establishing correlations among the composition, morphology and electrochemical performance. Particular attention has been paid to the post-mortem analysis of NIBs in order to understand why the same material behaves differently when applied as anode for LIBs and NIBs.

Materials and Methods

Three nickel oxide samples were synthesized by the freeze-drying method. For the sample designated NiO_air only $Ni(NO_3)_2 \cdot 6\ H_2O$ was dissolved in 25 ml of water. For the other two samples

$C_6H_8O_7 \cdot H_2O$ and $Ni(NO_3)_2 \cdot 6\ H_2O$ reagents were added in the molar ratios of 0.25:1 and 1:1, in order to produce composites with different carbon contents. The resulting solutions were subsequently frozen in a round-bottom flask that contained liquid nitrogen. Afterwards, the round bottom flasks were connected to the freeze-dryer for 48 h at a pressure of 3·10-1 mbar and a temperature of -80ºC to sublime the solvent. The as-obtained precursors were subjected to a single heat treatment at 400ºC for 6h. The heat treatment of the NiO_air sample was carried out in air while the other two samples were calcined in a nitrogen atmosphere. Subsequently, the products were ball-milled for 30 minutes.

A Perkin-Elmer 2400CHN analyzer was employed to determine the carbon content of the samples. Structural characterization of the samples was carried out using X-ray powder diffraction with a Bruker D8 Advance Vario diffractometer using CuKα radiation. The obtained diffractograms were profile-fitted using the FullProf program [17]. The morphologies of the materials were studied by Transmission Electron Microscopy (TEM) using a FEI TECNAI F30 and by a scanning electron microscope (JEOL JSM 7500F) and by Scanning Electron Microscopy (SEM) (JEOL JSM 7500F). Magnetic susceptibility measurements (dc) were carried out at 300K with a Quantum Design SQUID magnetometer. X-ray photoelectron spectra were (XPS) were obtained on a SPECS system equipped with a Phoibos 150 1D-DLD analyzer and a monochromatic AlK_α (1486.6 eV) source. Raman spectroscopy was carried out using a InVia Raman spectrometer using Ar^+ laser excitation with a wavelength of 514 nm.

2032 coin cells were assembled to evaluate the electrochemical performances of the samples. To prepare the electrodes, the active materials were mixed with conducting carbon black (Super P, Timcal) and polyvinylidene fluoride (PVDF) binder with weight ratios of 70:15:15 and dispersed in N-methyl-2-pyrrolidone (NMP) to form a slurry. The slurry was then cast onto Cu current collectors and dried at 120ºC in a vacuum oven overnight. For the lithium ion batteries, electrochemical cells with metallic lithium foils as counter electrodes, Celgard 2400 polypropylene separators and 1 M $LiPF_6$ in 50%-50% ethyl carbonate (EC) and dimethyl carbonate (DMC) as the electrolytic solution, were assembled in an Ar-filled glove box. For the sodium ion batteries, metallic sodium foils were used as counter electrodes. The electrolyte was 1 M $NaPF_6$ in 50%-50% ethyl carbonate (EC) and dimethyl carbonate (DMC) solution with 1 wt % FEC All the electrochemical and electrochemical measurements were carried out on a Bio-Logic VMP3 potentiostat/galvanostat at room temperature. Typical electrode loadings were 1.3 mg/cm2.

Results and Discussion

.Elemental analysis revealed that the samples contained an average amount of carbon of 0, 18 and 29%. Accordingly, the samples were called NiO_18%C, NiO_29%C and NiO_air as this material was calcined in air.

The structural characterization by XRD showed that for the NiO_air sample, all of the diffraction peaks could be indexed to pure phase cubic nickel oxide. No additional reflections were detected indicating the absence of impurities. In the case of NiO_18%C two weak reflections can be detected at $2\theta \approx 45^o$ and 53^o corresponding to metallic nickel (Powder Diffraction File 88-2326 PDF card). However, different from NiO_air and NiO_18%C samples, the diffraction maxima of NiO_29%C composite appears to have less intensity and higher broadening. Additionally, the reflections corresponding to metallic nickel have higher intensity in this sample than in the former ones. This could be attributed to the higher amount of carbon in this sample, as it probably has led to a more reducing atmosphere and consequently, a higher amount of Ni (II) has been reduced to Ni(0).

SEM images allowed asserting that the NiO_air sample is composed of irregularly shaped particles with a wide range of size (5-50 nm). In the same way, NiO_18%C and NiO_29%C composites seemed to contain nanoparticles homogeneously dispersed in the in situ generated carbon matrix. In order to further investigate that morphology, TEM measurements were carried out. **Figures 1a, 1b** and **1c** show transmission electron micrographs of the NiO_air, NiO_18%C and NiO_29%C samples. It can be deduced that the NiO_29%C composite is made up of 5-10 nm homogeneous spherical nanoparticles embedded in the in situ generated carbon matrix. The particle size of NiO_29%C sample was the smallest of all the samples as the high amount of carbon in this composite acted preventing the growth of particle size.

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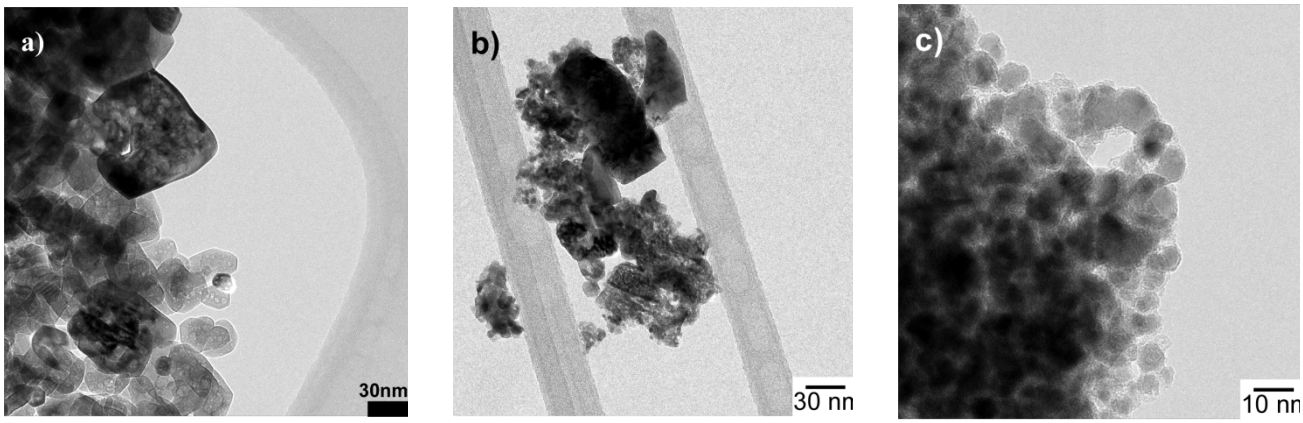

Figure 1.TEM images of a) NiO_air, b) NiO_18%C and c) NiO_29%C samples.

The magnetic hysteresis loops at room temperature of the NiO_air, NiO_18%C and NiO_29%C samples exhibited that the samples contain <1, 5 and 41% of metallic nickel, respectively. On the other hand, Raman spectroscopy measurements showed that NiO_18%C and NiO_29%C samples have a typical Raman spectrum of non-graphitic carbons. Both of them show two pronounced peaks, one located at ≈1600 cm^{-1} which corresponds to the G-band and is ascribed to the E_{2g} graphitic mode. The other band located at ≈1340 cm^{-1}, D-band, corresponds to a defect induced mode[18]. Thus, the presence of the D band indicates that the in situ generated carbon is a typically non-graphitizable carbon.

To evaluate the electrochemical performance, lithium half-cells containing NiO_air and NiO_18%C and NiO_29%C composite materials were discharged at current densities corresponding to C/10 and 1C rates.

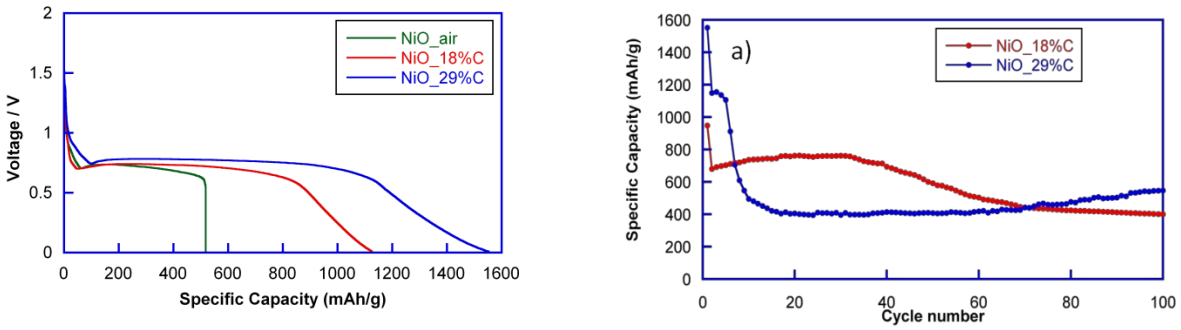

Figure 2. First discharge curves for NiO_air, NiO_18%C and NiO_29%C at C/10 and cyclability of the samples

As it can be seen, NiO_29%C composite is the one that shows the best electrochemical performance as it has a smaller particle size, a more homogeneous appearance and higher carbon and metallic nickel

contents. Due to the synergistic effect that these factors could produce, the electrochemical behavior of NiO_29%C is better in all aspects.

NiO_29%C composite was selected to test it versus metallic sodium due to its good lithium storage behavior. **Figure 3** shows the first two discharge-charge curves of NiO_29%C versus metallic sodium at C/10. As it can be observed, the capacity drastically decays from the third cycle on.

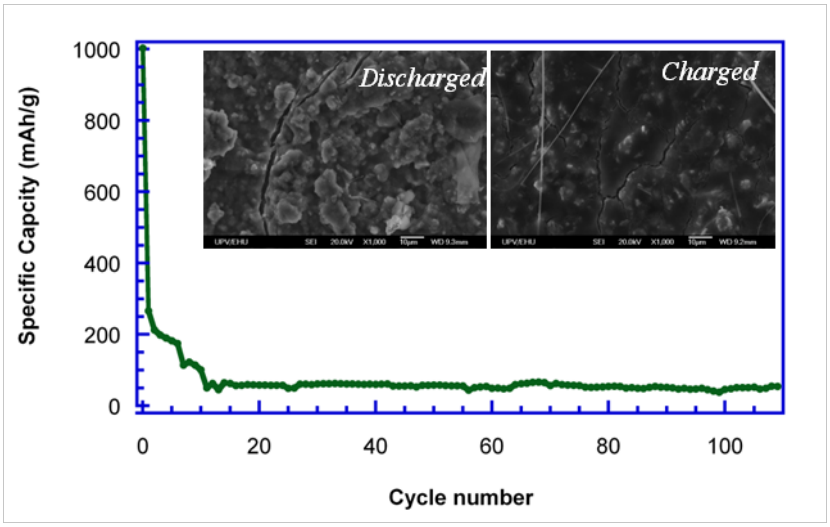

Figure 3. Cyclability of NiO_29%C sample and SEM micrographs of the discharged and charged electrode.

In order to investigate the origin of the capacity fade, a post-mortem study of the sodium half cells containing NiO_29%C was performed. Post-mortem analyses (ex.situ XRD, SEM, magnetic measurements, XPS and FTIR) revealed that the capacity decay could be mainly ascribed to the absence of a stable SEI upon cycling.

Conclusions

. NiO_air, NiO_18%C and NiO_29%C samples were successfully prepared by a freeze-drying method. X ray diffraction measurements for NiO_air sample showed that all of the diffraction peaks could be indexed to nickel oxide. For NiO_18%C and NiO_29%C, metallic nickel was detected as well as nickel oxide. The morphologic study demonstrated the heterogeneity of NiO_air sample with an average particle size of 5-50 nm. However, NiO_18%C and NiO_29%C are more homogeneous, have smaller particle size and present an in situ generated amorphous carbon matrix. The most significant result was the reduction of particle size with the increasing of carbon amount. Magnetic measurements allowed calculating the amount of metallic nickel for each sample. NiO_air, NiO_18%C and NiO_29%C samples were employed in LIBs and NiO_29%C composite was the one with the highest specific capacity, best cycleability, highest coulombic efficiency and best rate discharge capability. This fact could be ascribed to the higher amount of metallic nickel and carbon, the smaller particle size and the homogeneous character that this sample has in comparison to the other materials. On the other hand, the NIB with the NiO_29%C composite revealed a poor cycling stability. Post-mortem analyses (ex.situ XRD, SEM, magnetic measurements, XPS and FTIR) revealed that this fact could be mainly ascribed to the absence of a stable SEI upon cycling. In this regards, the surface reaction that occurs when discharging (reduced carbon) and charging ($NaCO_3R$) the electrode, implies a huge volume expansion causing the fracture of the electrode and leading therefore, to a poor electrochemical performance of the NIB. Additionally, the large amount of carbon that NiO_29%C

composite contains is another important factor to be considered since the storage of Na into carbon is very limited. Consequently, the diffusion pathways could be blocked promoting the deterioration of the kinetics of the conversion reaction.

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http://sciforum.net/conference/mol2net-03

Comments on Tetracarbonyl(pyrrolylimine) Complexes of Rhenium

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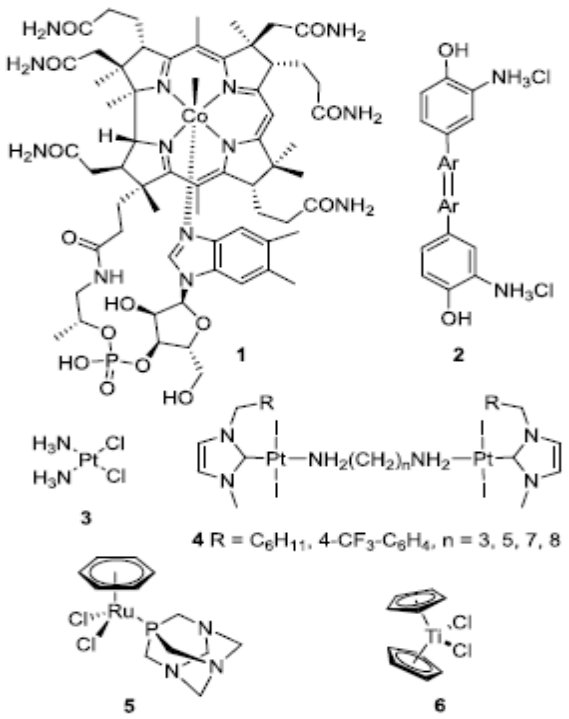
Graphical Abstract



Abstract. The synthesis of tetracarbonyl (pyrrolylimine) complexes of rhenium bearing chirality on the pyrrolyl ligands was reported. The reactivity of these compounds towards the substitution of one carbonyl ligand with triphenyl phosphine, tricyclohexyl phosphine and trimethyl phosphite was studied. The rhenium becoming a stereogenic center in that transformation, the resulting tricarbonyl species were obtained as mixtures of diastereomers, with diastereomeric excesses varying from 8 to 84%, according to the reaction conditions and the relative steric hindrances of the pyrrolylimine and the organophosphorus ligands.

Reference:

Curr Top Med Chem. 2017 Jul 19. https://www.ncbi.nlm.nih.gov/pubmed/28730956

Use of novel metallic nanoparticles for improvisation of electrical properties of PVDF-HFP polymer film

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Abstract

Nanomaterials of compound of copper and arsenic were formed in alcohol in a novel way. Incorporation of these materials by simple solution casting technique in poly vinylidene fluoride-co-hexafluoropropylene (PVDF-HFP) polymer films significantly improves their electrical properties.

The incorporation of the nanomaterial in the polymer matrix activates the transition of phase between α and β. This provides higher mobility of the charge carriers which participate in the interfacial polarization. These films have higher dielectric constant, ac conductivity and lower dissipation factor (tanδ) at room temperature, compared to the pure PVDF-HFP.

These nanocomposites are easy to fabricate and environment friendly and their presence in polymer matrix to get enhanced electrical properties will have a significant contribution in the present day research in electronics.

Poly(vinylidene fluoride) (PVDF) and its co-polymers like poly(vinylidene fluoride-hexa-fluoropropylene) (PVDF-HFP) are in great demand for their versatile and unique properties like flexibility, low processing temperature, low dielectric constant, high dielectric breakdown field etc., making them potential candidate for a broad range of applications in electronic industry.

In order to improve the capacitive performance of the polymer material, a great deal of effort had been devoted to develop PVDF-HFP composites by incorporating different metallic nanoparticles within the matrix. The effective dielectric permittivity of these metal nanoparticles (NPs) doped polymer composite are higher than that of the host polymer matrix. They also show enhancement of conductivity and decrement of tangent loss making them potential candidates as good capacitors and electric energy storage devices.

The compound of Copper and Arsenic (Cu-As) at different potencies were obtained from Hahnemann Publishing Company, India. The NP aspect of these potencies has been proved experimentally. The advantage of this method is that in this way the shape and size of the NPs can be regulated by the method of preparation. Moreover, these NPs are eco-friendly, inexpensive, nontoxic, easily available metallic nano fillers which can enhance the dielectric constant, conductivity and decreases the dissipation factor of polymer films by many folds.

These NPs were incorporated in the polymer PVDF-HFP (Sigma-Aldrich, USA) in dimethyl-sulfoxoide solution (Merck, India) and films were prepared by simple solution casting technique. In a typical synthesis, 100 mg of PVDF-HFP was added to 2 ml DMSO and mixed together under vigorous stirring at 60 °C for 3 h. Measured amount of Cu-As at different potencies were added to the solution. The volume wt% of all prepared NPs of different potencies is 23.08%. Cu-As PC were obtained by casting the whole mixture in clean dry petri dishes and evaporating the solvent in an incubated oven at 60 °C for 12 h. The films were then coated by silver paste on both sides for electrical measurements. The synthesized films had the thickness in the range of 48–54 μm as measured by using a digital screw gauge. The average density value of the fabricated films is 0.81 gm cm^{-3}.

These nanocomposites of PVDF-HFP/Cu-As (Cu-As PC) were studied by EDX (Fig.1). The enhancement of dielectric constant and conductivity and decrease of tangent loss of the nanocomposite films were observed by changing the potency and the observed values were compared with the pure PVDF-HFP film.

At lower frequency the easy orientation of dipoles as well as interfacial polarization contribute enhancement in dielectric constant (Fig. 2). As the frequency is increased further, dipole response is restricted and the dielectric constant has a saturation tendency. In this case, the internal individual dipoles contribute to the dielectric constant which is ideally the electronic polarisation effect (1).

Throughout the frequency range, tangent loss continuously decreases with increasing frequency for all films (Fig. 2). This rapid decrement may be due to intermolecular friction or vibration. At lower frequency range the easy orientation of dipoles for higher relaxation time contributes to higher tangent loss. As frequency increases, less polarization effect continues due to less relaxation time. So intermolecular friction or vibration diminishes which is responsible for saturated tangent loss. On the other hand after 100 KHz the tangent loss increases for all films which may be due to leakage current.

The ac conductivity increases exponentially with frequency for all the nanocomposite films (Fig.2). This increase in conductivity with frequency arises due to the presence of free ions in the polymer matrix that may increase the mobility of the ions which finds an easy path to move and hence increase the electrical conductivity. It has also been observed that the value of ac conductivity is higher for all nanocomposite films compared to the pure polymer film. The increase of $\sigma_{a.c}$ is due to the increased aspect ratio of metal ions (1).

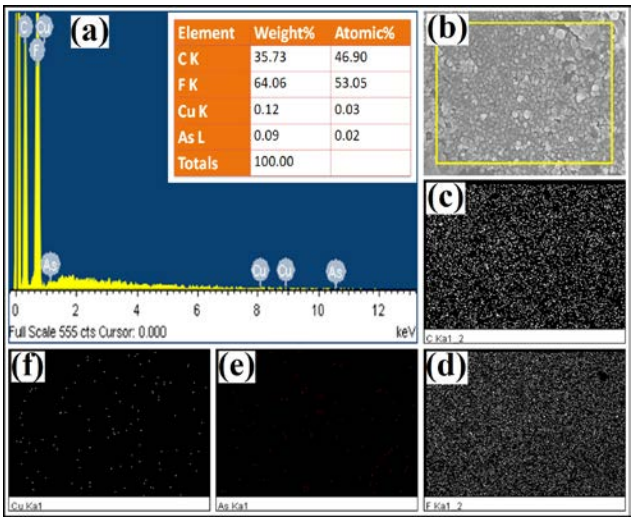

Figure 1. EDX analysis and elemental mapping of Cu-As PC

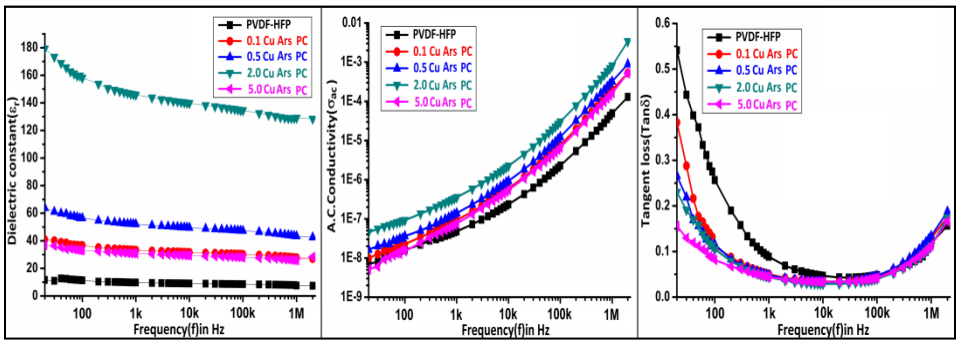

Figure 2. Electrical characteristics of Cu-Ars compound

We conclude that pure polymer films which have comparatively low dielectric constant can be modified into materials with enhanced dielectric constant and comparatively low tangent loss by making a composite with specially devised metal NP and hence can be a promising candidate for the fabrication of high charge- storing multilayer capacitors.

1. A. L. Gayen, D. Mondal, B. K. Paul, D. Roy, P. Bandyopadhyay, S. Manna, R. Basu, S. Das, D. S. Bhar, P. Nandy
Improvisation of electrical properties of PVDF-HFP: use of novel metallic nanoparticles
2017 J. Material Science: Materials in Electronics 28:14798–14808

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Standardization of commercial cinnamon essential oils by gas chromatography-mass spectrometry analysis

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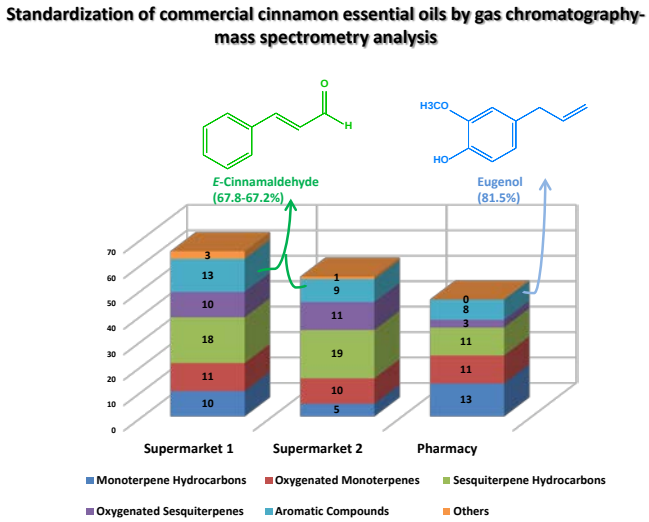
Graphical Abstract



Abstract

The chemical composition of seven *Cinnamomum zeylanicum* Blume essential oils traded as spices and medicinal items has been determined by gas chromatography-mass spectrometry analysis. Eighty-two compounds accounting for 95.39-99.03% of the total essential oil were identified. Qualitative and quantitative differences were found in the essential oils obtained from dried and powdered cinnamon bark purchased at supermarkets and cinnamon leaf essential oil from a pharmacy. The aromatic compound *E*-cinnamaldehyde (67.84±3.15%; 67.16±5.05%) was the principal component of the essential oil in commercial cinnamon bark employed as a spice; whereas eugenol was the main compound (81.51±0.21%), in commercial cinnamon leaf essential oil for medicinal purposes. The qualitative and quantitative differences in the analyzed essential oils can affect the organoleptic properties, mainly the spice's flavor as well as the pharmacological properties of the cinnamon (bark and leaf) essential oils.

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Direct spectrophotometric method to determine cell density of *Isochrysis galbana* in serial batch cultures from a larger scale fed-batch culture in exponential phase

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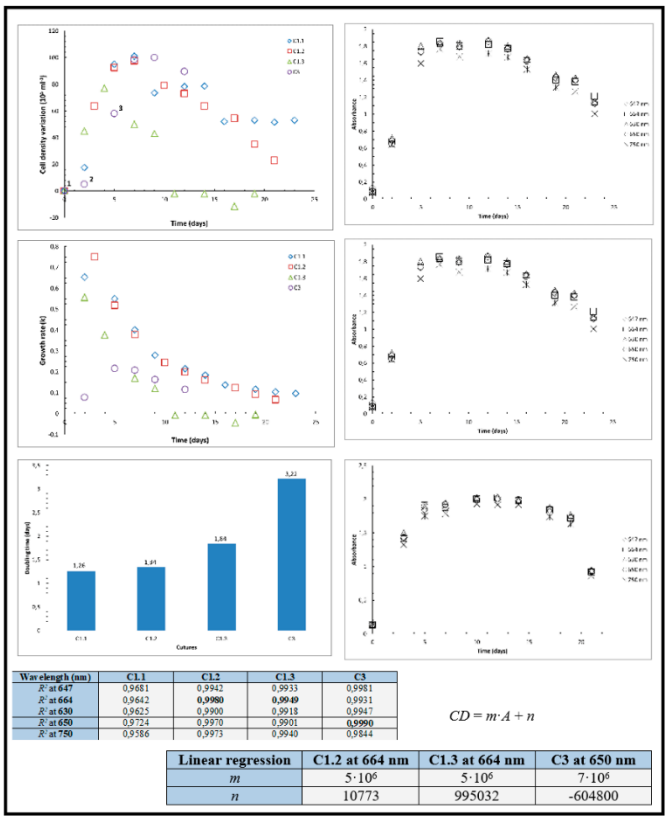
Graphical Abstract



Wav elength (nm)	C1.1	C1.2	C1.3	C3
R^2 at 647	0,9681	0,9942	0,9933	0,9981
R^2 at 664	0,9642	**0,9980**	**0,9949**	0,9931
R^2 at 630	0,9625	0,9900	0,9918	0,9947
R^2 at 650	0,9724	0,9970	0,9901	**0,9990**
R^2 at 750	0,9586	0,9973	0,9940	0,9844

$$CD = m \cdot A + n$$

Linear regression	C1.2 at 664 nm	C1.3 at 664 nm	C3 at 650 nm
m	$5 \cdot 10^6$	$5 \cdot 10^6$	$7 \cdot 10^6$
n	10773	995032	-604800

Abstract.

In this work, a very useful and accurate procedure, based on the spectrophotometric method published by the American Public Health Association in the Standards Methods for the Examination of Water and Wastewater, was developed to determine cell density of *Isochrysis galbana* performing a single direct absorbance measurement in exponential phase of growth, which is the desirable operating mode for any microalgae production plant. Thus, *Isochrysis galbana* was cultured in serial batch cultures from a larger scale fed-batch culture. The growth performance of this species of microalgae under laboratory conditions was analysed by spectrophotometry at different wavelengths and cell counting in a haemocytometer (Neubauer chamber) showing that doubling times and cell death increased with increasing initial cell density. Besides, it was demonstrated that the absorbance of these cultures followed a linear trend as a function of time and cell density during the exponential phase of growth, results in which the developed direct method is based on.

https://www.ucv.es/investigacion/publicaciones/catalogo-de-revistas/revista-nereis (NEREIS Nº9)

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MOL2NET, International Conference Series on Multidisciplinary Sciences
http://sciforum.net/conference/mol2net-03

Enhancement of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)'s properties for advanced industrial applications

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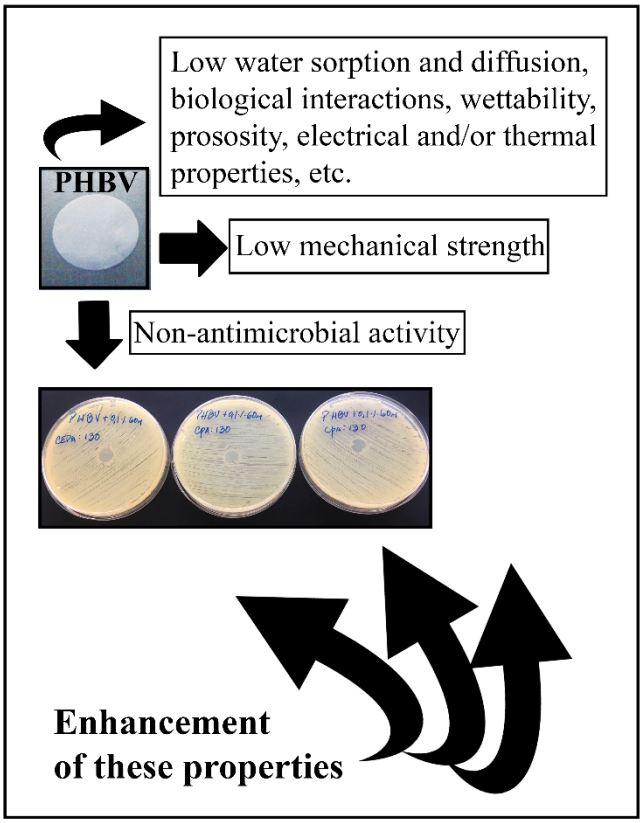
Graphical Abstract



Abstract.

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) is a very promising biodegradable polymer from the family of Polyhydroxyalkanoates (PHAs) with many potential applications in many important industrial fields such as biodegradable packaging, synthetic prosthesis, therapeutic delivery, wound dressing, 3D tissue scaffolds for tissue engineering, etc. due to their excellent biocompatibility, non-toxicity, and suitable large-scale industrial production. However, many of its potential uses required for these applications often are hindered by their low mechanical strength, non-antimicrobial activity, low water sorption and diffusion, biological interactions, porosity, electrical and/or thermal properties, among others. Thus, new advanced PHBHV-based composite materials have been developed as multicomponent systems in the form of composite or nanocomposite materials, which are expected to exhibit superior properties to increase the potential uses of these materials. Even though the great advances achieved so far, much research has to be conducted still in order to find new strategies to fabricate novel materials able to overcome many of these problems.

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MOL2NET, International Conference Series on Multidisciplinary Sciences
http://sciforum.net/conference/mol2net-03

Rural community's territorial system dynamics at the Anzu river valley in the Amazon territory to propose change scenario

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Graphical Abstract

Kichwa community's

Mestizos and *Kichwa*

Jungle and animal protection

Alliances and synergies

Abstract.

A *Kichwa* community's dynamics at the river Anzu valley, in the Ecuadorian Amazon territory, to propose change scenarios is analyzed. The Anzu valley, populated by sustainable mestizos and *Kichwa* communities, autonomous, in harmony with nature, visible in the different products that they process from environment resources, live in their legalized territories, where government entities recognized the *Kichwa* ancestral property. The *Kichwa* achieved to articulate strategies for the biodiversity resources use, whose beneficial repopulation and processing may allowed obtaining their maintenances, the payment of their labor and income families. The knowledge system and the people's cultural identity in social harmony with nature are revalued. The territory possession and its security, together with the ancestral knowledge, management systems understanding of the environment resources, which come from a cultural tradition, wisdom in the jungle associated with new technological forms that provide economic income through the local resources processing, gives communities the opportunity to renew their pride, which contributes to the consistent use of their environment. It recovers and intensifies the agro ecosystem management, gradually increases the promissory species sowing and repopulation, animals and plants. The desired scenario implies a sustainable, autonomous functioning, with alliances and synergies.

Keywords: *Kichwa* communities, Amazon territorial systems, sustainability.

Introduction

In the world, indigenous peoples have lived on the nature products, many peoples migrate when the area resources are depleted, until the natural environment is regenerated and can be used again. Indigenous peoples' livelihoods are altered by processes related to development, political decisions, natural resources exploitation, mining, urbanization, modernization, infrastructure development,

climate change and global warming (Dublin & Tanaka, 2014; Akerlof *et al*., 2010). As a plurinational-intercultural rights & justice State, Ecuador is made up of 15 original nations or indigenous nationalities, as well as Afro-Ecuadorian populations, montuvias (peasants from the coastal region), white and mestizo people (Constitución de la República del Ecuador, 2008).

There is now an international trend towards the indigenous people's collective rights recognition, whose main claims are the land recovery from which they were dispossessed; the collective rights to own, develop, use, control and occupy their lands and traditional resources involve the integrity and conservation of their natural habitat, including environmental protection. Some Latin American countries have made significant progress in this regard, through constitutionally recognizing the indigenous people's legal personality, which has not been possible in other countries. The right to respect, integrity and conservation of habitats and natural resources are closely linked to the indigenous peoples and environmental protection (Gaona, 2013).

The Amazon western portion, including Ecuador, is one of the most bio diverse areas on the planet, home to several indigenous peoples, it maintains intact portions of jungle and it has a high probability of stabilizing climate conditions face to global warming problems (Smith & Leiserowitz, 2014; Gainette, 2009).

In the present evolution analysis researching from Amazon community's development and its consequences in the Ecuadorian Amazonian territory, it is contextualizing the original indigenous nation territory of Ecuadorian Amazonian *Kichwa* and the conceptualizing of the change scenarios pertinence. The aim was to analyze a *Kichwa* community's dynamics at the river Anzu valley, in the Ecuadorian Amazon territory.

Materials and Methods

Six communities located in the ancestral territory of the original Ecuadorian *Kichwa* Amazonian nation are studied, from Northeaster Tzawata, until the Southwester Veinticuatro de Mayo communities (table 1), located in the low, medium and high levels of the Anzu River valley; the communities settled since the end of the 19th century, come from the Napo province. They are rural communities, representative of a common origin and express a vulnerability gap in relation to the initiatives developed by the local governments and the planning units.

Table 1. Location of selected communities in the study.

Community	Z [a]	Altitude	North	East	Parish	Canton	Province
Tzawata	18	508	9 869 264	179 523	Carlos Julio Arosemena	Carlos Julio Arosemena	Napo
Wayuri	17	1200	9 862 500	831 306	Mera	Mera	Pastaza
Flor de Bosque	18	752	9 858 789	170 876	Santa Clara	Santa Clara	Pastaza
Boayaku	18	808	9 854 478	168 082	Teniente Hugo Ortiz	Pastaza	Pastaza
Unión de Llandia	18	1099	9 851 931	169 383	Teniente Hugo Ortiz	Pastaza	Pastaza
Veinticuatro de Mayo	17	994	9 845 509	832 473	Fátima	Pastaza	Pastaza

[a] Universal Transverse Mercator South zone

The studied communities are located from 508 to 1 200 meter over sea level, in the eastern foothills of the central Andes, south of the sub-Andean zone identified as Napo Uprising; on hydromorphic alluvial soils with a forest vocation, volcanic origin and conservation purposes; soils formed by the dejection cones in the Amazon foothills, as a result of the fusion of plio-quaternary glaciers and volcanic and seismic activity (Senplades; Instituto Geográfico Militar, 2010). Rainfall is never less

than 4 000 mm per year and exceeds 6 000 mm per year; the average temperature is set between 20 & 24 ° C; the climate is tropical humid; the topography corresponds to broken and collapsed terrain, with slopes varying from relatively flat terrain in the lower areas to inclinations of 70 degrees or more at higher altitudes. Landscapes prevailing are permanent humid tropics (González and Salinas, 2010). The life area corresponds to pre-montane rainforest (Cañadas, 1983); plant formation is always Piedmont green forest (Sierra, 1999). The region contains just 5.06% of the national population, with densities from 2.82 to 15.38 inhabitants per km^2. The research goes through surveys to the families and their leaders, interviews to former settlers, and discussion in nine workshops.

Results and Discussion

The *Kichwa* communities have the *chakra* system installed in primary or secondary forest to take advantage of the organic fertility of the forest, it's cultivated for several years and is nourished with the system of *ushun* (crop regeneration); when it loses fertility, it becomes in *purun* containing the remaining planted species: fruit, medicinal, forest, like the *ayllu's* (family lineage) and the communities' work testimony, footprints in the territory for recognition of limits. This indigenous system in the vast Amazonian territory remains imbricated with the environmental system and provides subsistence sources and benefic species. According Escobar (2002) biodiversity conservation is only ensured with local cultures and communities focus. *Kichwa* culture is prevalent in the communities, with their systems of treatment of the environment, farms, cultural ceremonies of healing and evidences of the ancestral territories in petroglyphs.

The indigenous communities said that the jungle or *Pachamama* is their pharmacy, supermarket, school, security system and origin of power. Although they maintain large amounts of forests and compensation programs exist as "forest partners", they did not receive payments in cash or others for the environmental system maintenance, water protection, biodiversity conservation, Oil Company's compensation or mining companies, reforestation, agroforestry or any other. Only the Tzawata community received in 2012 a tourism group interested in learning about life in the community, from its own reality. It was investigated the benefit that the environmental system provided to families, by extracting products for domestic use and for the market, assigning a monetary quantification for each element used; for example for wood to cook, considered a free service of the forest, the family estimated an amount or cost in money on the assumption of how much can pay or how much can be sold the fuel wood unit. Table 2 indicates the products extracted from the forest in 2012 and their quantification in money; it is a quantification of the goods or products they use, although they do not mean cash income in all cases for all families.

Table 2. Products of the forest used in 2012.

Community	Forest products, medicinal, other uses, wild animals.	Annual familiar Income ($)
Tzawata	Fine woods of chuncho, ahuano, cedar, laurel; fibers; barks and medicines, leaves, guayusa, cat's claw; guanta; edible fruits of hungurahua.	520
Wayuri	Wood to cook; chonta edible fruit; Chugchuhuazo medicine and Guayusa.	215
Flor de Bosque	Cinnamon and laurel fine woods; soft pihue wood; wood to cook; animals armadillo, guatusa, guanta; chambira and pita fibers; turmeric and ishpingo species; cat's claw medicine, drago blood.	533
Boayaku	Cinnamon, chuncho and laurel fine woods; soft pihue wood; sajino; chambira fiber; mushukhuan medicine.	960
Unión de Llandia	Canelo hardwood; soft pihue wood.	813
Veinticuatro de Mayo	Canelo hardwood; soft pihue wood; wood to cook; palmito; guatusas; monkey	160

Land, forest and water are more than a resource for peasants and indigenous people, it is relevant as a subject, part of its culture and identity; any local development project considered within the framework of a strategy must be oriented towards the biodiversity conservation, support the production of communities beginning with traditional systems research in their orientation both to the market and to the domestic economy. The territorial system under study has great biodiversity, not intensively occupied according to its morphology, becomes a priority area for establishing local development projects, but these can't ignore the intrinsic values associated with biodiversity, such as the principles of local autonomy, knowledge, identity and economy (Escobar, 2010).

Table 3 presents the synthesis of environmental system strategic diagnosis, with the structural and conjuncture keys that define the current territorial model. However, it is necessary to emphasize that structural keys generalization based on the environmental system strategic diagnosis does not mean that the impacts and responses are common to all communities, which is evidenced in the differences reported in the variables analyzed in the surveys. In the knowledge axis, economy and food sovereignty, the action lines include the systematization, recovery and socialization of values and the management and conservation forms of the territory and its resources; modeling the dynamics of the local, family, community and market economy (Coraggio, 2013, Figueroa, 2013); the definition of symbolic action forms, values and new economic practices in relations within the communities and in front of the market. In this axis the possibility of undertaking agro ecological, plant therapeutic, tourism (Tuncay, 2013), heritage recognition and social, cultural and demographic studies can be evaluated, in a strategic relationship with the Amazon State University.

Table 3. Environmental system strategic diagnosis

Structural keys	
Potentials	**Weaknesses**
- Key position in the *Kichwa* ancestral territory, near services axis. - Plant cover compatible with assigned conservation vocation. - Richness landscapes, biodiversity and associated knowledge of species uses, fine and soft woods, fibers, medicinal plants, foods, rituals, cosmetics, wild animals and natural fishing. - Roads network do not reach all communities that does not allow the colonizing possession ambition over the territory.	- There is a lack of legality and there is uncertainty about the access scope, travel and use of the ancestral territory. - It is a weak existing resources use in landscapes and biodiversity. - Legal and illegal mining incursion. - Road network does not reach all communities, which makes it difficult to trade.
Short-term keys	
Threats	**Opportunities**
-Existing landscapes and resources degradation, due to contamination -Species extinction due to overuse, bad use, natural climate change and associated with contamination farms.	- Sustainability and change axes declaration of productive matrix in the national plans of development for the good life in Ecuador. - Global alarm for the biodiversity conservation.

Conclusions

Products extracted in the communities from the forest in 2012 and their quantification in money do not mean cash income by these ones, but jungle does offers products to families for domestic or merchant use. Communities with more knowledge that conserve jungle, have *Kichwa* tradition and that are near ways have more income than those that have minor jungle resources and those that are far away from

the roads. The reduction of the resources of the forest and the reduction of the cultural tradition causes scenarios of change toward the greater commodification of products.

Structural keys generalization based on the environmental system strategic diagnosis revels potentials use from the own jungle and local people knowledge, but it is possible replant richness resources that give incomes to families; however, the structural keys generalization not mean that the impacts and responses are common to all communities, which is evidenced in the differences reported in the variables analyzed in the surveys. Change scenarios needs too the strategic collaboration among communities, university and development actors.

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VARIATION OF CONCENTRATION OF SOLUBLE SOLIDS AND AGRICULTURAL YIELD WITH ORGANIC FERTILIZATION IN THREE VARIETIES OF SUGAR CANE (*SACCHARUM SP HYBRID*) FOR THE PRODUCTION OF PANELA IN THE CONDITIONS OF THE ECUADORIAN AMAZON

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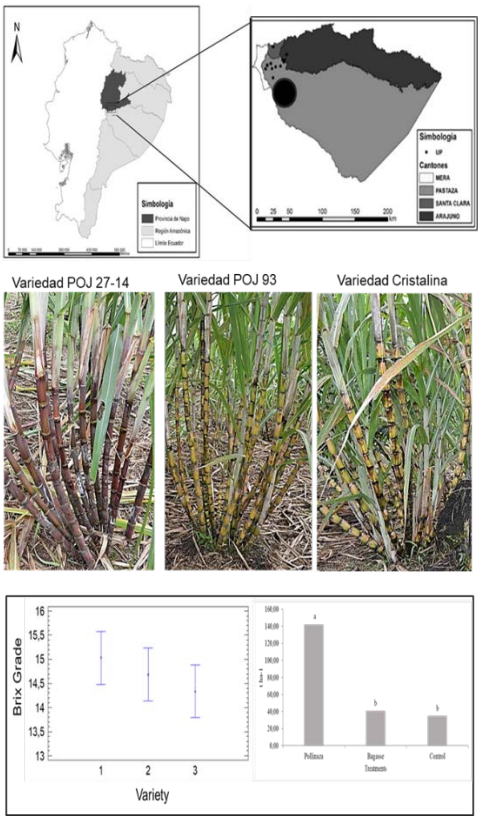
. Graphical Abstract



Abstract

The cultivation of sugarcane is an agricultural activity of great socio-economic importance in Ecuadorian Amazon. The study was carried out in the sugarcane production area of the municipality of Simon Bolivar, Pastaza province, Ecuadorian Amazon Region. We used a factorial design in random blocks with three replicates for a total of 27 experimental plots. The study factors were the varieties of sugarcane identified as: POJ 27-14, Crystalline and POJ 93 and two types of organic fertilizers: Pollinaza and sugarcane bagasse at a dose of 9060 kgha^{-1} compared with an absolute control without application of fertilizer. The analysis of combined variance and the Tukey's mean test with a significance level of 5% was performed to detect differences in mean between treatments. The results suggest that the concentration of sugars expressed in brix degrees did not show significant differences between the varieties, but it did show differences between the types of fertilization, being higher with the application of pollinaza with values of 15.9 degrees brix. The agricultural yield was affected according to the variety, registering higher values with variety POJ 27-14 with a yield of 107 t ha^{-1} in comparison with the other two varieties, which showed statistically similar yields.

Keywords: sugarcane, varieties, brix degrees, agricultural yield, Ecuadorian Amazon.

Introduction

The cultivation of sugarcane is an agricultural activity of great socio-economic importance in the world and in America was introduced by Christopher Columbus on his second trip to the continent. Sugarcane is an exhaustive crop and depletes the soil nutrients heavily. Continuous sugarcane cropping with the use of only inorganic fertilizers has led to depletion of essential available nutrients beside organic carbon in the soil (Kumar and Cham, 2013). In Ecuador, it is possible that the cultivation of the panela cane was brought from Colombia, a little before the middle of the sixteenth century, to settle in the hot valleys of the Interandina Region and in some sectors of the Litoral, to later be located in the Eastern and Western foothills of the Andes (Suquilanda, 2004).

In Ecuador this crop constitutes a relevant sector of the economy, since 20% is destined to the panela production and 80% of the total planted area is destined for the sugar production and ethyl alcohol from the juice of cane.

In Ecuadorian amazon region there are provinces that stand out for having sugarcane crops and panela production is one of the main agricultural activities of Pastaza province (ASOCAP, 2012). However, in this province there is not sufficient information on the productive characteristics (agricultural yield and brix degrees) of the different varieties of sugar cane that are grown, as well as the effect of organic fertilizers on this crop. The genetic improvement of sugarcane for panelera agroindustry is oriented to the substitution of genetic materials with low productive potential, through the introduction and evaluation of high yielding varieties and good agroindustrial performance, adaptable to the management conditions of the panela zones (López-Lopera and Tamayo Velez, 2017).

In this context, the need arises to study the agricultural behavior of three varieties of sugarcane, identified as: 1) POJ 27-14; 2) POJ 93 and 3) Crystalline, in the climatic conditions of the Simon Bolívar parish, Pastaza province with the application of organic fertilizer.

Materials and Methods

The investigation was carried out in the "San Carlos" farm, located in the Oswaldo Hurtado enclosure of the Vía Puyo - Macas km 29 belonging to the Simon Bolívar parish of the Pastaza province (figure 1), at a height of 1071 masl, with an average temperature of 29.5 °C, precipitation of 3000 to 4000 mm per year, relative humidity of 86% with the following geographic coordinates: 18184794E and 9817282N.

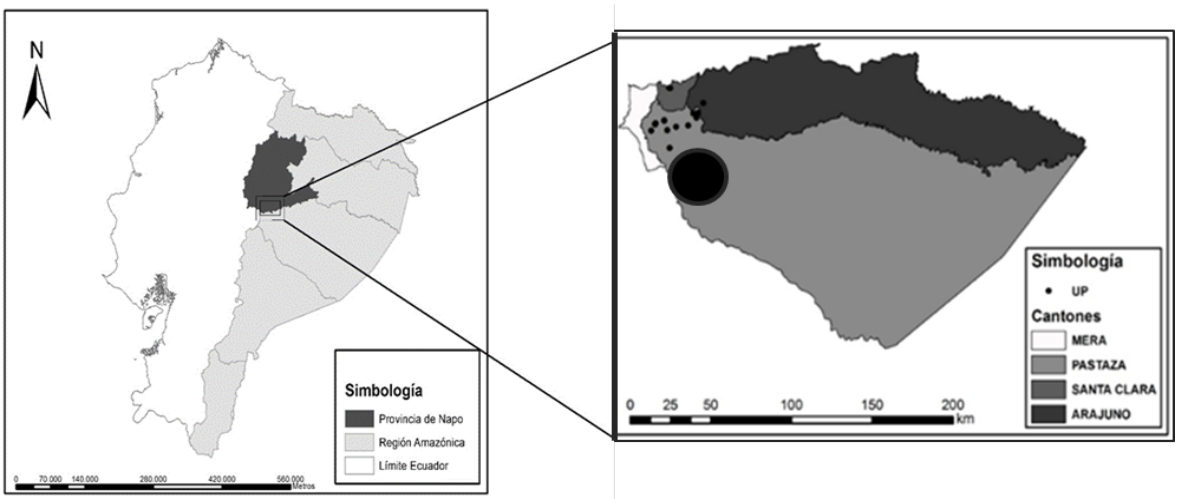


Figure 1: Map of the province of Pastaza. a) Sector where the experiment was developed.

Three varieties of sugarcane (POJ 27-14, Cristalina and POJ-93) and three levels of fertilization were studied, which consisted of two organic fertilizers, poultry litter and bagasse plus a control where fertilization was not carried out. A factorial design was carried out in randomized complete blocks, combining 3 varieties by 3 levels of fertilization (3 x 3). Hence, 9 treatments and three replications were generated for a total of 27 experimental plots.

The Brix Degrees were determined with the aid of a refractometer according to Osorio, 2007 and agricultural yield in t ha^{-1} for which the molly stems of two seedlings (in 3 m^2) were cut and weighed in a balance, to later calculate the yield on a hectare scale.

An analysis of variance was applied to the variables studied and the Tukey test was used to determine differences between the means for the level of significance of ($P < 0.05$). When there was no interaction between the factors, they were studied independently.

Results and Discussion

Concentration of sugar according to varieties and organic fertilization

Figure 2 shows that there is no statistical difference between the varieties in relation to the concentration of sugars, expressed in brix degrees. This may be due to the fact that sugar concentrations are highly influenced by the prevailing climatic conditions in the stage of maturity of the crop, mainly the temperatures and humidity that were the same for the three varieties; however, for the fertilizer factor there is a statistical difference between the poultry litter with the bagasse and control (figure 3), being the value of 15.9 obtained with the larger poultry carcass. There is no statistical difference between the bagasse and the control.

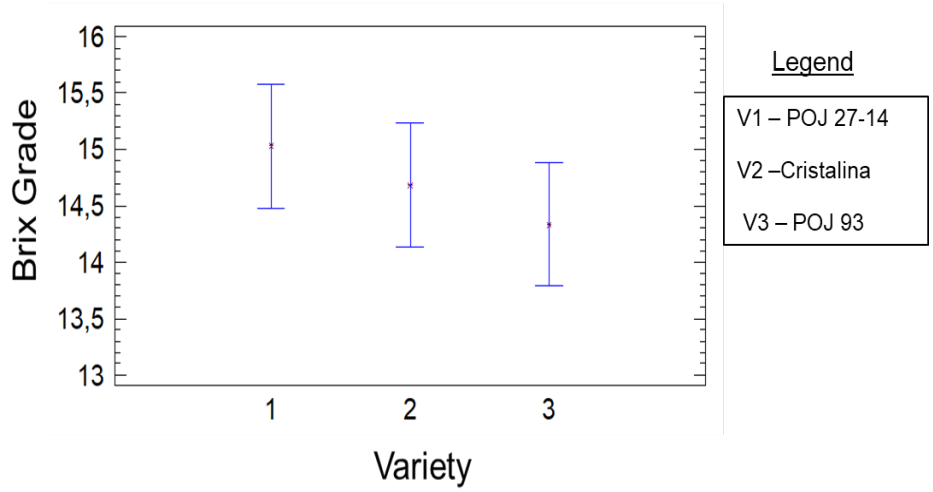


Figure 2. Brix grades in sugarcane according to varieties 300 days after planting. Simón Bolívar-Pastaza Parish. (Tuckey $p < 0.05$)

Patiño (2011), reports for the Cristalina variety an average of 14.20 degrees Brix at harvest age. These values are similar to those of our research, where a value of 14.7 is obtained (figure 2). Hernández et al., 2008, did not find significant differences in the yield of stems and the quality of the juices was not affected, when comparing vinasse, cachaza and chemical fertilization. On the other hand, Arreola-Enríquez, et al., (2004), explain that the quality of the juice (degrees Brix, sucrose, purity and fiber) showed no effect between the treatments, so it can be deduced that with 10 and 15 t -1 of organo-mineral

fertilizer of cachaça it is possible to increase the yield of sugarcane without affecting the quality of the juice.

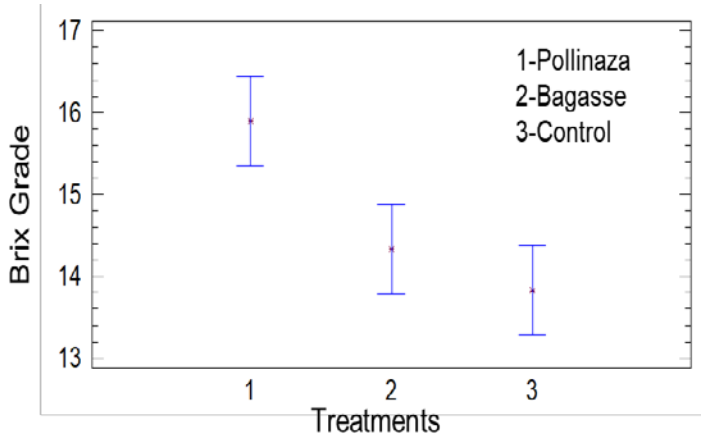


Figure 3. Brix grades in sugar cane according to organic fertilization 300 days after planting. Simon Bolívar-Pastaza Parish. (Tuckey p <0.05)

Variation of agricultural yield according to varieties.

Figure 4 shows the average yield in t ha^{-1} of the varieties POJ 27-14, Cristalina and POJ 93 without considering the fertilization. It can be seen that in POJ 27-14 a yield of 107 t ha^{-1} is obtained, which is statistically higher than that obtained in the other two varieties, followed by the Cristalina variety, and without statistical differences between Crystalline and POJ-93. Patiño (2011) for the Cristalina variety obtained an agricultural yield of 78.35 t ha^{-1} in the Morona Santiago province, whose was higher than the 59 t ha^{-1} of our research. On the other hand, the variety POJ 27-14 with an average of 107 t ha^{-1} was superior to the results obtained by Garcia (2006) in the variety POJ 28-78 with 88.4 t ha^{-1} of yield. Also the results of the variety POJ 93 were superior with yield of 51 t ha^{-1} to those reported by ASOCAP (2012), who state that in recent years the yield of the variety POJ 93 in the province of Pastaza has declined to 40 t ha^{-1}.

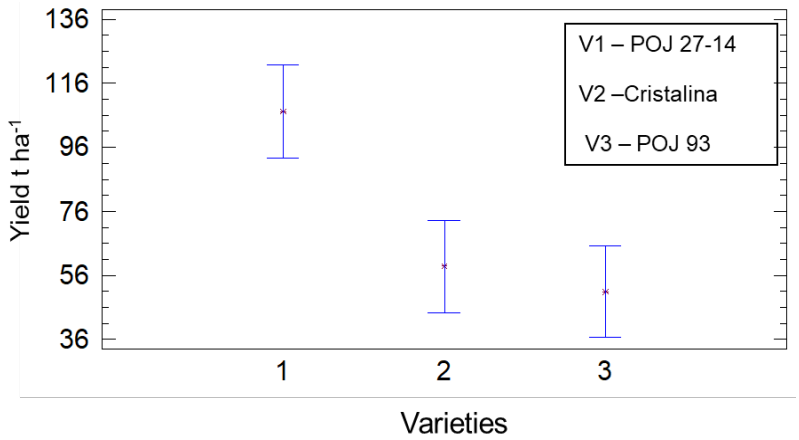


Figure 4. Agricultural yield t.ha-1 in sugarcane according to varieties at 300 days after planting. Simón Bolívar-Pastaza Parish. (Tuckey p <0.05)

Variation of agricultural yield according to organic fertilization

In the fertilizer factor, when a pollinaza is applied, a higher yield is obtained with values of 142 t.ha^{-1} that differs statistically from those obtained with the bagasse and absolute control, however the bagasse and the control do not differ statistically in themselves, being numerically superior when bagasse is applied (figure 5). According to Arreola-Enriquez, et al., (2004) cane yield increased significantly when fertilized with 10 t ha^{-1} of mulch organ-mineral fertilizer (84.6 t ha^{-1}), in comparison with the absolute control and chemical fertilization (35 and 52 t ha^{-1}), respectively.

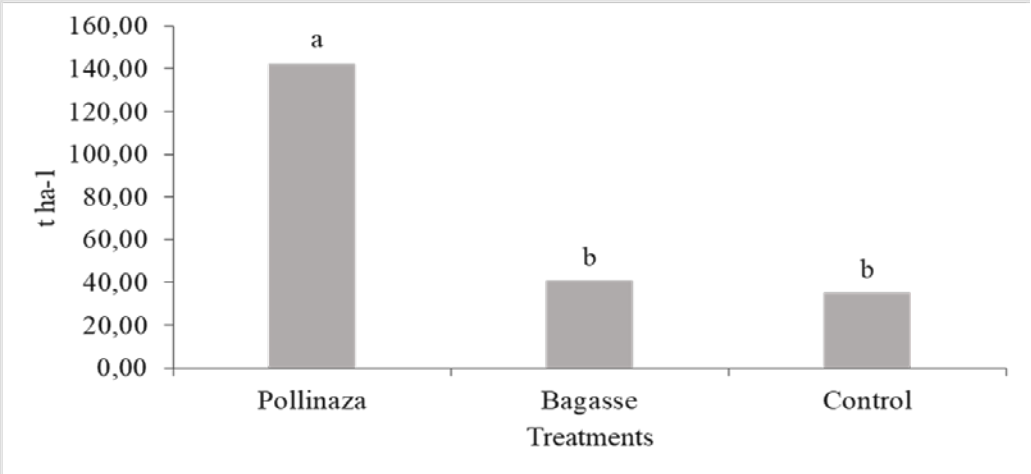


Figure 5: Yield in t ha^{-1} depending on the fertilization treatment, Simon Bolívar-Pastaza.

Conclusions

- The monitoring of these materials POJ 27-14; POJ 93 and Cristalina allowed the identification of varieties with agronomic potential, for soil and climate conditions of the area of study. The use of the pollinaza as organic fertilization, showed better agricultural yield and concentration of soluble solids for the three materials evaluated.
- In general terms, according to the conditions in which the research was developed the variety POJ 27-14 showed the better behavior.

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SOIL STRUCTURE AND CARBON SEQUESTRATION AS ECOSYTEM SERVICES UNDER DIFFERENT LAND USES IN THE ECUADORIAN AMAZON REGION

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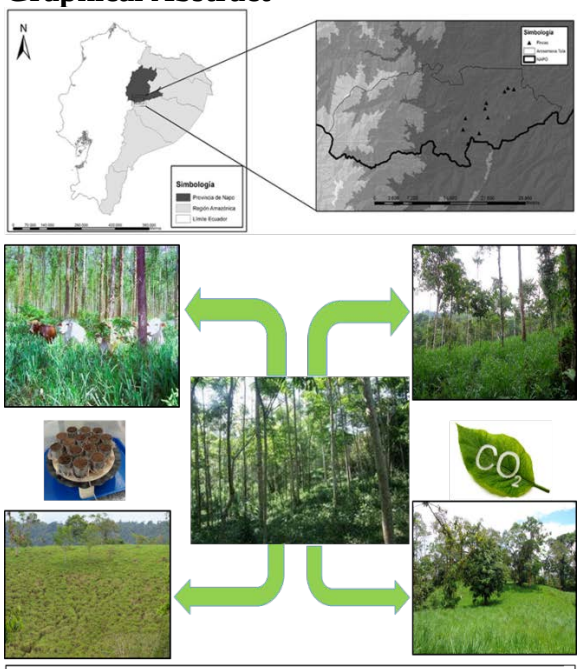
Graphical Abstract



Structural indexes/Carbon Stock	AFSs GGWT	GGWTHT	AFSs DGWT	DGWTHT	Secondary Forest
	Depth 10-30 cm				
Bulk density (BD)	0.57b	0.82a	0.81a	0.84a	0.44b
saturated hydraulic conductivity (Ksat) cm h^{-1}	6.27b	10.34b	0.78c	10.69b	25.51a
Total porosity (TP)	81.89a	72.63b	73.15b	69.79c	83.14a
Aeration porosity (AP)	9.51a	9.18 a	9.19 a	9.50 a	12.31a
Retention porosity (RP)	72.47a	63.45a	63.97a	60.29a	70.83a
Soil C stocks (Mg C ha^{-1})					
0-30 cm	49.44b	41.03c	46.91b	36.75c	51.49a
Reduced soil C stock (Mg C ha^{-1})	-2.05 (4 %)	-10.46 (20 %)	-4.58 (9 %)	-14.74 (29 %)	---

Abstract

The identification of the factors that cause changes in agroecosystems and the services they provide is essential to design management that minimizes negative impacts on the environment. In this sense, the objective of this research was to characterize some structural indices and the carbon sequestration potential under different land uses in the Ecuadorian Amazon region. In each selected land use, we collected disturbed an undisturbed soil samples within depths from 0-10 and 10-30 cm. From these samples were evaluated some structural indexes such as the bulk density (BD), saturated hydraulic conductivity (K_{sat}), total porosity. (Tp), aeration porosity (Ap), retention porosity (Rp) and total organic carbon (TOC). The results showed significant differences by treatment and depth, obtaining the best physical condition in the Forest and in some uses of grass with trees. Regardless of land use, the structural conditions evaluated through the structural indexes exhibited better results in the surface horizon, which is strongly associated with the content of organic matter. It is shown that the reduction of greenhouse gas emissions (CO_2) is associated with the increase and protection of organic matter with agroforestry systems.

Key words: *Multivariate analysis, fertility, impact, Amazon Region, land use*

Introduction

The soil resource represents a fundamental subcomponent of natural ecosystems and agroecosystems due to the multiple functions attributed to it and the relationships it maintains with the rest of the components. The soils of the tropics are relevant for the global C cycle due to the magnitude of their area and the production of biomass (Gardi et al., 2014). In tropical regions, extensive conversion of forests to pastures and agricultural intensification are typically identified as the most important promoters of land use change, with consequent loss of fertility, quality and biodiversity (Bravo et al., 2017; Valera et al., 2016; Vallejo-Quintero, 2013). Moreover, the intensification of agricultural production and the management systems with a focus on monoculture have profound effects on the ecosystem services provided by the soil and its biodiversity (Altieri and Nicholls, 2013). Soils and their biodiversity are the engine of all production systems and most of the environmental services of terrestrial ecosystems are provided by this resource. For example, primary production, nutrient recycling, water and carbon storage, detoxification, pest and plant disease control, and flood control are highly dependent on the quality of the soil and the integrity of its functioning biological (Lavelle, 2009). In this context, it has been pointed out that of the 24 largest environmental services registered in the Millennium Ecosystem Assessment, 12 are produced by the soil to a large extent and 5 depend very much on it (Lavelle, 2009, MEA, 2005). Therefore, the identification of the factors that cause changes in agroecosystems and the services they provide is essential to design management that minimizes negative impacts on the environment.

Ecosystem services can be defined “the capacity of natural processes and components to provide goods and services that satisfy human needs, directly or indirectly” and the Millennium Ecosystem Assessment (MEA, 2005) describing four categories being: supporting, provisioning, regulating and cultural service. In this perspective, agroecosystems can be suppliers and consumers of ecosystem services and humans value these systems chiefly for their provisioning services, and these highly managed ecosystems are designed to provide food, forage, fibre, bioenergy and pharmaceuticals. However, the functioning of agroecosystems depends strongly on a set of ecosystem services provided by the natural resources of untouched ecosystems (Zhang et al., 2007).

Soil structure along with genetic biodiversity for use in crop and livestock breeding, soil fertility, nutrient recycling and water supply are considered as support services (Zhang et al., 2007). While carbon sequestration is classified as a regulatory service due to the effect it has on climate regulation through greenhouse gas emissions (Power, 2010).

Soil structure and aggregate stability are important to improving soil fertility, increasing productivity, enhancing porosity and decreasing erodibility (Bronick and Lal, 2005). The structure of soils is composed by primary and secondary particles. Primary particles are individual units of sand, silt and clay, while the secondary particles result from the arrangement and binding of primary particles into aggregates by the effect of organic compounds and inorganic cementing agent’s suelo (Alvarez y Taboada, 2008).

Soil structure and fertility play a large role in determining where different kinds of farming take place and the quantity and quality of agricultural output (Zhang et al., 2007). Earthworms and macro and micro invertebrates increase soil structure via burrows or casts and enhance soil fertility through partial digestion and communition of soil organic matter (Edwards, 2004). Moreover, well-aerated soils with abundant organic matter are fundamental to nutrient acquisition by crops, as well as water retention (Zhang et al., 2007). Also, soil pore structure, soil aggregation and decomposition of organic matter are influenced by the activities of bacteria, fungi and macrofauna, such as earthworms, termites and other

invertebrates (Power, 2010; Martinez et al., 2008). Management practices in agroecosystems can degrade, maintain or improve soil structure (Bravo et al., 2017; Pla, 2010), as well as affect soil microbial communities as bioindicators sensitive to changes in land use (Vargas-Machuca, 2010). For example, agricultural management practices as mechanical ploughing, disking, cultivating and harvesting can degraded soil structure (Power, 2010), but other management practices as cover crops, incorporation of crop residues and agroforestry system reduce erosion and runoff and can maintain soil organic matter, fertility by minimizing the loss of nutrients and keeping them available to crops (Bravo et al., 2016; Espinoza-Dominguez, 2012) Together these practices conserve a suite of ecosystem services to agriculture from the soil (Power, 2010).

Soil organic carbon (SOC) is related to the sustainability of agricultural systems affecting soil properties related to the sustained yield of crops. However, the amount of COS does not only depend on local environmental conditions, but it is strongly affected by soil management (Martinez et al., 2008). Soil carbon sequestration thus provides additional ecosystem services to agriculture itself, by conserving soil structure and fertility, improving soil quality increasing the use efficiency of agronomic inputs, and improving water quality by filtration and denaturing of pollutants (Lal 2008). Therefore, the identification of management systems or land uses for carbon capture and the improvement of soil structural conditions can restore the functionality and productivity of the soil resource. Appropriate management practices can reverse degradation, reducing CO_2 emissions to the atmosphere and providing a series of benefits not only in the mitigation of climate change, but also in desertification and erosion control, water quality, food security and Soil fertility increasing the absorption of water in the soil as corrective measures for global warming (Bravo et al., 2016; Lal, 2008). Numerous works both globally and nationally show the capacity of agroforestry systems to store organic carbon in the soil, which reflects its potential to mitigate climate change, ranging from 24 to 70 Mg ha^{-1} (Bravo et al., 2016; Deng et al., 2016; Somarriba et al., 2012; Jadan et al., 2012). Despite hundreds of field studies and at least a dozen literature reviews, there is still considerable disagreement about the direction and magnitude of changes in C stocks in the soil with the change in land use (Deng et al. al., 2016).

With this perspective, the objective of this work was to characterize the structure and carbon stored in the soil as ecosystem services under different land uses in the Ecuadorian Amazon region.

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Materials and Methods

The work was carried out in municipality of Carlos Julio Arosemena Tola, Napo province, Ecuador (Figure 1). The climate is characteristic of a humid tropical forest, with an altitude of between 500 and 600 masl. The average annual rainfall is 3000mm, an evapotranspiration of 150mm, temperatures between 23.4 to 25°C and a relative humidity of 86% (Uvidia et al., 2015). The soils belong to the inceptisol order and are recent, shallow and generally acidic soils without well-defined horizons but with high organic matter content, low natural fertility (low potassium, calcium and phosphorus content) and high iron contents (Bravo et al. 2017, Nieto and Caicedo, 2012). Land uses related to livestock systems were selected, which were compared with the primary forest as reference system, describe as: GGWFT: Gramalote grass with trees; GGWTHT: Gramalote grass without trees; DGWT: Dali Grass with trees; DGWTHT: Dali Grass without trees and SF: Secondary Forest.

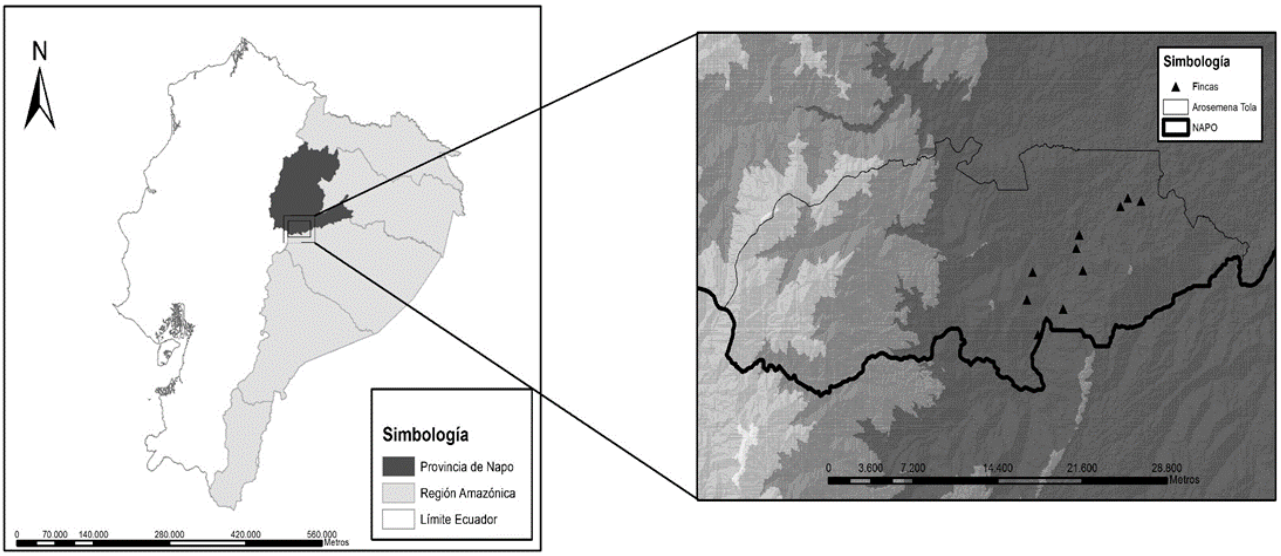

Figure 1. Relative location of selected land uses in municipality of Arosemena Tola, Napo province, Ecuador.

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Soil sampling, determination of structural indices and total organic carbon.

The impact of land-use change on structural indices and total organic carbon was assessed using a systematic sampling scheme, establishing a transect of five sampling points for each selected land use. We collected disturbed an undisturbed soil samples at 0-10 and 10-30 cm depth. From these samples undisturbed were determined, Bulk Density (BD) using the cylinder method (Blake and Hartge, 1986); the saturated hydraulic conductivity (K_{sat}) by the variable loading method (Pla, 2010); and the pore size distribution (Tp: total porosity), aeration porosity (Ap: pores of radius> 15µm) and retention porosity (Rp) using the table of saturation tension and to a matric potential of -10kPa (Gee and Bauder, 1986). Total organic carbon (TOC) was analyzed by Walkey-Black method (Nelson and Sommer, 1982).

Results and Discussion

Table 1 shows the results of the average values of structural indices and carbon sequestration in the different land uses and the two soil depths considered. As can be seen, the structural indexes showed significant differences ($P < 0.05$), resulting in better physical conditions the surface horizon indistinctly of land use. When comparing the different land uses and the two depths considered, a good physical behavior is observed, which is reflected in the evaluated structural indexes (BD, K_{sat}, Tp, Ap, Rp) with better values in the superficial horizon, especially the use with secondary forest. Bulk density (Da) varied significantly ($P < 0.05$), obtaining the lowest value in the Secondary Forest (SF,) (0.34 Mg m^{-3}) and GGWT (0.43 Mg m^{-3}), while the rest of the selected land uses reached the highest densities. Regardless of the land use, the value of the bulk density increased with depth (10-30 cm), reaching values between 0.81 to 084 Mg m^{-3}. Bulk density represents a very important variable of great agricultural significance whose values must be interpreted according to the textural class. In general, the textural classes determined in the field for the selected land uses varied between clayey and clay loam, categorized as fine textures. Therefore, when comparing the values obtained from BD with any depth and land use with the value indicated as critical of 1.3 Mg m^{-3} for this type of texture, it can be noted that there is no soil compaction (Pla, 2010) and therefore a good functioning that favors a greater possibility of exploration of the volume of soil by the roots of the plants. In addition, bulk density has a strong influence on the development of roots, the resistance to penetration, the movement of water, air, the content of nutrients and their availability (Alvarez and Taboada, 2008). Sometimes the most important effects of land use

change are associated with the change in pore geometry, which even without large variations in density, determine strong changes in the soil hydrological behavior (Bravo, et al., 2015). When we analyzed the value of saturated hydraulic conductivity (Ksat), associated with soil permeability, high values were recorded in all uses, well above the critical limit of 0.5 cm.h^{-1}, (Pla (2010), in special, in the Forest and GGWT (Table1).

This behavior is related to the textural and structural condition that favors the penetration and movement of water in the soil profile. In general, the hydraulic behavior of the soil, presented the highest values of Ksat in the surface horizon (0-10 cm), which decreases with depth (layer of 10 to 30 cm) to reach values close to 0.78 cm h-1 in some land uses (DGWT). The values of the total porosity (Tp) were much related to bulk density, suggesting that the higher the density, the lower the porosity. Total porosity (Tp) was high in all the land uses studied (greater than 60%), with a large fraction of the total volume represented by the retention pores (Rp), which gives these soils a high moisture retention capacity, indistinctly of the land use. On the other hand, the volume of aeration pores (macropores (Ap)> 15 μm) that actively contribute to the water flow (Bravo et al., 2008; Alvarez and Taboada, 2008), they are in smaller proportions.

Table 1. Structural indexes and carbon sequestration under different land uses systems.

	Depth 0-10 cm				
	AFSs		AFSs		Secondary
Structural indexes	GGWT	GGWTHT	DGWT	DGWTHT	Forest
Bulk density (BD) Mg m^{-3}	0.43**b**	0.56**a**	0.53**a**	0.56**a**	0.34**b**
saturated hydraulic conductivity (K_{sat}) cm h^{-1}	33.48**b**	39.80**b**	17.42**c**	13.10**c**	49.74**a**
Total porosity (TP) %	85.01**a**	82.01**a**	80.47**a**	84.12**a**	86.77**a**
Aeration porosity (AP) %	13.66**b**	14.92**b**	13.87**b**	15.74**b**	18.77**a**
Retention porosity (RP) %	71.41**a**	67.09**a**	66.60**a**	68.38**a**	68.00**a**
	Depth 10-30 cm				
Structural indexes/Carbon	AFSs		AFSs		Secondary
Stock	GGWT	GGWTHT	DGWT	DGWTHT	Forest
Bulk density (BD)	0.57**b**	0.82**a**	0.81**a**	0.84**a**	0.44**b**
saturated hydraulic conductivity (Ksat) cm h^{-1}	6.27**b**	10.34**b**	0.78**c**	10.69**b**	25.51**a**
Total porosity (TP)	81.89**a**	72.63**b**	73.15**b**	69.79**c**	83.14**a**
Aeration porosity (AP)	9.51**a**	9.18 **a**	9.19 **a**	9.50 **a**	12.31**a**
Retention porosity (RP)	72.47**a**	63.45**a**	63.97**a**	60.29**a**	70.83**a**
Soil C stocks (Mg C ha^{-1})					
0-30 cm	49.44**b**	41.03**c**	46.91**b**	36.75**c**	51.49**a**
Reduced soil C stock (Mg C ha^{-1})	-2.05 (4 %)	-10.46 (20 %)	-4.58 (9 %)	-14.74 (29 %)	---

Agroforestry systems (AFSs); GGWT: Gramalote grass with trees; GGWTHT: Gramalote grass withoth trees; DGWT: Dali Grass with trees; DGWTHT: Dali Grass without trees. Significant differences of the means according to Tukey's adjustment (P< 0.05) in the same row are indicated with different letters.

In general, all land use showed adequate physical condition, reflected by the average values obtained in the structural indexes with low values of bulk density (BD), high values of total porosity (Tp), aeration porosity (Ap), retention porosity (Rp) and saturated hydraulic conductivity (Ksat) (Table 1). Moreover, due to the peculiarities of the Ecuadorian Amazon region (EAR), characterised by soils with high organic matter content, a type of granular structure is generated that allows one to obtain higher values of Ksat and Ap, which improves the soil's rate of infiltration and its capacity (Bravo et al., 2017). However, this situation changes with the depth where the values are decreasing, showing limiting values from 10 cm around 9% for most land uses with the exception of the soil under secondary forest (Table 1). A reduction of the infiltration rate, as a consequence of the decrease of macroporosity in high precipitation conditions (>3000 mm), landscapes with high slopes common in the Ecuadorian Amazon region, represent one of the main causes of the activation processes of water erosion commonly observed in the area (Bravo et al; 2017). Our results suggest that the studied structural indices such as bulk density (BD), total porosity (Tp), aeration porosity (Ap), retention porosity (Rp), saturated hydraulic conductivity (K_{sat}), help to characterize different processes in the soil (compaction, aeration, infiltration), which may be affected by the change of land use. In addition, the role of the soil resource as a regulator of the ecosystem and its contribution to the mitigation of global climate change is reinforced (Bravo et al., 2016, Lal, 2008).

Effect of land use change on soil carbon stocks

Some authors point out that the soil C sequestration due to land change use is likely to be affected by multiple factors such as climatic conditions, soil texture, site preparation and management, vegetation type, land use history, etc. (Deng et al., 2016). In our case, the land history use in the ecuadorian amazon region is with forest which has allowed to store large amounts of soil carbon stocks (Bravo et al., 2017). Land use change, can cause a changes in soil C stock (Table 1), showing significantly higher values in the use with secondary forest and GGWT 51.49 and 49.44 Mg C ha^{-1} respective in compared to the rest of the land uses. Despite the history of use, in all cases there is a decrease in the carbon reserves in the soil when converting forest to livestock systems, with a higher proportion in those systems without trees, with values that fluctuated from 2.05 (4%) in GGWT to -14.74 (29%) in DGWHT, as shown in Table 1. Similar results have been reported by other researchers who report a decrease in the carbon stock in the soil between 8 to 42% when there is conversion of forest to livestock systems and cultivation (Powers et al., 2011)

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Conclusions

The structural indexes studied such as bulk density (BD), total porosity (Tp), aeration porosity (Ap), retention porosity (Rp), saturated hydraulic conductivity (K_{sat}), help to characterize different processes in the soil (compaction, aeration, infiltration), which may be affected by land use change. All this, together with the potential for carbon sequestration, reinforces the role of the soil resource as a regulator of the ecosystem and its contribution to the mitigation of global climate change.

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Conflicts of Interest

The authors declare no conflict of interest".

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MOL2NET, International Conference Series on Multidisciplinary Sciences
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A short overview on the use of metabolomic multi-platforms in biomedical research

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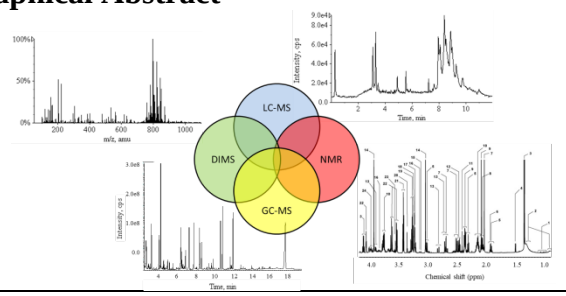
Graphical Abstract



Abstract.

The integration of various analytical platforms is emerging as a very interesting alternative with the aim to maximize coverage in metabolomics. In this work, we present a concise review of the most commonly used metabolomic multi-platforms in biomedical research.

Metabolomics is a very interesting option in biomedical research because of its potential to investigate metabolic alterations associated with disease pathogenesis and progression. However, due to the complexity of the human metabolome, metabolomic analysis usually requires the combination of complementary analytical platforms to maximize metabolite coverage.

The integration of reversed-phase liquid chromatography (RP-LC) and gas chromatography (GC) coupled to mass spectrometry (MS) is the most commonly employed alternative, since RP-LC-MS provides a lot of information about the presence of low polar compounds (mainly lipids), while GC-MS allows monitoring low molecular weight metabolites [1-3]. On this basis, the combination of reversed-phase and hydrophilic interaction liquid chromatography (HILIC) is also an interesting strategy, since the coupling HILIC-MS allows analyzing polar compounds in biological samples without the recourse of the derivatization step needed in gas chromatography [4-6]. Alternatively, capillary electrophoresis (CE) can also resolve the polar metabolome, but its combination with RP-LC-MS has only been proposed in a few works [7-8]. On the other hand, nuclear magnetic resonance (NMR) also complements the analytical performance of previously described techniques based on MS, since NMR can be applied for high-throughput metabolomic fingerprinting of biological samples, prior to in depth analysis by GC-MS, LC-MS or CE-MS [9-11]. In this line, direct mass spectrometry analysis can also be employed for a preliminary metabolic screening, and then complemented with conventional hyphenated approaches [12-13].

To conclude, it should be noted that none analytical platform is able to accomplish a global characterization of the metabolome in a single analysis, so the application of metabolomic multi-platforms is necessary. In the author's opinion, an interesting alternative is the combination of direct mass spectrometry analysis, to carry out a "first pass" screening, with RP-LC-MS (focused on lipid compounds) and GC-MS (for the analysis of the low molecular weight fraction of the metabolome).

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Multi-scaleanalysisof structural variability of Caryophyllaceae saponins by a simplex machine learning approach

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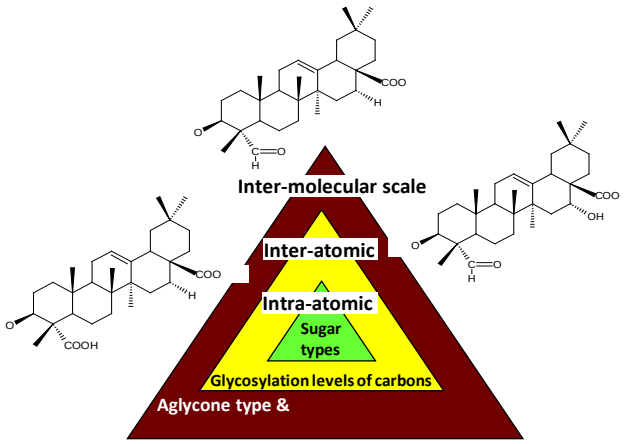
Graphical Abstract



Abstract. A mass conservation law-based chemometric approach was developed to extract smoothed processes governing inter- and intra-molecular variability of structural diversity in metabolic pools. The approach consisted of a machine-learning method using simplex rule to calculate a complete set of smoothed barycentric molecules from iterated linear combinations between molecular classes (glycosylation classes). An application to four glycosylation levels (*GLs*) of Caryophyllaceae saponins highlighted aglycone-dependent variations of glycosylations, especially for gypsogenic acid (*GA*) which showed high 28-glucosylation levels. Quillaic acid (*QA*) and gypsogenin (*Gyp*) showed closer variation ranges of *GLs*, but differed by relationships between glycosylated carbons toward different sugars. Relative *GLs* of carbons C3 and C28 showed associative (positive), competitive (negative) or independent (unsensitive) trends conditioned by the aglycone type (*GA*, *Gyp*) and molecular (total) *GLs* (the four classes): 28-glucosylation and 28-xylosylation showed negative global trends in *Gyp* vs *GLs*-depending trends in *QA*. Also, relative levels of 3-galactosylation and 3-xylosylation varied by unsensitive ways in *Gyp* vs positive trends in *QA*. These preliminary

	results revealed higher metabolic tensions (competitions) between considered glycosylations in *Gyp* vs more associative processes in *QA*. In conclusion, glycosylations of *GA* and *QA* were relatively distant whereas *Gyp*(common precursor) occupied intermediate position.

Introduction

The Caryophyllaceae plant family was proved to be a wide source of saponins essentially based on three triterpenic skeleton (aglycones or sapogenins) including gypsogenin (*Gyp*), quillaic acid (*QA*) and gypsogenic acid (*GA*) [1].Apart from the sapogenin type, structural variability of Caryophyllaceae saponins showed multi-factorial and multi-scale aspects due to different glycosylation levels (*GLs*) and glycosylation types essentially occurring at the carbons C3 and C28.

By considering a wide dataset of 205 Caryophyllaceae saponins based on*Gyp*, *QA* and*GA*with different *GL*(2 to 9), a machine learning approach was applied to extract key information on inter- and intra-molecular regulatory processes governing the observed structural diversityin relation to aglycones (a), glycosylation levelsand types (b, c) and substitution carbons(d) [2]. *In silico* combinations between saponin structures belonging to different molecular classes (*GLs*) provided a complete set of simulated theoretical molecules from which significant trends within and between glycosylated carbons were revealed to govern structural variability at inter-molecular scale. This helped to better understand hierarchical and sequential glycosylation orders responsible for diversification of saponins in Caryophyllaceae.

Materials and Methods

Machine learning approach was applied to the three aglycones separately(*Gyp*, *QA*, *GA*). It consisted in combining structural variabilities of saponins belonging to *q*molecular classes (concerning one aglycone)representing*q*increasing glycosylation ranges:for *Gyp* and *QA*, saponins were stratified into *q*=4 classes of glycosylation levels (*GLs*)(*GLs*= 1, 2, 3, 4) representing saponins with 3-4, 5-6, 7 and 8-9 substituted sugars, respectively; for GA, *q*=3 classes were considered (*GLs* = 1, 2, 3) corresponding to saponins with 3, 4, 5 substituted sugars, respectively. Saponins of different *GL* classes wereinitially characterized by the relative *GLs* of different sugars substituted at different carbons (C3, C16, C23, C28).Combinations between the *q*molecular classes were applied using Scheffé's simplex matrix (*N* rows x *q* columns) which provides a complete set of *N* mixturesvarying gradually by different weights w_j(from 0/5 to 5/5) of the *q*mixed *GL*classes*j* (with Σw_j=1)[2].

In output of each combination, a barycentric molecular profile was calculated by averaging the relative levels of glycosylation (*G*) profiles of the *n* randomly sampled contributive saponins. The mixture design was iterated 30 times by bootstrap technique then the 30 resulting response matrices (containing *N*elementary barycentric*G*-profiles) were averaged leading to a final response matrix containing *N*smoothed barycentric *G*-profiles and representing a deep regulatory machinery of the whole studied structural system.

The smoothed response matrix was used for graphical analysis of regulatory trends between glycosylated carbons.For two given glycosylated carbons, different regulatory trends were highlighted by considering successions of weight ellipses associated to different *GL* classes [2].

Results and Discussion

Data smoothing by simplexmachine learning approach helped to highlight regulatory processes of glycosylation in Caryophyllaceae saponins at inter-molecular, inter-atomic and intra-atomic scales. Illustrations are provided by xylose (*Xyl*), glucose (*Glc*) and galactose (*Gal*) substituted at carbons C3 and/or C28.

Inter-molecular scale variations 1 (aglycone effect). The three simplex plots associated to the three aglycones-based saponins showed strong differentiation spaces of relationships between the same glycosylated carbons (**Figure 1a, b**). Illustrations are given for 28-*Xyl* vs 28-*Glc* (**Figure1**) and 3-*Xyl* vs 3-*Gal* (**Figure2**):

GA was markedly distant from *Gyp* and *QA* indicating some specific glycosylation orders (in *GA*). This could be linked to the occurrence of two carboxylic groups in *GA*(23- and 28-COOH) vs only one (28-COOH) in both *QA* and *Gyp*. Specific space of *GA* was characterized by strong relative 28-*Glc* levels (0.65-0.75) without competing (comparable) levels from other sugars in both C3 and C28 (**Figure1a**)[1, 3, 4]. *Gyp* occupied intermediate position between *GA* and *QA* whereas *QA* was the most distant from *GA* (**Figure1a, 2b**).Relative locations of different aglycones-based saponins in simulated graphicswere compatible with the metabolism: *Gyp* is metabolically precursor of both *QA* and *GA* (by 16- and 23-hydroxylation, respectively); this is compatible with intermediate position of *Gyp*between *QA* and *GA* (competing for *Gyp*) [3].

Although *Gyp* and *QA* plots showed spatial neighboring (compared to *GA*), they differed by dispersions, inclinations and internal organization of corresponding clouds of points: relationship between 3-*Xyl* and 3-*Gal* showed significantly higher dispersion in *QA* than in *Gyp* (**Figure1b**). This aspect indicated wider regulation range of 3-*Gal* in *QA* compared to *Gyp*[3]. However, for (28-*Xyl* vs 28-*Glc*), the two aglycones showed opposite relationship leading to inversely inclined clouds of points: (global positive trend in *QA* against negative trend in *Gyp*) (**Figure1a**).

Inter-molecular scale variation 2 (molecular glycosylation effect). For a same aglycone, global variation trends between substituted sugars significantly varied with molecular *GLs*:

In *Gyp*, 28-*Glc* showed a strong peak under low molecular *GL* (*GL*=1) followed by rapid decrease to minimum in higher *GLs* (*GL*=2, 3, 4) (**Figure 1b**). This highlightedstrong contribution of early 28-*Glc* in ramification process (monodesmosylation) of*Gyp* leading to preliminary structural diversification of saponins [3, 5, 6]. In *QA*, 28-*Glc* seemed to play intermediate modulatory role due to its low relative levels in *GLs* = 1 and 4 (**figure 1c**).

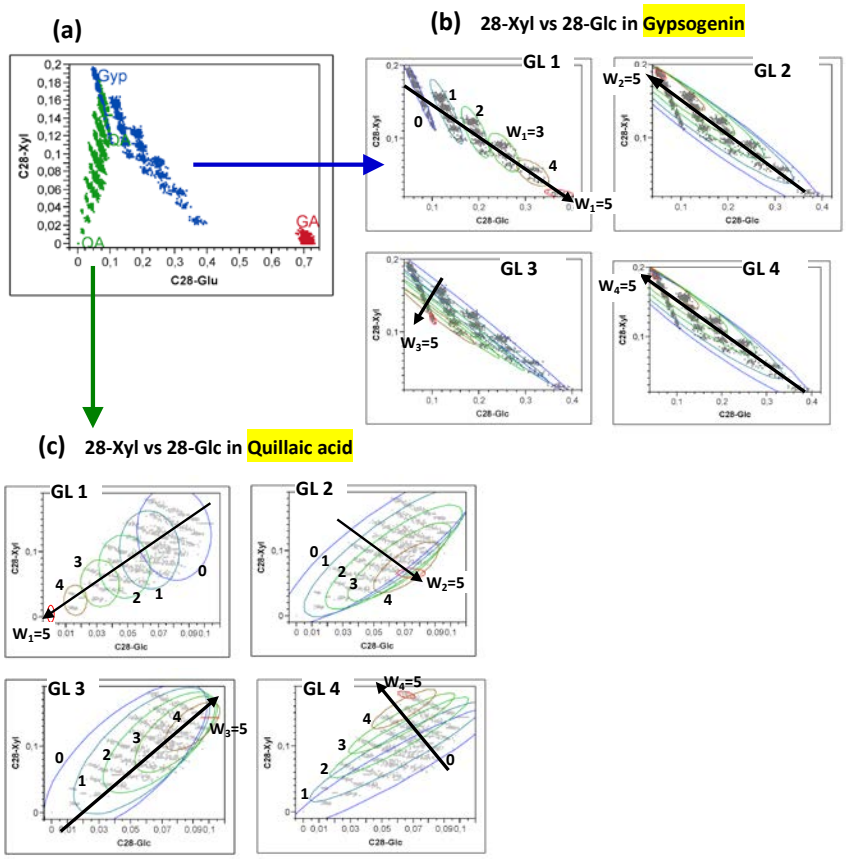


Figure 1. Multidirectional smoothed plots given by simplex machine learning for aglycone-dependent relationships between C28-xylosylation (28-*Xyl*) and C28-glucosylation (28-*Glc*). (**a**) Three plots corresponding to quillaic acid (*QA*), gypsogenin (*Gyp*) and gypsogenic acid (*GA*). (**b**, **c**) Four plots associated to four glycosylation levels (*GLs*) in *Gyp* and *QA*, respectively.

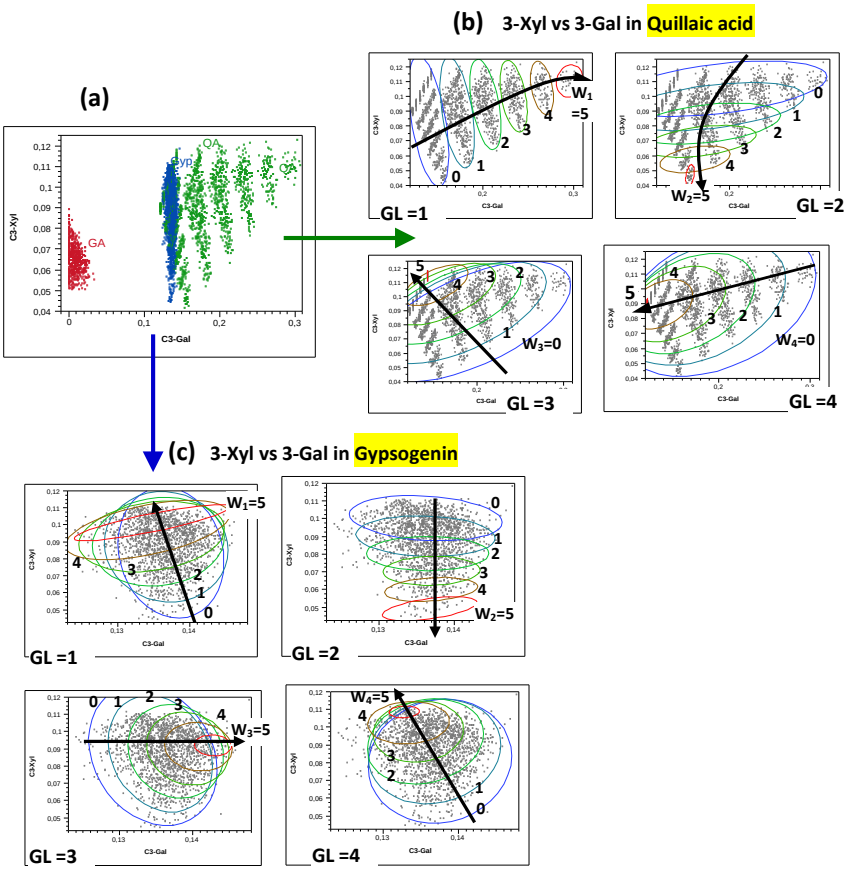


Figure 2. Multidirectional smoothed plots given by simplex machine learning for aglycone-dependent relationships between C3-xylosylation (3-*Xyl*) and C3-galoactosylation (28-*Gal*). (**a**) Three plots corresponding to quillaic acid (*QA*), gypsogenin (*Gyp*) and gypsogenic acid (*GA*). (**b**, **c**) Four plots associated to four glycosylation levels (*GLs*) in *QA* and *Gyp*, respectively.

However, in *Gyp*, relative levels of 28-*Xyl* showed alternated states with molecular *GLs*: global minimal regulations in the less glycosylated saponins (*GL*=1), followed by maximal global regulations in *GL*=2 and 4 via slight decrease in *GL*=3 (**Figure 1b**). This highlighted key role of 28-*Xyl* in

elongation process for the synthesis of *Gyp*-based saponins with high *GL*s. Also, in *QA*, 28-*Xyl* showed gradual (step-by-step) increases of relative levels with molecular *GLs*. This was indicative of key role of xylosylation in elongation process at C28 in *QA* (**Figure 1c**).

Inter-atomic scale variation. By considering xylosylation between C3 and C28 in *QA*, 3-xylosylation seemed to be initially favored while 28-*Xyl* was at its lowest levels (in *GL*=1) (**Figures 1c, 2b**). This could be indicative of key role of 3-xylosylation in molecular ramification of *QA* [3]. For higher *GL* (2 to 4), relative level of 28-*Xyl* showed continuous increase vs alternated variation for 3-*Xyl*. This highlighted increasing affinity of C28 vs alternated affinity of C3 for xylosylation with *GLs* in *QA*.

Intra-atomic scale variation. By considering the inclinations of weight ellipses of different *GLs*, further regulatory processes were highlighted between 28-*Xyl* and 28-*Glc* conditionally to the aglycone type (*Gyp* vs *QA*) (**Figures 1a, 2**):

In *Gyp*, weights' ellipses showed negative inclinations indicating systematic competitions between *Xyl* and *Glc* for substitution at C28 in all the *GLs* (**Fig. 1b**). Such a competition could suggest the implication of different glycosyl-transferases (*GT*) in the two glycosylations of C28 [3]. Hypothesis on specific *GT* in *Gyp* could be also locally indicated by not clearly inclined weight ellipses in the relationship 3-*Xyl* vs 3-*Gal*.

However, in *QA*, weights' ellipses in (28-*Xyl* vs 28-*Glc*) showed positive inclinations indicating some associative process between these two sugars for their 28-substitution despite some global negative trends (for *GLs*=2, 4). Such associative substitution process could occur under the effect of a same *GT* having a promiscuity character and leading to sequential glycosylations by different sugars [5, 7-10]. Also, weight ellipses in 3-*Xyl* vs 3-*Gal* showed some positive inclination (except in *GL*=1) leading to further indication about intra-atomic associations between considered glycosyls in favor of shared (promiscuity) *GT* hypothesis.

Conclusions

Simplex-based machine learning applied to structural variability of Caryophyllaceae saponins highlighted strong differentiation in metabolic glycosylation governed by the aglycone type, molecular *GL* and substituted carbon. *GA* was strongly characterized by high levels of 28-*Glc* followed by *Gyp* then *QA*. Effect of *GLs* was partially associated to key role of 28-*Xyl* in elongation in both *Gyp* and *QA*. At inter-atomic scale, preliminary 28-*Glc* and 3-*Xyl* seemed to be responsible for molecular ramification in *Gyp* and *QA*, respectively. At intra-atomic scale, 28-*Xyl* and 28-*Glc* showed competition in *Gyp* and association in *QA* that could be indicative of different and shared *GTs*, respectively. Finally, metabolic tensions for glycosylation seemed to decrease from *GA* to *QA* via *Gyp*.

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Computational Study (*In Silico*) of the Antimicrobial and Toxicological Potential of Isoeugenol

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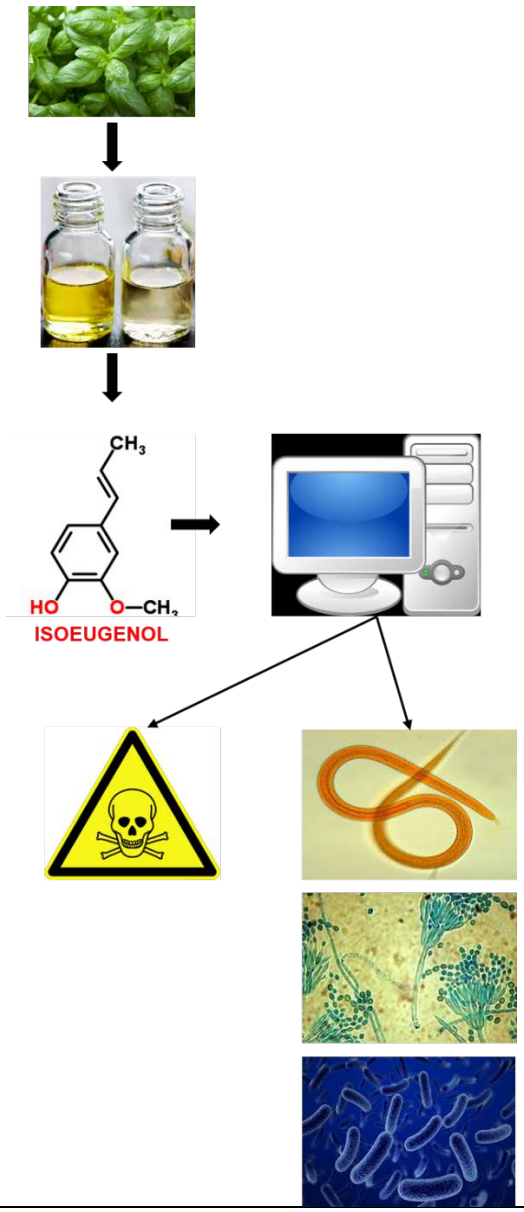
Graphical Abstract



Abstract.

The search for new compounds with antifungal activity has become very important, mainly due to the large increase of fungal infections and also the appearance of antifungal resistant strains available in the market, as well as for use for pest control. Medicinal plants represent an alternative for the substitution of these synthetic fungicides for natural products, since they have a large quantity and variety of secondary metabolites with biological properties, among them, phenylpropanoids. Therefore, the present study aims to investigate the in silico antimicrobial and toxicological potential of 2-Methoxy-4-propenylphenol (isoeugenol) through computational analysis. For this, the Prediction of Activity Spectra for Substances (PASS online), Molinspiration and Osiris software were used. PASS online showed that isoeugenol has the opportunity to present antiseptic (Pa: 0.571 and Pi: 0.009), antifungal (Pa: 0.492 and Pi: 0.032), antibacterial (Pa: 0.379 and Pi: 0.035), antimycobacterial (Pa: and Pi: 0.022) and antihelmintic (Pa: 0.335 and Pi: 0.028), mainly against nematoda (Pi: 0.562 and Pa: 0.009). Molinspiration showed that the phytoconstituent has good potential for oral bioavailability, with nDLH = 1, nALH = 2, mass = 164.2 Da and

	cLogP = 2.38. In the analysis with the Osiris program, it was demonstrated that isoeugenol has low irritant (1) and tumorigenic risk (1), and high mutagenic risk and high reproductive toxicity. In view of this instilled study, it was possible to verify that the compound is a potential candidate for in vitro and in vivo studies of antimicrobial and toxicological action and to prove the data obtained from the computational analysis.

Introduction

The occurrence of antifungal resistance cases used in clinical practice has made the search for new molecules with potential biological activity increasingly subject to studies related to antimicrobial activity [1-2]. Thus, the search for new substances capable of combating fungal infections, or even improving the action of antifungal agents commonly used in the clinic, may be a promising path to fight against resistance and reduce the limitations of conventional treatment, such as adverse effects and high toxicity [3], not only in human use, but also in pest control in agriculture.

Alternatively, we have plants as sources of new biologically active compounds. The great quantity and diversity of secondary plant metabolites has been attracting the interest of researchers from different areas of science, who see in plants a promising source for the discovery of molecules with potential human use, with significant commercial value in the pharmaceutical, food, agronomic and cosmetic [4]. These studies are important for the discovery of compounds with biological activities, since their structural diversity of these substances is greater than that presented by most of the combinatorial strategies carried out on heterocyclic compounds [5]. These characteristics give these compounds a greater possibility of becoming tools in the fight against diseases, among them microbial infections.

To help in this search for new molecules, medical chemistry emerges as a major tool. The aim of medical chemistry is the planning, discovery, identification, preparation and understanding of the molecular action functioning of biologically active compounds [6]. Medical chemistry also takes into account metabolism, chemical structure and pharmacological activity [7].

The use of computational tools brought more speed and efficiency in the development of new drugs. The *in silico* drug discovery process involves the identification of the target of interest (receptor structure), the choice of compounds from inhibitor bases, evaluation of protein interaction with possible inhibitors, and calculation of pharmacokinetic and pharmacodynamic properties of possible inhibitors [6].

Given this perspective and knowing that the antifungal activity of natural compounds has been scientifically attributed to essential oils, extracts, coumarins, terpenes, flavonoids, amides, alkaloids and even phenylpropanoids [8-11], the aim of this study was to investigate the *in silico* antimicrobial and toxicological potential of 2-Methoxy-4-propenylphenol (isoeugenol) phenylpropanoide by means of computational analysis.

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Materials and Methods *(optional)*

The computational analyzes of the antimicrobial activities and toxicological risk of isoeugenol were carried out using the online PASS software, Molinspiration Molecule Viewer and Osíris.

The "Prediction of Activity Spectra for Substances" - PASS online is software designed to evaluate the overall biological potential of an organic molecule on the human organism, according to its structural arrangement. The spectrum of biological activities of a chemical compound is the set of different types of biological activities, which reflect the results of interaction of the compound with various biological entities. In this way, the online PASS provides several facets of the biological action of a compound, obtaining the indexes Pa (probability of being active) and Pi (probability of being inactive) by estimating the categorization of a potential compound to belong to the subclass of active or inactive compounds, respectively [12].

The Molinspiration Molecule Viewer software allows the perception of molecules using sophisticated Bayesian statistics, which combine the structures and properties of the representative active compound in the specific target with the structures of inactive molecules, to recognize typical substructure characteristics of the active molecules. This program is capable of evaluating the molecule providing several parameters, among them the ability to predict the probability of the compound to act on certain pharmacological targets [13-14].

To verify the toxicity and theoretical pharmacokinetic parameters of isoeugenol, the compound was subjected to the in silico study of ADMET parameters (absorption, distribution, metabolism, excretion and toxicity) using the Osiris program. This online tool is able to predict the toxicity of the substance by comparing its chemical structure with molecular fragments whose defined toxicity is found in a database. The toxicity results are expressed as mutagenic, tumorigenic, irritability and effects on the reproductive system [15].

After analysis, the software provided the potential of druglikeness and drug-score that are related to topological descriptors, and other properties such as cLogP and molecular mass, in addition to theoretical analysis of mutagenic, tumorigenic, irritant and reproduction effects. The results obtained were then classified from a scale of 1 to 3, where: 1 means absence of toxicity, 2 moderate toxicity and 3 severe toxicity. In addition to reporting the possible toxicity of a molecule, Osiris is able to report important physicochemical parameters in predicting the theoretical oral bioavailability of the drug under study. These parameters are: partition coefficient (water / oil) - cLogP, molecular weight, number of hydrogen acceptors - nALH, number of hydrogen donors - nDLH [15].

To verify whether the compound can be planned for oral administration, an analysis based on the "Rule of Five" was performed, as described by Lipinski [16]. In this rule, if the molecule shows scores of at least 3 parameters meeting the requirements (cLogP $\leq$ 5, molecular weight <500 daltons; nALH $\leq$ 10; nDLH $\leq$ 5), the molecule will theoretically present a good oral bioavailability.

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Results and Discussion *(optional)*

After analysis of the probable biological activities of isoeugenol through the online PASS, more than 522 activities with more than 30% of the probability of being active (Pa> 30%) were obtained, of this total, 49 activities have a high probability of occurrence > 70%). The computational study revealed the activities most likely to be exerted by the compound. Among the 522 activities with a chance of being performed by isoeugenol, we highlight the antimicrobials: antiseptic (Pa: 0.571 and Pi: 0.009), antifungal (Pa: 0.492 and Pi: 0.032), antibacterial (Pa: 0.379 and Pi: 0.035), antimycobacterial (Pa: 0.478 and Pi: 0.022) and antihelmintic (Pa: 0.335 and Pi: 0.028), mainly against nematoda (Pi: 0.562 and Pa: 0.009).

These antimicrobial activities can be attributed to several characteristics of isoeugenol reported in the literature. Bhatia et al. [17] found that the antifungal activity of isoeugenol against strains of

Candida spp. is due to the ability to inhibit H + pumping through plasma membrane ATPase and alteration of fungal membrane permeability. These data are supported by the results of the online PASS analysis, which demonstrates that the phytoconstituent has a considerable chance of exerting instability in the fungal plasma membrane as a membrane permeability potencilizer (Pa: 0,408 and Pi: 0,054) and membrane integrity antagonist (Pa: 0.316 and Pi: 0.105). These data support the hypothesis advocated by Sikemma et al. [18] and Gill et al. [19] that attribute to isoeugenol the destabilizing action of the fungal membrane due to its chemical structure.

Next, the molecular properties of isoeugenol, based on molecular descriptors using Lipinski's rule of five, were calculated in the Molinspiration Online Property Calculation Toolkit software (**Table 1**). Lipinski's rule of five establishes some structural parameters relevant to the theoretical prediction of the oral bioavailability profile, which is added to the absorption and permeability of possible drugs and depends on five parameters: (1) number of hydrogen bonding groups (nALH) less than or equal to 10; (2) number of donor hydrogen bonding groups (nDLH) less than or equal to 5; (3) molecular mass (MM) less than or equal to 500 g / mol; (4) octanol-water partition coefficient (milog P) less than or equal to 5; (5) polar surface area (PSA) less than or equal to 140 Å. Molecules that violate more than one of these rules may have problems with bioavailability.

Table 1 - The theoretical analysis of the physico-chemical properties of isoeugenol required for theoretical oral bioavailability compared to the standard of the Lipinski's rule of five - Osiris

Phytoconstituent	**Parameters for Bioavailability Evaluation**			
	nDLH	**nALH**	**Da**	**cLogP**
Isoeugenol	1	2	164.20	2.38
Padrão da "Regra dos cinco" Lipinski	= 5	= 10	< 500	< 5

nDLH: Number of hydrogen donors; nALH: Number of hydrogen acceptors; Da: Molecular weight; cLogP: Partition coefficient water: oil.

Analyzing the results obtained in Osiris through Lipinski's "Rule of Five" [16], it was verified that isoeugenol presents a good theoretical oral bioavailability, since all the physicochemical parameters evaluated for this molecule presented within the cut established by the "Rule of Five" Lipinski.

The study of drug likeness and drug score was performed using the Osiris Property Explorer program (**Table 2**). Drugcore values between 0.1 and 1.0 and a positive value for drug likeness indicate that the molecule evaluated contains frequent groups in commercial drugs. The theoretical toxic effects evaluated were mutagenicity, tumorogenicity, irritant effects and human reproduction through color (red = high risk, yellow = moderate risk and green = no risk).

Table 2. Risk of toxicity of isoeugenol compared to standard antifungal (voriconazole) calculated using Osiris Property Explorer software.

Compounds	**Toxicity risk**			
	MUT	TUMO	IRRI	REP

Isoeugenol				
Voriconazole				

: Nontoxic; : Highlytoxic; MUT: Mutagenic; TUMO: Tumorigenic; IRRI: Irritant; REP: Reproductive effective.

According to the in silico toxicity analysis of the compound, it has been found that it does not present a risk of being irritant and tumorigenic. Although it has demonstrated a high risk of being mutagenic and presenting reproductive toxic effects, these data do not exclude it from being a drug candidate, since other antimicrobial drugs, such as itraconazole, also present a high tumorigenic and mutagenic risk [20].

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Conclusions

The in silico study identified the antimicrobial potential of isoeugenol, as was expected according to the literature, and identified the phytoconstituent toxic profile likely to be developed by the compound when in contact with the organism. Thus, this preliminary computational analysis allows us to continue the studies of isoeugenol in other experimental models.

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In Vitro Antifungal Effect of Isoeugenol Against *Penicillium citrinum* Strains

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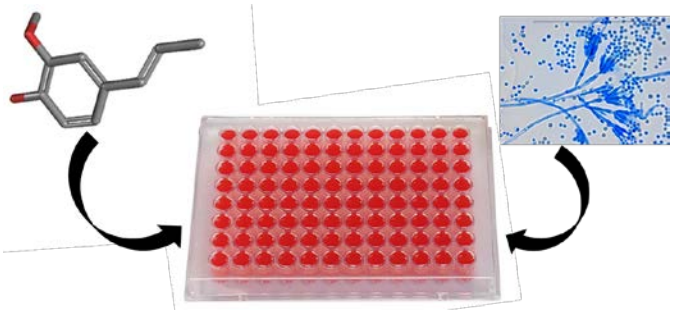
Graphical Abstract



Abstract.

Human mycoses have controversial treatment, since the available antifungal drugs besides favoring the appearance of resistant isolates, can present great toxicity to the human organism. This fact has driven the search for more efficient, safe and natural therapeutic alternatives with the objective of reversing this scenario of resistance presented by pathogenic fungi, for this, new natural and effective alternatives, such as the use of plant extracts, natural compounds and semi-synthetic, have been extensively investigated for the development of new drugs. In view of this scenario, this study aims to evaluate the antifungal activity of isoeugenol in vitro against strains of *Penicillium citrinum*. For this, the broth microdilution technique was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC) of isoeugenol and voriconazole. The concentrations by which the strains were submitted, 1024 µg/mL to 0.5 µg/mL, were obtained by means of serial dilution at a ratio of two, so that in the first row of the plate is the highest concentration and in the last , the lowest concentration. Finally, 10 µL of the fungal inoculum of each isolate was added to the wells, where each strain was placed on a plate

column. MIC was defined as the lowest concentration capable of inhibiting fungal growth visually verified by maintaining the original coloration of the medium. After reading the MIC, aliquots of 20 μL of the supernatant from the wells where complete inhibition of fungal growth in the microdilution plates were observed were plated on Sabouraud dextrose agar plates. Plates were incubated, and MFC was considered the lowest concentration at which growth was less than 3 colonies. The assays were performed in triplicate and the geometric mean was calculated. The CIM of isoeugenol varied between 256 and 32 μg/mL, being the highest MIC value for the LM-21 strain and the lowest value for the LM-02, LM-03, LM-08, LM-155, LM- 157 and LM-161. The MIC range of voriconazole was 0.5 to 256 μg/mL. The MFC of isoeugenol varied between 64-512 μg/mL and for voriconazole it was 2 μg/mL and above 1024 μg/mL. In this way, we can conclude that isoeugenol presented an antifungal effect, which enables it as a potential antifungal drug, requiring complementary tests that clarify the mechanism of action involved in its antimicrobial activity.

Introduction

The indiscriminate use of antifungal compounds led to the emergence of multiresistant microorganisms for the drugs used in the medical routine. Antifungals considered first choice, especially for dermatophyte and nondermatophytic fungi and new opportunistic pathogenic fungi isolated from immunocompromised patients, have become intrinsically resistant, with resistance developed in response to drug exposure during pharmacological treatment [1-4].

This problem is further accentuated, since treatment with antifungal drugs available in addition to favoring the emergence of resistant isolates can present great toxicity to the human organism. Furthermore, the increase in the number of immunocompromised patients, associated with a greater resistance of fungal strains, poses a challenge to the pharmaceutical industry in the search for new therapeutic agents that present greater safety, efficacy and low toxicity [5-6].

Given this scenario, there was a greater search for more efficient, safe and natural therapeutic alternatives. As alternatives, plants are extremely rich sources of molecules with medicinal potential, however, only about 25% of the medicines produced in Brazil are of vegetable origin. However, the academic interest in this topic has been growing, especially due to indications of empirical origin [7-9]. Among the secondary metabolites produced by plants, we have the essential oils. They are volatile compounds extracted from plants, often as part of the natural defense of plants against microorganisms.

There is still a lot to learn about the mode of action of essential oils, but a common and important feature is their hydrophobicity, which allows interaction with the cell membrane, leading to leakage of important ions and other compounds [10].

Isoeugenol is an essential oil of the subgroup of phenylpropanoids, being chemically designated as 2-methoxy-4- (1-propenyl) phenol, it is naturally present in a naturally occurring phenolic constituent of clove oil, monkey orange, basil, the petunia flower [11-12]. It's used in perfumes, soaps, detergents, air purifiers and as a flavoring agent in cosmetics and food products. In addition, isoeugenol has a propenyl moiety and beneficial properties such as antioxidants and anti-inflammatory [13-16].

The aim of the present study was to verify the in vitro antifungal effect of isoeugenol against strains of *Penicillium citrinum*. The objective of the present study was to verify the in vitro antifungal effect of isoeugenol in the literature on the antimicrobial activities of essential oils and of the various biological properties of phytoconstituents.

Materials and Methods

Isoeugenol and voriconazole were purchased from Sigma-Aldrich® (São Paulo, SP, Brazil). The solutions were prepared at the time of the tests, dissolving them first in 5% dimethylsulfoxide (DMSO) and 2% Tween 80 (Sigma-Aldrich®, São Paulo, Brazil), and using sterile distilled water to perform the dilutions employed in the tests.

The fungal strains used were of the species Penicillium citrinum of clinical origin (LM-02, LM-03, LM-04, LM-08, LM-30, LM-145, LM-155, LM-157, LM-161, LM -171 and LM-278) and standard INCQS 40011 (National Institute for Quality Assurance in Health) and performed an inoculum a fungal suspension and adjusted 0.5 scale Mc Farland, which corresponds to approximately 1-5 x 106 CFU / mL [17-19].

For the determination of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of isoeugenol and voriconazole were carried out using the broth microdilution technique [17-20]. Sterile, capped 96-well plates were used. At each well in the plate, 100 µl of doubly concentrated RPMI-1640 was added. Then 100 µl of the solution of the doubly concentrated products were dispensed into the wells of the first row of the plate. The concentrations by which the strains were submitted, 1024 µg / mL to 0.5 µg / mL, were obtained by means of serial dilution at a ratio of two, so that in the first row of the plate is the highest concentration and in the last , the lowest concentration. Finally, 10 µL of the fungal inoculum of each isolate was added to the wells, where each strain was placed on a plate column.

The plates were then aseptically closed and incubated at the temperature and time suitable for the reading. Subsequently, the results were observed observing the change of the RPMI medium. MIC was defined as the lowest concentration capable of inhibiting fungal growth visually verified by maintaining the original (pink) coloration of the medium. The assays were performed in triplicate.

After reading the CIM, aliquots of 20 µL of the supernatant from the wells where complete inhibition of fungal growth in the microdilution plates were observed were seeded on Sabouraud dextrose agar plates and incubated. CFM was considered the lowest concentration in which growth was less than 3 colonies (approximately 99 to 99.5% of death activity). The assays were performed in triplicate and the geometric mean was calculated [21].

Results and Discussion

According to results obtained from the Minimum Inhibitory Concentration test described in Table 1, we can observe that the MIC of isoeugenol ranged between 256 and 32 μg/mL among the strains, being the highest MIC value for the LM-21 strain and the lowest value for LM-02, LM-03, LM-08, LM-155, LM-157 and LM-161 strains.

Table 1 - Minimum Inhibitory Concentration and Minimum Fungicide Concentration values for isoeugenol and voriconazole against *Penicillium citrinum* strains.

Strains of *Penicillium citrinum*	Isoeugenol (μg/mL)		Voriconazol (μg/mL)		*C1	**C2
	MIC	MFC	MIC	MFC		
INCQS 40011	64	64	256	+	+	-
LM-02	32	64	64	128	+	-
LM-03	32	64	0,5	2	+	-
LM-04	256	512	+	+	+	-
LM-08	32	64	0,5	+	+	-
LM-30	128	256	8	16	+	-
LM-145	64	128	32	32	+	-
LM-155	32	64	64	64	+	-
LM-157	32	64	1	4	+	-
LM-161	32	64	2	8	+	-
LM-171	64	64	2	8	+	-
LM-278	64	128	256	+	+	-

* C1 - Control of microbial growth: wells containing RPMI-1640 broth, DMSO (5%), Tween 80 (2%) and the inoculum of each strain, in the absence of the phytoconstituent or antifungal. ** C2 - Culture medium sterility control: wells containing RPMI-1640 broth, DMSO (5%), Tween 80 (2%), in the absence of the phytoconstituent or antifungal. (+): fungal growth. (-): absence of fungal growth.

After the MIC of isoeugenol was found for the strains of *P. citrinum*, the Minimum Fungicide Concentration (CFM) was determined. As described in Tables 2 and 4, we can observe that isoeugenol presented a CFM at the concentration of 2 μg/mL for 8.33% of the strains, from 4 μg/mL to 16.7% of the strains, 8 μg/mL for 33.33% of the strains, from 16 μg/mL to 41.67% of the strains, from 32 μg / mL to 50% of the strains, from 64 μg/mL to 58.33% of the strains, from 128 μg/mL to 66.67% of the strains and 512 μg/mL to 100% of the samples.

Pizzolitto et al. [22] also screened strains of the filamentous fungus *Aspergillus parasiticus* using the phytochemicals: thymol, carvacrol, eugenol, isoeugenol, creosol, m-Creosol, p-Creosol, o-Cresol and phenol. In this study, they found that isoeugenol had a MIC of 206 μg/mL, which was lower than those found in the other compounds tested, showing its potency against the microorganism, including carvacrol. This result corroborates the MIC values obtained in this study, since the highest MIC value for Penicillium citrinum was 256 μg/mL.

For the control of the experiments, voriconazole was used as standard antifungal. Although there are studies in the literature using voriconazole in several species of fungi, this study unprecedentedly carried out the determination and characterization of the antifungal activity of the substance against standard and clinical strains of *Penicillium citrinum*.

In the experiments performed in this study, the MIC of voriconazole had a considerable variation, showing a great difference of sensitivity between the strains used. The MIC range of antifungal was 0.5 to 256 μg/mL, and for LM-21 strain it was not possible to define the minimum inhibitory concentration because it was higher than 1024 μg/mL and the lowest MIC value was for strain LM-03 (Table 1).

Regarding CFM, we can observe that voriconazole presented a variation of 2 μg/mL at values higher than 1,024 μg/mL among the strains used, establishing a CFM at the concentration of 2 μg/mL for 8.33% of the strains. 4 μg/mL for 16.67% of the strains, from 8 μg/mL to 33.33% of the strains, from 16 μg/mL to 41.67% of the strains, from 32 μg / mL to 50% of the strains. 64 μg/mL for 58.33% of the strains and from 128 μg/mL to 66.67% of the strains.

It is important to note that for 33.33% of the samples it was not possible to detect CFM because there was growth above 1024 μg/mL (Table 1). Voriconazole was first introduced in clinical use in 1995 and approved by the Food and Drug Administration (FDA) in the United States in May 2002 and in Brazil in July 2012 [23-25].

It is a new anti-fungal agent of the triazole class that shows promise for the treatment of a broad spectrum of fungal pathogens, including species of *Aspergillus, Candida, Cryptococcus neoformans, Penicillium marneffei, Scedosporium apiospermum* among others [26-35].

Conclusions

The demonstration of the antifungal effect of the phenylpropanoic acid isoeugenol on strains of *P. citrinum* in vitro makes the molecule a candidate for a possible drug to be used as an agent in the fight against agricultural pests and in infections against other microorganisms.

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THERMOALKALINE PRETREATMENT INFLUENCE ON ANAEROBIC BIODEGRADABILITY OF FILTER CAKE FOR METHANE PRODUCTION

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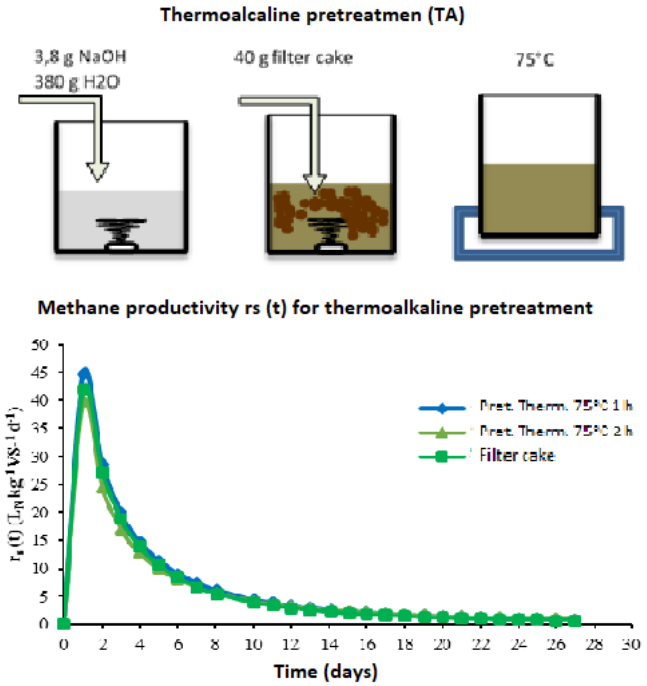
Graphical Abstract



Abstract.

Currently millions of tons of solid waste are produced worldwide, receiving only a small part of some kind of treatment, the rest are used indiscriminately pollute the environment. Complexity of these wastes requires the study of alternative methods to help improve the efficiency of the stabilization process. Thermoalkaline pretreatment study was carried out taking into account the nature of filter cake from sugar manufacturing process. Sodium hydroxide NaOH was used as chemical agent and temperature conditions of 75 0C evaluated for different times. This research was conducted in order to determine the effect of pretreatment of filter cake to increase the yield of methane.

The physical-chemical characterization of filter cake from “Melanio Hernández” Sugar Mill (Sancti Spiritus, Cuba), it was carried out through analysis of total solids (ST), volatile solids (SV) and pH. Methane yield parameter for anaerobic digestion in mesophilic conditions (37 ± 1 C) was determined. It was demonstrated that filter cake is a solid residue of sugar industry in this sector with high energy potential due to its organic content for the production of methane. Pretreatment at 1 hour had greatest increase in methane potential with respect to the untreated filter cake. These results demonstrate the relevance of the thermoalkaline pretreatment severity in terms of time and sodium loading to obtain the optimum anaerobic biodegradability of this biomass.

Introduction

The world today faces an energy crisis due to the indiscriminate use of conventional fuels (oil, natural gas and coal); and becoming more serious by the non-renewable nature of these resources and its excessive use in different countries day ago. Within the ways that humanity has to alleviate energy and environmental problems, there is a greater use of renewable energy sources, among which the use of biomass stands out.

Power generation from biomass is a renewable source with the greatest potential in Cuba, from residual cattle and pigs, production of sugar, alcohol, coffee pulping and landfills, which are today, as a whole, a means of environmental pollution. [1] (Contreras Velásquez, 2006).

Filter cake is the main residue of the cane sugar industry, producing 30 to 50 kg per ton of processed raw material, which represents between 3 and 5% of the ground cane. This percentage and its composition vary with the agroecological characteristics of the area, with the crop harvested, factory efficiency, method of clarification used, among other factors [2] (Cárdenas, G.; S. Guzmán, 1983). This high content of insoluble organic matter 85% [3] (Sánchez et al., 2005), relatively low cost and large volumes generated make this biomass an attractive source for bioconversion processes.

Main uses are reported to filter cake have been as a soil in sugarcane agriculture, cattle feeding and extraction of waxes and oils . Even with the multiple uses that the filter cake has, large quantities of this waste remain unused, which leads to serious contamination problems in the areas destined for disposal and only a small part receives some type of treatment. An effective and practical method that is applied is to subject this residual to dehydration by heat, obtaining as a result a more stable and easy to use material called melote [4] (Sarria, P, Solano, A, Preston, TR, 1990).

In Cuba, the anaerobic decomposition of filter cake has been used as an alternative treatment [5] (Cruz, 1991, [6] González et al., 1995). For this, large volumes of water are used (volume ratio 1: 4, filter cake: water) in order to dilute the high content of suspended solids. Although there are advances in the study of solid waste treatment at the international level [7] (Zheng and Zhao et al., 2014), there are still aspects to be clarified about the possibility of a previous stage of treatment, which allows the final stabilization

of complex organic material. without the need for large dilutions. Moreover, when it is known the applicability of hydrolysis for various purposes in materials with a high carbohydrate content [8] (Rodríguez-Vázquez et al., 1992), which constitutes the limiting step in the anaerobic digestion process. Currentl y, there are different methods of pretreatment including mechanical, physical, thermal and chemical, as well as biological methods . In the thermoalkaline pretreatment several works confirm the aforementioned [9] (Teghammar et al., 2010). [10] Gossett et al. (1982) concluded that lignin pretreated by thermo-alkaline treatment at concentrations above one g L-1 had a greater inhibitory effect for methanogens.

In general, the selection of parameters during pretreatment is an important aspect for productivity and / or methane yield in all pretreatment methods addressed. In the case of the thermoalkaline pretreatment with NaOH, it could be thought of using the cleaning waters of the equipment containing soda to pretreat the filter cake and to reduce the pretreatment in terms of water and the purchase of this chemical for its subsequent conversion to bioethanol or biogas, which offer alternative solutions to use of economic and environmental potential of the biomass.

The objective of this investigation was to determine the effect of the thermoalkaline pretreatment with NaOH on the biodegradability of filter cake to increase the methane yield.

Materials and Methods

The filter cake used in the experiments was collected during the 2015 harvest in "Melanio Hernández" Sugar Mill, province of Sancti Spiritus, Cuba. The filter cake was air dried for 72 hours, and then stored at 4 °C in nylon bags.

- Analytical methods

Physical-chemical characterization of the filter cake consisted in the analysis of total solids (ST), volatile solids (SV) and pH, according to the standard methods [11] (APHA, 2012).

The pH was measured with a Crison 52-11 electrode, connected to a Crison GLP 22 pH / mV meter. The resolution of the reading is 0.01 pH units and the accuracy of ± 0.01. Calibration was performed with standard CRISON buffer solutions of pH 7.02 and 4.00 at 20 °C. The samples were mixed with water at a 1:10 ratio and stirred at 150 rpm for 20 minutes (VDI 4630 2005).

- Thermoalkaline pretreatment with sodium hydroxide (NaOH)

Thermoalkaline pretreatment with NaOH was carried out in two times, the first two repetitions in a time of 1 hour and the other two in 2 hours. To the mixture was added 3.8 g of sodium hydroxide, 40 g of filter cake and 380 g of water to keep the dilution of 10 parts thereof were hermetically sealed and covered with aluminum foil to prevent heat loss to the environment and placed in a thermoreactor, reaching the required temperature after 29 minutes of having put them in the equipment.

- Production and determination of methane potential.

Trials were carried out in batch, in triplicate, with the pretreated filter cake and without pretreatment in polyethylene reactors of 2 L capacity, at a constant temperature of 37 ± 1ºC. The reactors were placed in an incubator to keep the temperature constant.

For the test batch was used as an inoculum source one digestate plant family biogas hog manure substrate. The substrate mass was determined from equation 1.

$$p_i = \frac{m_i \cdot c_i}{m_s \cdot c_s} \qquad (1)$$

Where:
mi: inoculum mass (kg)
ms: substrate mass (kg)
ci: volatile solids concentration of inoculum (gkg-1)
cs: volatile solids concentration of substrate (gkg-1)

Volatile solids concentration of inoculum and of substrate were determined according to standard methods (APHA, 2012).
In the experiment, a control reactor was used (bottle with inoculum without substrate), with the objective of subtracting in the determination the methane formed from the organic matter contributed by the inoculum. All the bottles were shaken manually once a day to favor contact between the substrate and the microorganisms, re-suspend the sediments and break the layer of floating material.
The reactors were connected to graduated cylinders filled with 3% NaOH solution in order to dissolve the CO_2 content. Cumulative methane production was measured by liquid displacement. The gas pressure is calculated based on the height of liquid column and subtracted from atmospheric pressure before normalization (273 K and 101.29 kPa). The methane volume was standardized. The digestion process was stopped at 21 days when there was no more than 1% by volume of methane daily.
To refer to the volume of gas produced v1, measured at ambient temperature and pressure T_1 and p_1, at standard conditions T_0 and p_0 as standardized volume v_0, equation 2 was applied according to manual VDI-4630 (2006). Before normalizing the volume of methane was subtracted the volume produced in control reactor (inoculum without substrate).

$$v_0 = v_1 \cdot \frac{(p_1 - p_w) \cdot T_0}{p_0 \cdot T_1} \qquad (2)$$

Where:
v_0: Normalized methane volume (Nm3)
v_1: Volume of ethane measured at temperature T_1 and pressure p_1 (m3)
p_1: Pressure at which methane (mbar) was measured
p_w: Vapor pressure of water at temperature T_1 (mbar)
T_1: Temperature at which methane was measured (K)
p_0: Normal pressure (1013.25 mbar)
T_0: Normal temperature (273.15 K)

Methane potential, defined as the amount of methane generated per amount of substrate was determined according to equation 3 (VDI-4630, 2006), during a time of digestion. The values obtained are represented in a cumulative biogas yield curve and B (mLNgSV-1) over time.

$$y_B = \frac{\sum_{t=0}^{t=21-29} V_B}{m_s} \qquad (3)$$

Where:
VB: Cumulative methane volume during the digestion time t (Nm3)

ms: Mass of substrate added to the reactor in terms of volatile solids (kgSV)

Another parameter of interest in the evaluation of the discontinuous anaerobic digestion process is the specific productivity of methane rs (t). His determination was performed using the Hill model [12] (Mahnert, 2007) according to (Equation 8).

$$r_{s(t)} = y'_{CH4(t)} = y_{CH4max} \cdot \frac{b.c^b.t^{b-1}}{(c^b + t^b)^2} \qquad (8)$$

Where:

y_{CH4} (t): Cumulative methane production (mLNg-1SV-1)

y_{CH4max}: Maximum methane yield (mLNg-1SV-1)

t: Digestion time (d)

b, c: Model coefficients

Results and Discussion

Filter cake used for biological pretreatment and anaerobic digestion is characterized in triplicate (Table 1). These results are similar to those reported by other authors (Sánchez et al., 2005; [13] Meunchang et al, 2005; [14] Baez-Smith, 2008; [15] Radjaram and Saravanane, 2011; [16] Lopez, 2013).

Table 1 shows the high content of organic matter, given by the values of volatile fractions, SV / ST and SV / MF of 80.38 and 76.32%, respectively.

The drawback inherent treatment of filter cake, is given by the intrinsic characteristics of the material, wherein the carbon source is largely insoluble form which hinders its metabolization. So that the microorganisms can assimilate the organic matter, this has to be in dissolved form, so that, if part of a complex waste, as it is this type of waste, it is necessary an initial stage of pre-treatment to help to the formation of simpler molecules that can be easily used by microorganisms in the subsequent biological treatment. This, together with the favorable conditions of pH and content of organic matter, will favor the final stabilization process.

Table 1. Chemical characterization of filter cake

Parameters	Unit	Filter cake	Values reported
pH		5.4±0.06	7,5[a], (4,5 - 5)[c], 7,7[d], 5,5[f]
ST	%MF	9.95 ±2.01	10[a], 9,09[b], 29[c], 20[e], 6,28[f]
SV	%ST	80.38±5.02	83,91[f]
SV	% MF	76.32 ±5.22	

Data are expressed as the mean value ± standard deviation. All percentages are on a dry basis, except for TS.

[a] Rouf et al., (2010); [b]López-González, 2013; [c] Radjaram y Saravanane (2011); [d] Meunchang et al., (2005); [e] Baez-Smith (2008); [f] Sánchez et al., (1996).

pH was 5.4, a value that is in the range reported by Meunchang et al., (2005), Radjaram and Saravanane (2011); Rouf et al., (2010); Sánchez et al., (1996). pH variation is due to the process of generation of filter cake, collection method, preservation and determination. Chemical composition of the filter cake

depends on a variety of factors including the variety of sugar cane, soil, nutrient, clarification process adopted, filtering operation, and other environmental factors (Velarde et al., 2004).

- Methane productivity

Specific productivity of methane behavior rs (t), according to model used by Mähnert 2007 is showing in figure. 1. The rs (t) increased the first days reaching the maximum value before day 2 with a maximum value of 44.73LN kg-1VS-1 d-1. Most of time the productivity of reactors was lower than that of untreated filter cake of 0-9 days, which indicates a longer delay phase.

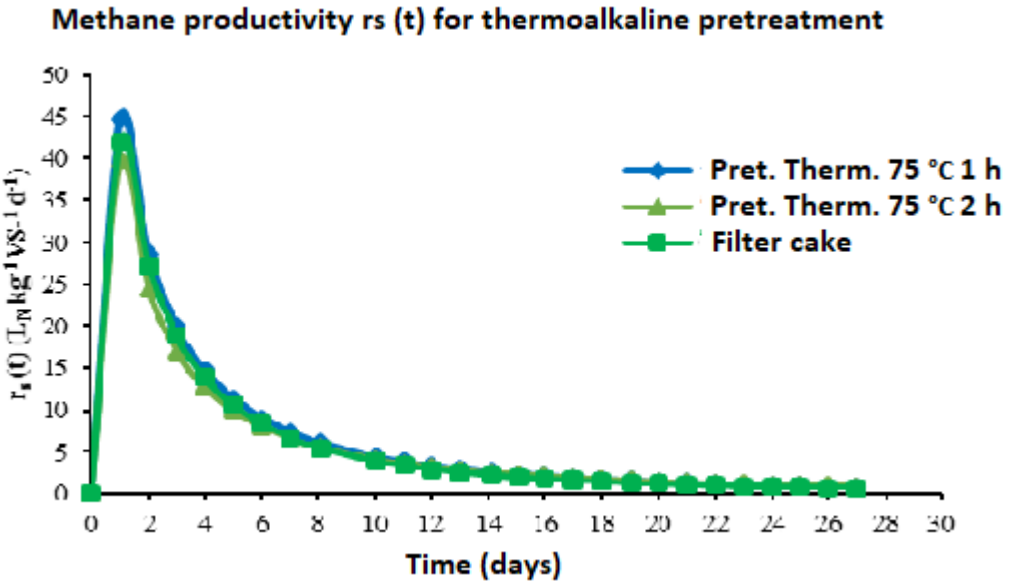


Figure 1 Specific Productivity of Methane rs (t) for pretreatment thermoalkaline

Conclusions

1. Filter cake is a solid residue of the sugar industry with high energy potential for the production of methane by its organic content.
2. The severity in the alkaline pretreatment in concentration and time drastically reduces the benefit of methane yield, which indicates the formation of unwanted byproducts.

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Application of molecular topology to the prediction and optimization of mosquito repellent activity of N-acyl-piperidine derivatives

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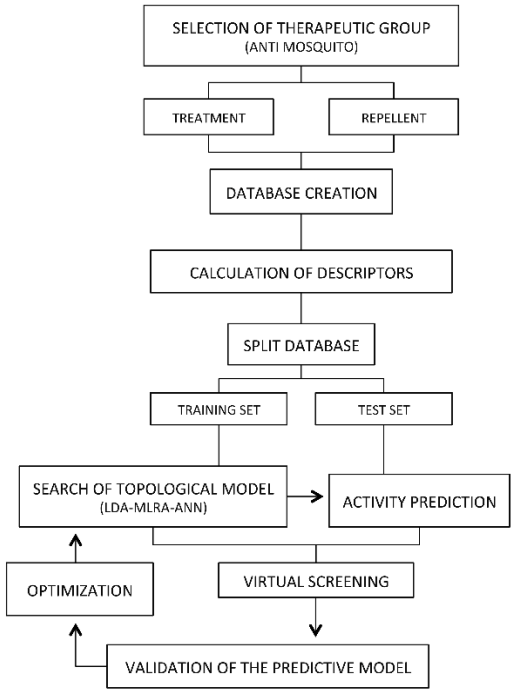
Graphical Abstract



Steps to follow in the search of QSAR prediction models by molecular topology

Abstract.

A topological-mathematical model has been developed based on Multilinear Regression Analysis in order to search new active molecules with mosquito repellent activity. The molecular characterization was performed using topological indexes and a 5-variable model was chosen for prediction of protection times (R^2 = 0.8457 and Q^2 = 0.7486). The model was validated by an internal leave-one-out type cross-validation and a randomization test. The results confirmed the predictive power for the property under study. Finally, after carrying out a virtual screening, new compounds have been proposed with expected higher potency as mosquito repellents.

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Exploring different strategies to improve the magnetic response of cobalt doped ferrite nanoparticles

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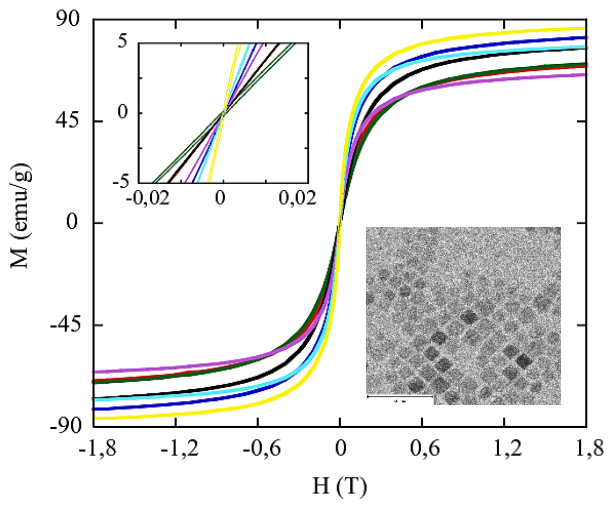
Graphical Abstract



Abstract.

With the aim of studying the structural and magnetic properties of cobalt doped magnetite nanoparticles ($Fe_{3-x}Co_xO4$), several samples were synthetized by thermal decomposition method using different cobalt concentrations (0 – 5%) at different condition reflux time (30 – 120 min). In this work, we demonstrate that the synthetic parameters highly influence. Both, the morphology and the cobalt concentration, obtaining higher saturation magnetization values for 2 hours reflux. An exhaustive magnetic characterization by means of magnetization and electronic magnetic resonance has established conditions to improve the magnetic response of doped nanoparticles.

Introduction *(optional)*

The application of magnetic nanoparticles in therapies based on localized magnetic hyperthermia depends directly on their size, composition and biocompatibility. Hyperthermia therapy is based on the fact that tumor cells are more thermosensitive than healthy cells, so that raising the temperature to a certain value could selectively damage the cancer cells [1]. They must present sufficient size to generate the necessary heat in the localized area and produce the necrosis always maintaining its superparamagnetic character. Certainly, the magnetic saturation derived from the composition of the material will be primarily responsible for the generation of heat in magnetic hyperthermia, being one of the key factor that defines the possible efficacy of such technique [2].

With the aim of studying the structural and magnetic properties of cobalt doped magnetite nanoparticles ($Fe_{3-x}Co_xO_4$), different cobalt concentrations (0 – 5%) and different synthesis conditions have been employed to prepare the nanostructures. Nanoparticles have been structural and morphological characterized and magnetic properties have been studied by measuring magnetization at different temperatures and applied fields. Electron Magnetic Resonance (EMR) measurements have been carried out as this technique can provide useful information on particle evaluation, shape and surface effects or inter-particle interactions.

Materials and Methods

Several samples were synthetized by thermal decomposition of iron (III) acetylacetonate and cobalt (II) acetylacetonate in a solvent of a high boiling point and in the presence of oleic acid, oleylamine and 1, 2-hexadecanediol [3 – 4]. The mixture was first heated at 200 ºC for 30 min (nucleation phase) and then was ramped with a heating rate of about 2º/min to the reaction temperature of 295ªC in argon atmosphere. Synthesis was performed at various times to investigate the morphological evolution of the NPs. After the solution was cooled to room temperature, the resulting nanoparticles were precipitated with ethanol and magnetic separated using a magnet.

Results and Discussion

The structural characterization of samples was made by X-ray diffraction (XRD). The intensity and peak positions of the diffraction patterns are in good agreement with face-centred cubic phase of magnetite (Fd-3m, JCPDS No. 89-0691). No traces of any kind of detectable impurities were observed. The main effect of changing the reflux times is related to the amount of cobalt introduced in the ferrite lattice, as confirmed by ICP analysis. Longer reaction times give rise to an increase in the effectiveness of doping due to the higher temperatures needed for the decomposition of the Co-precursor in comparison with the Fe one. Mass evolution observed in thermogravimetric analysis under Ar flow has been similar for all samples. The first great mass loss (of around 10%) occurs between 300 and 420 ° C, which can be associated to the decomposition of weakly attached surface functional coating or capping groups. The second mass loss of about 20%, between 550 and 650 ° C is related with the final decomposition of ligands surrounding the magnetic core. The total amount of organic matter is between 28 - 44%. TEM micrographs of all samples show well-dispersed homogeneous nanoparticles with sizes between 6 and 11 nm and morphologies depending on the reaction times, varying from spheric, cuboctahedral to cubic.

Magnetic studies have been performed by macroscopic magnetometry at different temperatures and fields. Measurements of magnetization versus temperature after Cooling at Zero Field (ZFC) and Field (FC) show the usual characteristics of a superparamagnetic (SPM) behaviour. The substituted samples blocking temperatures, T_B, vary in the 46 – 110 K interval depending on size, anisotropy constant and Co content, in good accord with the Stoner-Wohlfarth theory [5]. The SPM character of samples has been additionally confirmed by the absence of coercive field (H_C) in the hysteresis loops recorded at R.T. Magnetization values obtained at R.T. vary between 65 and 86 $emu.g^{-1}$, corresponding the maximum values to samples with the highest Co contents and with the highest times of reflux temperatures. Nevertheless, variations of the saturation values from one sample to another has been analyzed and interpreted taking into account the modulation of antiferromagnetic interactions between T_d and O_h sites occupied by magnetic cations. EMR spectra recorded at R.T in colloidal medium complete the information concerning the magnetic characteristics of the different samples. The spectra exhibit anisotropic lines different than the previously observed nearly Gaussian lines, characteristic of magnetite nanoparticles [3]. This fact corroborates the formation of cobalt doped ferrite nanoparticles.

Conclusions

This work provides a deeper insight into the obtaining of cobalt doped ferrite nanoparticles with $Fe_{3-x}Co_xO_4$ general formula. The ability to tune the amount of cobalt in the ferrite lattice is due to variations of reaction times during the synthesis. These changes do not seem to affect significantly the average particle size or the size distribution. Magnetic data confirm the dependence of blocking temperatures with the cobalt content. Thus, higher magnetizations are related to longer reflux time and, consequently, with higher amounts of cobalt in the ferrite nanoparticles.

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Evaluation of the Interference of Solvents Used in the Evaluation of Antimicrobial Activity of Liposoluble Natural Compounds

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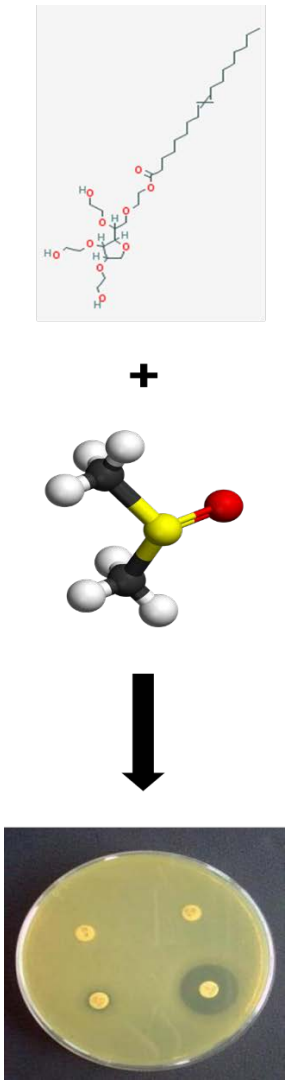
Graphical Abstract



Abstract.

Because it is an activity already consolidated throughout the ages, the use of compounds from plants has been well studied and tested for definition or proof of its antibacterial activity. Despite the difficulties encountered as the solubility of essential oils, there are compounds that help in experiments, they are called solvents and emulsifiers and the most used in phytotherapeutic tests are: ethyl acetate, acetone, ethyl alcohol, methyl alcohol, neutral detergent (phosphates free), dimethylsulfoxide (DMSO), triton X-I00 and polysorbate 80 (tween 80). In view of this fact, the present work seeks to identify concentrations of polysorbate 80 (Tween 80) and dimethylsulfoxide (DMSO) capable of performing an antibacterial activity, due to its wide use in the scientific environment, against the following bacterial strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 25922 and *Enterococcus faecalis* ATCC 29212. The evaluation of the activity of the compounds was performed by the diffusion disc method. This method is the one recommended by the Clinical and Laboratory Standards Institute and is based

	on the diffusion through the agar of a reagent impregnated in a disc of filter paper and the diffusion of the same leads to the formation of a halo of inhibition of growth of the micro-organisms whose diameter is inversely proportional to the minimum inhibitory concentration. This method is qualitative, that is, it allows to classify the bacterial sample as susceptible, intermediate or resistant to antimicrobial. The tests were carried out with different concentrations of the reagents to determine the antimicrobial effect of the studied solvents. The experiments were run in triplicate at all concentrations using the compounds in combination (DMSO + Tween). The incubation was done in a greenhouse at 35 ± 2 ° C, for a period of 24 hours. The tests were performed and the results expressed in mm by the arithmetic mean of the diameter of the inhibition halos, formed around the discs. As results, no values were determined that determined antimicrobial activity, and it is not possible to determine MIC when the formed halo is equal to or less than 6 mm or when there is no formation thereof. In view of the results, it can be observed that the compounds may not present activities against the microorganisms tested or, due to their physico-chemical characteristics, suffer some interference, such as the diffusion difficulty in the agar, its insolubility in water and chemical complexity.

Introduction

The use of medicinal plants for the treatment, cure and prevention of diseases is one of the oldest forms of medicinal practice of mankind. As early as the early 1990s, the World Health Organization (WHO) reported that 65-80% of the population in developing countries depended on medicinal plants as the only form of access to basic health care [1].

In the scientific field, extracts and essential oils from plants are used as natural sources of new compounds to combat bacterial infections [2]. However, the estimation of the antibacterial activities of many plant-derived compounds is hampered due to their low solubility in water. Solubilizers, such as surfactants and solvents, have been used to solve this problem, but it may be difficult to distinguish the contribution in the antimicrobial activity of the solubilizer from the compounds under investigation [3]. Among the most used solvents and emulsifiers in phytomedicine tests are: ethyl acetate, acetone, ethyl alcohol, methyl alcohol, phosphates free, dimethylsulfoxide (DMSO), triton X-I00 and tween 80 [4].

Dimethyl sulfoxide (DMSO) is the organic solvent most commonly used in biochemical and cellular assays during drug discovery programs [7]; is an aprotic solvent of universal use with the ability to permeate biological membranes, and therefore is commonly used to obtain the appropriate biological availability of hydrophobic toxic substances [8]. Mi et al [9] also emphasizes that the popularity of DMSO in both the pharmaceutical and antimicrobial industries is due to several factors, including: (i) low toxicity, (ii) organic and inorganic dissolution capacity (iii) the ability to remain in a liquid state over a wide temperature range (e.g., 19Â ° C to 189Â ° C), (iv) ability to improve cell membrane permeability, and (v) miscibility in water and a wide range of organic solvents.

However, surfactants may interact with organisms and drugs affecting the in vitro activity of antimicrobial agents. According to Gomez-Lopez et al. [10] the surfactant could modify the solubility of the antifungal, developed in a medium and aid in the precipitation of the agent, leading to the increase of MIC. According to Hammer et al. [11], when using an emulsifying agent, it is necessary to take into account the possible interactions between this agent and the components of the essential oil, besides the possible antimicrobial activities that can be presented by the same. For them, these effects may vary according to the ratio of essential oil and emulsifier, which makes it essential to use this association appropriately.

Considering the reports presented, and taking into account also the fact that, to date, no standard amount of these agents has been defined, the present study aims to standardize the minimum inhibitory concentrations of Tween 80 and DMSO, considering its wide use in the scientific milieu, against the following bacterial strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 25922 and *Enterococcus faecalis* ATCC 29212.

Materials and Methods

Diffusion tests were performed with different concentrations of polysorbate 80, (ween 80), and dimethylsulfoxide (DMSO) capable of performing an antibacterial activity of the five microorganisms. Therefore, to test all concentrations, three petri dishes were used for each strain tested. The incubation was done in an oven at 35º C, for a period of 24 hours.

The microorganisms used for the tests were bacterial strains: Gram positive *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212; Gram negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Proteus mirabilis* ATCC 25922.

The tests were performed and the results expressed in mm by the arithmetic mean of the diameter of the inhibition halos formed around the discs during the disk diffusion test. Then, five microorganisms were suspended, the turbidity degree of which was 0.5 McFarland scale, corresponding to 1 x 10^8 CFU / mL, which was spread with the aid of a swab. After sowing each disc was impregnated with different concentrations of the reactants and pressed against the plate in order to ensure complete contact with the agar surface, being applied individually and evenly distributed, so that the distance from the center of the disc to the edge not exceed 24 mm. Plates containing Müller-Hinton Agar were inverted and placed in an oven at 35 ° C for 24 hours after application of the disks therein.

Results and Discussion

The disc diffusion test is accepted by the FDA (Food and Drug Administration) and established by CLSI (Clinical and Laboratory Standards Institute). This method was idealized by Bauer et al. in 1966, and since then it has been one of the methods most used in clinical microbiology laboratories in

Brazil to test antimicrobial susceptibility. The principle of this method is based on the diffusion through agar of an antimicrobial agent impregnated on a filter paper disc, which leads to the formation of a bacterial growth inhibiting halo whose diameter is inversely proportional to the minimum inhibitory concentration. This method is qualitative, that is, it allows to classify the bacterial sample as susceptible, intermediate or resistant to antimicrobial [9-11].

The reading of the results was performed by measuring the diameter of the halos, in mm, formed around the disks containing the reagents that when greater than 6 mm becomes visible and indicates susceptibility of the microorganism to the substance tested [2].

Tables 1 and **2** were designed with the purpose of indicating the sizes of inhibition halos that were or were not formed by the microorganisms studied, whose values represent the arithmetic mean of the results in triplicate.

Table 1: Verification of the antimicrobial activity of the association of DMSO + TWEEN 80 against gram positive strains in different concentrations in the fusion disc method.

Bacterial strains	Substances / Halo (mm)							
	AMC	DMSO/Tween 80 (%)						
		40/32	20/16	10/8	5/4	2,5/2	1,25/1	0,625/0,5
S. aureus ATCC 25923	30	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E. faecalis ATCC 29212	22	Ø	Ø	Ø	Ø	Ø	Ø	Ø

AMC: Amikacin disc (30 μg), Ø absence of inhibition halo of bacterial growth.

As noted in **Table 1**, it can be noted that there was no inhibition of growth of the tested microorganisms. This shows that a reaction may have occurred between the solvents, dimethylsulfoxide and polysorbate 80, not allowing them to exert their action on the bacterial strains tested. In addition, several other factors may have contributed to this result, such as the chemical characteristics of the solvents that may hinder the dispersion in the culture medium, as well as other interferences of the test adopted.

Other interferences were reported by Nascimento [8], who found that the disc diffusion method may have several interferents, such as oil volatility, diffusion difficulty in agar, its insolubility in water and chemical complexity.

Table 2 shows the results of the tests on the gram negative bacteria *E. coli*, *P. mirabilis* and *P. aeruginosa*, where it was also evident the absence of interference in the compounds for the bacterial strains.

Table 2: Verification of the antimicrobial activity of the association of DMSO + TWEEN 80 against gram negative strains in different concentrations in the fusion disc method.

Bacterial strains	Substances / Halo (mm)							
	AMC	DMSO/Tween 80 (%)						
		40/32	20/16	10/8	5/4	2,5/2	1,25/1	0,625/0,5
E. coli	29	Ø	Ø	Ø	Ø	Ø	Ø	Ø

ATCC 25922							
P. mirabilis ATCC 25933	22	Ø	Ø	Ø	Ø	Ø	Ø	Ø
P. aeruginosa ATCC 27853	33	Ø	Ø	Ø	Ø	Ø	Ø	Ø

AMC: Amikacin disc (30 μg), Ø absence of inhibition halo of bacterial growth.

Gram negative microorganisms have a more complex morphology than gram positive bacteria, that is, they have an extra membrane (external membrane), which hinders the action of solvents, become an obstacle to their reach and action. In addition, gram positive microorganisms differ in the organization of their structures that are found externally to the plasma membrane where there is a structure, which is thick and has a large layer of peptideoglycan called the outer membrane [12, 13]. Another difficulty in the analysis of the results obtained in this experiment is that there is no standardization for the technique to be used in this type of assay for said reagents.

In a study developed by Estrela [14], it was observed that substances with different capacities of diffusion and dissociation using the techniques of liquid medium and diffusion in agar, and observed that the first one would be more effective precisely because of the difficulty that some substances have to diffuse on agar.

Conclusions

From the results found, it became evident the need to standardize the concentrations in use of the appropriate reagents, since several experiments are carried out using these microorganisms with variations in the concentrations of the same ones, without knowing that they can interfere in some way in the result of the experiment.

New studies should be carried out, since this is a pioneer in this line of research, seeking to identify the concentrations of solvents used that may cause interference or even inhibit the growth of the mentioned microorganisms or others, including using other methodologies with less interference.

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Antibacterial Potential Of The Alpha-pinene Positive Enantiomer Against The Strain *Proteus mirabilis*

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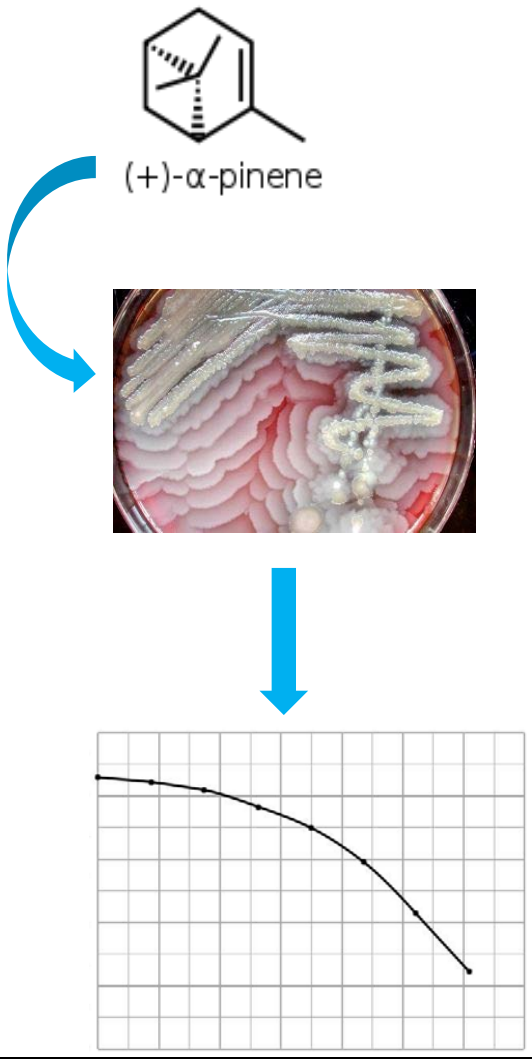
Graphical Abstract



Abstract.

Essential oils are complex mixtures of volatile aromatic compounds derived from the secondary metabolism of plants, and which have several chemical components responsible for their therapeutic and organoleptic properties, among them, the class of terpenes, specifically alpha-pinene, a compound organic, which acts as an antibacterial agent. Among the multiresistant strains, the gram-negative species *Proteus mirabilis* is responsible for causing urinary tract infections. Thus, the present study aims to evaluate the antibacterial potential of (+) - alpha-pinene against the ATTC (American Type Culture Collection) standard strain of *Proteus mirabilis* ATCC 25933. Knowing the sensitivity of the bacterial strain, after the test of microdilution in broth, we intend to evaluate the antibacterial potential, ie the time by which the compound was able to act to eliminate the bacterial strain. For this, the present work used the methodology of Time Killing (Bacterial). In this sense, the strain was initially peeled into Mueller Hinton Agar (AMH) 24 hours prior to the test. For the analysis of the death curve in the time intervals 0, 2, 4, 8 and 24 hours of the MIC and MICx2 of the α-pinene, MIC of the amikacin and negative control, that is, the culture medium plus the bacterial inoculum. A 10 µL aliquot of the well contents and diluted in a 0.9% physiological solution

	was withdrawn, thereby forming a suspension, and an aliquot of 10 μL of this new dilution was then withdrawn and plated on plates containing Mueller Hinton agar with the aid of a Drigalski handle. This procedure was repeated at times t0, t2, t4, t8 and t24. The plates were then incubated at 35 ± 2 ° C for 24 hours and the number of colony forming units (CFU) counted, adjusting with the dilution factor used in each procedure. It is worth noting that this work is a pioneer in the evaluation of the antibacterial activity of the positive enantiomer of α-pinene, considering that there are no reports in the literature of studies against this bacterial. The MIC of amikacin was shown to be able to totally inhibit the growth of the strain within the first two hours, and the alpha-pinene MIC inhibited the growth of *P. mirabilis* after 24 hours. Inhibition occurred progressively at times 2, 4 and 8 hours until its total inhibitiomiran at 24 hours. However, the MICx2 of the alpha-pinene was also able to inhibit the total growth of the strain, but in a less time, requiring 8 hours for its total effect. It is therefore observed that the test substance, (+) - alpha-pinene has therapeutic potential to treat infections resulting from this bacterial strain. It is hoped that this study may support the development of future research to better elucidate the mechanism of action, the clinical safety of the substance as well as the toxicity of the compound. Thus, it may be a naturally occurring compound used as a new therapeutic option in opportunistic infections caused by *Proteus mirabilis*.

Introduction

Research into new antimicrobial agents is necessary because of the emergence of resistant microorganisms and fatal opportunistic infections. Among the bacterial strains, the strain of *Proteus mirabilis*, a gram-negative anaerobic bacterial species responsible for causing opportunistic infections in the Urinary Tract stands out [1].

UTI is defined as a condition where the urinary tract is infected by pathogens that cause inflammation. It is one of the most prevalent pathologies in all age groups. It is particularly important in young, sexually active women because of the high prevalence. It is the major cause of sepsis in hospitalized patients. In fact, urinary tract infection can be considered as a syndromic diagnosis, which encompasses several clinical conditions such as asymptomatic bacteriuria, urethritis, cystitis, pyelonephritis, prostatitis, renal and peri-renal abscess, in various contexts of presentation [2-3].

The genus Proteus is divided into 5 species: *Proteus vulgaris, Proteus mirabilis, Proteus penneri, Proteus myxofaciens* and *Proteus hauseri*. These microorganisms are usually found in the intestinal microbiota of man and animals, soil and polluted water. The *Proteus mirabilis* strain is a Gram-negative bacterium, belonging to the family Enterobacteriaceae, where the movements of its flagella through the surface of the solid medium have a "veil" appearance [4].

In this context, essential oils are complex mixtures of volatile aromatic compounds derived from the secondary metabolism of plants and have several chemical components responsible for their therapeutic and organoleptic properties, among them there is a class called monoterpene such as alpha pinene [5].

In this way, searching for natural alternatives to combat multiresistant strains, essential oils such as: *Juniperus phoenicea, Salvia officinalis, Cupressus sempervirens, Mutellina purpurea, Thymus vulgaris,* which have monoterpene alpha-pinene, which has antibacterial activity [6]. -7]. In this sense, this research becomes relevant as it seeks a new therapeutic alternative, using the phytoconstituent in clinical practice, using it alone or in association with antibiotics, making it possible to effectively expand the arsenal to combat bacterial infections.

Thus, the development of research of this kind can contribute significantly to the development of the health field worldwide, finding more effective substances in the race against resistance and the appearance of pathogenic microorganisms. Therefore, it is intended with this study to evaluate the antibacterial potential of the alpha-pinene compound against *Proteus mirabilis* (ATCC 25933).

Materials and Methods

The laboratory tests were carried out from February to September 2017. The test substance, the phytoconstituent (+) - alpha-pinene obtained from Sigma-Aldrich do Brasil Ltda and acquired with its own resources. It is noteworthy that the compound was prepared at the time of the tests, using two solvents in the dissolution of the phytoconstituent, such as: 1% Tween 80 and Dimethyl sulfoxide (DMSO) in a proportion of 5%, in addition to the sterile distilled water to reach the concentrations.

In this context, the gram-negative *Proteus mirabilis* ATCC 25933 standard strain was used for the antimicrobial tests in the antibacterial assay using the Time-Kill method. Therefore, in the execution of the tests, it used the culture media Müller-Hinton Agar and Müller-Hinton Broth (HIMEDIA, India). Before using them, the media were solubilized in distilled water and autoclaved at 121° C for 15 minutes.

In addition, after the incubation period, the bacterial inoculum was prepared, where a direct suspension, in saline, of selected isolated colonies was made. The suspension was then adjusted to show turbidity similar to the McFarland 0.5 scale, which corresponds to 1 x 10^8 CFU/mL [8].

For assay performance, the strains were first primed on Mueller Hinton Agar 24 hours prior to the test. For the analysis of the death curve of *P. mirabilis* in the time interval t = 0 hour, t = 2 hours, t = 4 hours, t = 8 hours et = 24, of the MICs and MICx2 of the α-pinene, amicacin MIC and negative control (culture medium + bacterial inoculum). An aliquot of 10 μL was withdrawn from the well contents and seeded on plates containing Müller Hinton agar with the aid of a Drigalski loop. This procedure was repeated at times t0, t2, t4, t8 and t24. The plates were then incubated at 35 ± 2 ° C for 24 hours and the number of colony forming units (CFU) counted, adjusting with the dilution factor used in each procedure. The results were presented in log 10 CFU/mL as a function of time to verify the rate and extent of antibacterial activity at the various concentrations of (+) - α - pinene.

All experiments were performed in triplicate and the results were expressed as the mean ± standard error of the mean (e.p.m). Differences between groups were assessed by the paired t-test. Differences were considered significant when $p < 0.05$.

Results and Discussion

Currently, there are several methods to evaluate the antibacterial activity of natural products, and the most known methods include agar diffusion method, macrodilution method and microdilution. Thus,

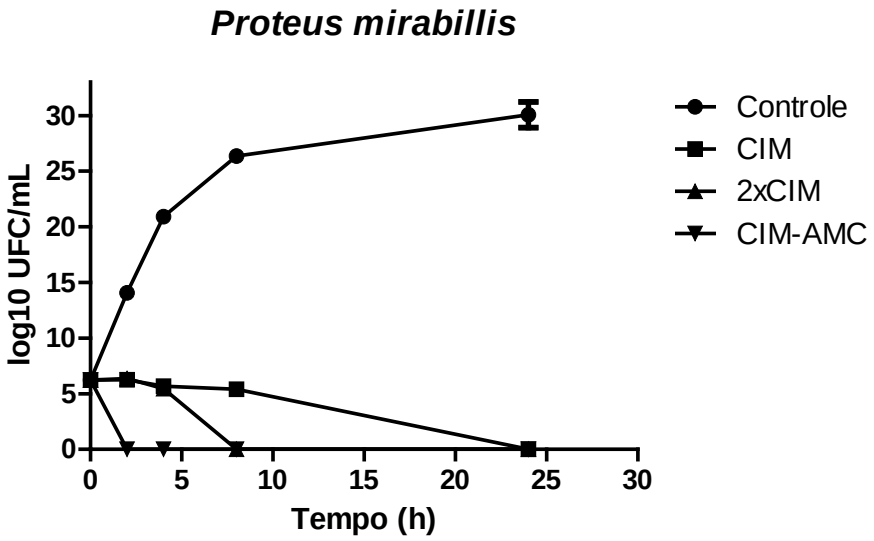
a screening was performed to evaluate the activity of alpha-pinene against the strain of *P. mirabilis* by disc diffusion methodology with concentrations ranging from 160 to 5 μL/mL and found that there was no halo formation of visible inhibition to the naked eye, so the researchers considered it resistant to all concentrations used [9].

This evidences the need to use different methodologies to evaluate a phytoconstituent, since another study using broth microdilution methodology was able to determine the MIC of alpha-pinene, which was 80 μL/mL, whereas for the disc- this determination was not possible [10].

Thus, by attempting to evaluate the antibacterial potential of the compound, i.e. the time by which it begins to develop its effect against the strain, the kinetics methodology of death was employed and it was found that the amicacin MIC was able of totally inhibiting the growth of the strain between the first two hours, which was already expected, since this antibiotic has a good action spectrum, that is, they act reaching a large number of microorganisms at the therapeutic doses.

As for the alpha-pinene MIC, it was found that it inhibited the growth of *P. mirabilis* after 24 hours. Inhibition occurred progressively at times 2, 4 and 8 hours until its total inhibition at 24 hours. However, the MICx2 of alpha-pinene was also able to inhibit the total growth of the strain, but in a shorter time, thus requiring only 8 hours for its total effect, as represented in **Graphic 1**.

Graphic 1: Death curve of the *P. mirabilis* strain (ATCC 25933) against the action of the (+) - α-pinene MIC and MICx2 concentrations and positive and negative controls.



A study using *S. officinalis* essential oil had alpha-pinene in its composition and found that the growth of the *P. mirabilis* strain was inhibited after 1h of contact with concentrations of 5 and 10 μL/mL, but the bacteria recovered its growth after 24h [11]. Proving that the compound acted faster, however, it was not able to permanently eliminate the bacterial species. Another research developed to evaluate the antimicrobial effects of the different isomers and enantiomers of the monoterpenes as (+) α-pinene and (+) β-Pinene against the gram-negative bacteria species, *Proteus vulgaris* and *C. albicans* fungus, where it was possible to verify that Time-Kill curves showed that (+) -α-pinene and (+) - β-pinene were highly toxic to *C. albicans*, killing 100% of the inoculum in 1 h. In contrast, the bactericidal effect occurred after 6h. In combination with commercial antimicrobials, ciprofloxacin plus (+) -α-pinene or (+) - β-pinene showed synergistic activity against *P. vulgaris* strain, whereas an indifferent effect against all fungi was detected when amphotericin B was combined with positive pinene enantiomers [12].

It is worth noting that this work is a pioneer in the study of the antibacterial effect of the positive enantiomer of α-pinene against the species of *Proteus mirabilis* ATCC 25933, since there is no information in the literature of research on the subject, using the methodologies used for this phytoconstituent.

Conclusions

According to the methodology used, it is concluded that the positive enantiomer of alpha-pinene has therapeutic potential to act against the bacterial species of *Proteus mirabilis* (ATCC 25933). In addition, due to the pioneering nature of the present study, there was a shortage of research that used the kinetic methodology of death, which interfered in the deepening of the discussions. Therefore, it is suggested that further studies conducted new studies using other methodologies, in order to determine more precisely the antimicrobial activity and the existence of possible toxic effects of this phytoconstituent. In addition, it is expected that this study may contribute to the development of future research related to the study of antimicrobial activity of natural products.

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Potent antioxidant activity of *Kigelia africana* flower fractions on cell-free systems.

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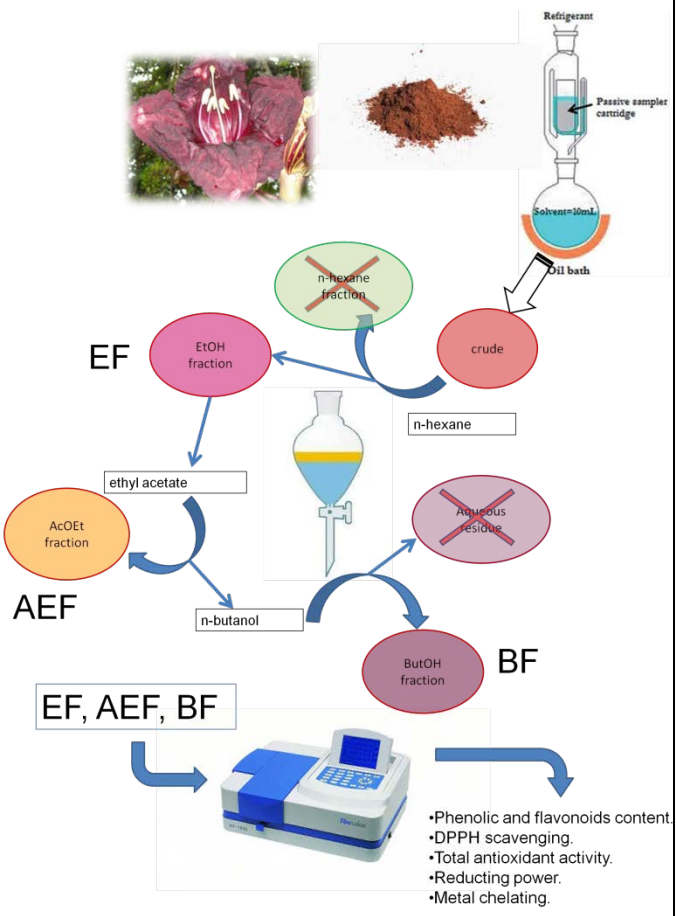
Graphical Abstract



Abstract

The aim of this work is to explore the antioxidant properties of three organic fractions obtained from *Kigelia africana* flowers on several cell-free systems. The vegetal material was subject to extraction with ethanol (90%) by soxhlet apparatus. Ethanolic **(EF)**, ethylacetate **(EAF)** and buthanolic **(BF)** fractions were obtained from crude ethanolic solution by liquid-liquid extraction procedures. Total Phenolic content (TPC) and Total Flavonoids content (TFC) were determined by Folin-Ciocalteu and $AlCl_3$ spectrophotometric methods respectively. The antioxidant and radical scavenging profile was assessed through 2, 2 – diphenyl – 1 – picrylhydrazyl (DPPH), Reducting power, Total antioxidant activity and Metal Chelating tests. Quercetin, rutin, gallic acid, hesperidin, ascorbic acid and Na_2EDTA were used as references. The antioxidant potency was strongly related with TPC and TFC values. This study reveals for the first time the antioxidant properties of *K. africana* flower fractions on cell-free systems. **Key Words:** Antioxidant, DPPH, *K. africana*.

Introduction

Many active compounds from medicinal plants, especially polyphenols and flavonoids, exhibit potential use as antioxidant agent against oxidative damage and cardiovascular disease (1), the first death cause in the world (2). The relation between oxidative stress and many human diseases as cancer, obesity, autism, arthritis, enteritis, hepatitis, diabetes mellitus, Parkinson disease, Alzheimer, cataracts, chronic renal disease, atherosclerosis and ageing are well documented (3-12).

Kigelia africana (Lam.) Benth. of Bignoniaceae family is an african medicinal tree from tropical zones that has been used as remedy in folkloric and natural medicines. The plant is used traditionally for numerous diseases such as psoriasis, eczema, wounds healing, fungal infections, rheumatism, diarrhea and stomach ailments. It is also use for skin care (13, 14). Some studies reported the antioxidants properties of *K. africana* aerial parts (15, 16). Nevertheless, the antioxidant potential of *K. africana* flowers not has been reported (13).

Materials and Methods

Fresh flowers of *K. africana* were collected in the Botanical Garden of the Central University of Las Villas. Plant sample was identified as *Kigelia africana* (Lam.) Benth. (Bignoniaceae) by a taxonomic expert of above Institution. The vegetal material was subject to extraction with ethanol (90%) by soxhlet apparatus. Ethanolic **(EF)**, ethylacetate **(EAF)** and buthanolic **(BF)** fractions were obtained from crude ethanolic solution by liquid-liquid extraction procedures.

The qualitative phytochemical analysis was carried out according to the ferric chloride, Shinoda, Baljet, *Bornträger*, Drangendorff, Kedde and Lieberman-Burchard tests as previous reported with slight modifications (17). For quantitative purposes, total phenolic content (TPC) was determined by Folin-Ciocalteu spectrophotometric method, reported as µg galic acid equivalents/mg dry extract (µgGAE/mgdE). **T**otal flavonoids content (TFC) was also determined by $AlCl_3$ spectrophotometric method, reported as µg quercetin equivalents/ mg dry extract (µgQE/mgdE) (18).

The antioxidant and radical scavenging profile was assessed through free radical scavenging (DPPH), reducting power (potassium ferricyanide), total antioxidant activity (phosphomolibdene) and metal chelating (Fe^{++}-Ferrozine) tests. Different doses of each fraction (1-400 µg/ml) were tested and the results were taken for constructing the respective concentration-effect curve. Quercetin, rutin, gallic acid, hesperidin, ascorbic acid and Na_2EDTA were used as references.

The IC_{50} (or EC_{50}) was calculated for each fraction or reference from concentration-effect curves using linear and non-linear regression.

The potency score (PS) was calculated individually for each substance in all tests according to the follow expression:

$$PS = \left(\frac{Ca50}{Ci50}\right);$$

Where: Ca_{50}, quercetin IC_{50} or EC_{50} value in a particular test; Ci_{50}, fraction or reference (not quercetin) IC_{50} or EC_{50} value in the same test.

Results and Discussion

Positive results were found for phenols, flavonoids, coumarins, and alkaloids in all fractions. The qualitative phytochemical analysis reveals that quinones are not present (or in a few amount only) in BF, however triterpens/steroids were detected only in this fraction but not in EF and EAF (table 1).

Table 1: Phytochemical screening of *K. africana* flowers fractions.

Classes of phytochemicals	Assay	Fractions		
		EF	**EAF**	**BF**
Phenols and tannins	$FeCl_3$	++	++	++
Flavonoids	Shinoda	+ (red[a])	+ (orange[b])	+ (red[a])
Coumarins	Baljet	+	+	+
Quinones	*Bornträger*	+	++	-
Alkaloids	Drangendorff	++	+	++
Cardiac glycosides	Kedde	-	-	-
Triterpenes and/or steroids	Lieberman-Burchard	-	-	+

+: positive, -: negative, ++: strong, a: flavonols?, b: flavones?.

Phenols and flavonoids are plant secondary products that may contribute to the natural antioxidant system against negative redox balance in human diseases (19). The total amount and particular chemical characteristics of these metabolites are relevant at this point, including the role as prooxidant agent (4, 19-22). The total phenolic content (TPC) and total flavonoids content (TFC) found for EAF (µGAE/mgdE = 523.31±23.40; µQE/mgdE = 43.57±3.46) were highest ($p<0.05$) than BF (µGAE/mgdE = 290.66±35.15; µQE/mgdE = 32.29±1.41) and EF (µAGE/mgdE = 116.02±13.47; µQE/mgdE = 14.25±1.36). According to these results, it is possible that the antioxidant potency score will show a direct relation with TPC and TFC.

Table 2: Potency score and IC_{50}, EC_{50} values for fractions and references. The values expressed as statistic mean ± standard deviation of sixth experiments.

Antioxidant	**DPPH**	**PS**	**TAA**	**PS**	**RP**	**PS**
	CI_{50}(µg/ml)		**CE_{50}(µg/ml)**		**CE_{50}(µg/ml)**	
quercetin	0.57±0.03	1	9.32±0.26	1	2.43±0.14	1
rutin	1.48±0.20	0.39	181.89±2.85	0.05	6.03±0.14	0.4
ascorbic acid	3.10±0.08	0.18	10.83±0.06	0.86	2.78±0.02	0.87
hesperidin	ND	-	> 400	-	ND	-
EAF	4.96±0.25[a,b,c,e,f]	0.11	28.99±0.62 [a,b,c,e,f]	0.32	5.16±0.26 [a,b,c,e,f]	0.47
BF	7.09±0.46[a,b,c,d,f]	0.08	99.36±1.88 [a,b,c,d,f]	0.09	5.94±0.32 [a,b,c,d,f]	0.41
EF	13.57±0.67[a,b,c,d,e]	0.04	204.55±10.14 [a,b,c,d,e]	0.05	20.02±1.19 [a,b,c,d,e]	0.12

PS: potency score, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, TAA: total antioxidant activity, RP: reducting power.

[a,b,c,d,e] statistically significant ($p<0.05$), a:quercetin, b: rutin, c: ascorbic acid, d: EAF, e: BF, f: EF.

In fact, the antioxidant profile of three fractions was in accordance with theirs TPC and TFC values (table 2). EAF exert the best antioxidant effect, which was similar to ascorbic acid and rutin. However, while DPPH scavenging, reducting power and total antioxidant activity tests revealed good results, the

metal chelating capacity was very low for all of them (~ ≤ 35%) (data not shown). The Na_2EDTA, unsurprising, showed a potent iron chelating effect (IC_{50} = 4.02±0.1 µg/ml).

Conclusions

This study reveals for the first time the antioxidant and free radical scavenging properties of *K. africana* flower fractions on cell-free systems. TPC and TFC for these fractions were also reported.

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4,6,6-trimethylbicyclo[3.1.1]hept-3-ene: Analysis of the Inhibitory Effect of Monoterpere on *Pseudomonas aeruginosa* Strain

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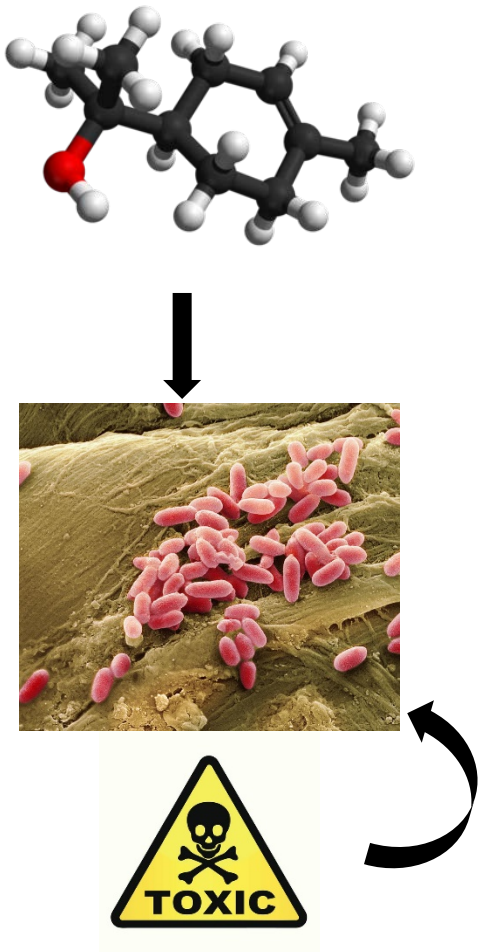
Graphical Abstract



Abstract.

Pseudomonas aeruginosa is a ubiquitous gram-negative non-fermentative bacterial species that exhibits natural resistance to some antibiotics and antiseptics, in addition to having a high expression of virulence factors, being responsible for causing, mainly, opportunistic infections in the hospital environment. It affects the respiratory tract causing about 80% of hospital pneumonias, being able to reach skin, soft tissues, eyes, ears, bones and the urinary tract. The treatment of nosocomial infections caused by *P. aeruginosa* is based on several classes of drugs, such as: Cephalosporins, Carbapenems, Aminoglycosides, among others. However, studies point to the existence of multiresistant species, including reserve drugs, such as imipenem, thus generating a public health problem. In addition, this year the World Health Organization has released a list of ten challenging multi-resistant microorganisms that require new antibiotics, and secondly the species *Pseudomonas aeruginosa* carbapenem-resistant. Given this panorama of bacteria resistant to

multiple commercially available antibiotics, it is necessary to study new compounds with antibacterial activity. As a possibility to combat bacterial infections, the action of a natural product, the positive enantiomer of 4,6,6-trimethylbicyclo [3.1.1] hept-3-ene, also known as (+) - α-pinene, before the *Pseudomonas aeruginosa* strain ATCC 27853, using methodologies standardized by the Manual Clinical and Laboratory Standards Institute. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration, and Nature Classification of Compound Effect were determined according to MBC/MIC ratio. The (+) - α-pinene was dissolved in 1% Tween 80, 5% DMSO and distilled water. In broth microdilution, the MIC was determined for the P. aeruginosa strain, at a concentration of 40 μL/mL, being characterized as bacteriostatic and the concentration 4 times higher than MIC was demonstrated to be bactericidal. This experiment made it possible to observe the action of the phytoconstituent on the species of *Pseudomonas aeruginosa*, emphasizing the need for permanent studies to determine the mechanism of action and toxicity of (+) - a - pinene allowing its future use against opportunistic infections caused by *Pseudomonas aeruginosa*.

Introduction

Pseudomonas aeruginosa is a ubiquitous gram-negative non-fermentative bacterial species that exhibits natural resistance to some antibiotics and antiseptics, in addition to having a high expression of virulence factors, being responsible for mainly causing opportunistic infections in the hospital environment. It affects the respiratory tract causing about 80% of hospital pneumonias, being able to reach skin, soft tissues, eyes, ears, bones and the urinary tract [2]. The treatment of nosocomial infections caused by *P. aeruginosa* is based on several classes of drugs, such as penicillins, cephalosporins, carbapenems, aminoglycosides and quinolones. However, studies point to the existence of multiresistant species, including reserve drugs, such as imipenem, thus generating a public health problem [3-4].

In addition, this year the World Health Organization has released a list of ten multiresistant microorganisms that require new antibiotics, and secondly the species *P. aeruginosa* carbapenem-resistant. Therefore, in view of the challenge of developing new antibiotics for the growing number of super-resistant microorganisms in the hospital environment, it is necessary to research natural products with antibacterial activity to aid in the fight against superbugs [6]. Oily compounds derived from plants

are composed of several substances, including monoterpenes, which are hydrocarbons present in these natural products, which act as antimicrobial agents with therapeutic potential, highlighting the 4,6,6-trimethylbicyclo[3.1.1]hept-3-ene or also known as α-pinene, which can be observed in several proportions, including as major compound, as occurs in the oils of *Satiria trimera, Juniperus phoenicea, Cupressus sempervirens*, among other oils [8].

The objective of this study was to analyze the inhibitory effect of (+) - α - pinene against the strain *Pseudomonas aeruginosa* ATCC 27853, by determining the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and classification of the nature of the effect of the compound according to the MBC/MIC ratio.

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Materials and Methods

The phytoconstituent, (+) - α-pinene, used in this experiment was obtained from Sigma-Aldrich do Brasil Ltda., And the solutions were prepared at the time of the tests, dissolving them first in 1% Tween 80 and DMSO in a ratio of up to 5%, and using sterile distilled water to achieve the desired concentrations.

The determination of Minimum Inhibitory Concentration was obtained by the broth microdilution technique with 96-well plates. The phytoconstituent concentrations used in this assay were 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 μL/mL. As a positive control, amikacin was used as control of sterility of the medium. Wells were used only with the culture medium, as well as the viability test of the bacterial strain with wells with culture medium plus bacterial inoculum [10]. The plates were aseptically closed and incubated at 35 ° C for 24 hours.

After the incubation period, the results were read with the addition of 20 μL of sodium resazurin solution (0.01%; w / v) (SIGMA), recognized as a colorimetric oxide-reduction indicator for bacteria. The experiments were performed in triplicate and the result was expressed by the arithmetic mean of the MICs obtained in the three trials [11].

The Minimum Bactericidal Concentration (MBC) was obtained by sowing aliquots of 20 μL of the dilutions corresponding to MIC and two immediately higher, CIMx2 and MICx4, from the contents of the wells of the microdilution plates, in Petri dishes containing Agar Müller-Hinton, which were scattered with the aid of a Drigalsky handle [12]. After sowing, the plates were incubated in an oven at 35 ° C for 24 hours. According to CLSI, CBM is considered the lowest concentration that prevented the visible growth of bacteria or allowed the formation of up to 3 Colony Forming Units (CFU). It is noteworthy that all experiments were performed in triplicate.

Results and Discussion

In this study, MIC was defined as the lowest concentration capable of visually inhibiting the bacterial growth observed in the orifices when compared to control growth [10].

Throughout the reading of the results, it can be identified that the phytoconstituent MIC was 40 μL / mL, twice the MIC equal to 80 μL/mL and four times the MIC equal to 160 μL/mL. Meanwhile, the MIC of amikacin was 1 μg/mL, and therefore the species studied was sensitive to the positive control used in the study, since according to CLSI, *P. aeruginosa* strain is considered to be susceptible to amikacin if the MIC is present, if less than or equal to 18 μg/mL. In addition, evaluating the wells with the controls allowed to guarantee the safety of the results, since the feasibility of the studied strain was verified and the sterility of the culture medium was confirmed.

Then, the determination of the minimum bactericidal concentration (MBC) was performed, which, after reading the results, was 40 μL/mL, and therefore the (+) - α-pinene MIC was bacteriostatic, since in the inoculated plates it was possible to visualize the formation of three more colonies for the MIC, MICx2 concentrations of the phytoconstituent. In addition, for the concentration 160 μL/mL there was no formation of visible colonies to the naked eye, being therefore bactericidal [12]. The MBC: MIC ratio was also applied, the result of which was 4: 1 (**Table 1**), characterizing the nature of the compound's effect as bacteriostatic [17].

Table 1: CIM and CBM values and classification of the nature of the antibacterial effect of 4,6,6-trimethylbicyclo[3.1.1]hept-3-ene [(+) – α – pineno]

Microorganism	**(+) – α – pineno (μL/mL*)***		**MBC:MIC**	**Effect**
	MIC	**MBC**		
Pseudomonas aeruginosa ATCC 27853	40 μL/mL	160 μL/mL	4:1	Bacteriostática

This study is unprecedented in the evaluation of the antibacterial activity of the positive enantiomer of 4,6,6-trimethylbicyclo [3.1.1] hept-3-ene, (+) - α-pinene, against the bacterial strain of *P. aeruginosa* ATCC 27853, that there is no information in the research literature on the subject using the methodologies used for this phytoconstituent.

In the literature, Farias et al. Carried out a study with (+) - α-pinene in 2017, with concentrations ranging from 160 to 5 μL/mL, using the disk diffusion technique for *P. aeruginosa* ATCC 27853. For this strain there was no formation of an inhibition halo visible to the naked eye, so the researchers considered it resistant to all concentrations used. This shows the importance of performing other techniques for the evaluation of an organic compound, since in the present work, using the broth microdilution method, it was possible to determine the MIC (+) - α - pinene, 40 μL/mL, whereas for the disc-diffusion test this determination was not possible.

In 2010, researchers evaluated the oils of *Juniperus phoenicea* L. and *Cupressus serpemvirens* L., which mostly contain α - pinene, however, in this study it was not possible to determine the phytochemical MIC for *P. aeruginosa* ATCC 27853, seen which, according to the authors, proved to be resistant [9].

Meanwhile, in 2013, in a study on the chemical composition and evaluation of antimicrobial properties of *Cupressus lusitanica* Mill. Essential oil, whose composition presents α - pinene, for bacterial strains, the researchers determined the MIC of the oil at 10% to 31.25 μg/mL for *P. aeruginosa* ATCC 27853, and its CBM with the same value for the oil with > 10% concentration [19].

Conclusions

After the experiment, it can be concluded that 4,6,6-trimethylbicyclo [3.1.1] hept-3-ene presents antibacterial activity against the *P. aeruginosa* ATCC 27853 strain, according to the broth microdilution test, and that this action is bacteriostatic. Therefore, it is recommended to continue the studies on the mechanisms of action and toxicity of the compound so that, in the future, it can be used as a new therapeutic alternative against opportunistic infections caused by *Pseudomonas aeruginosa.*

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Antioxidant activity of 5-FU and new fluorinated uracil derivates

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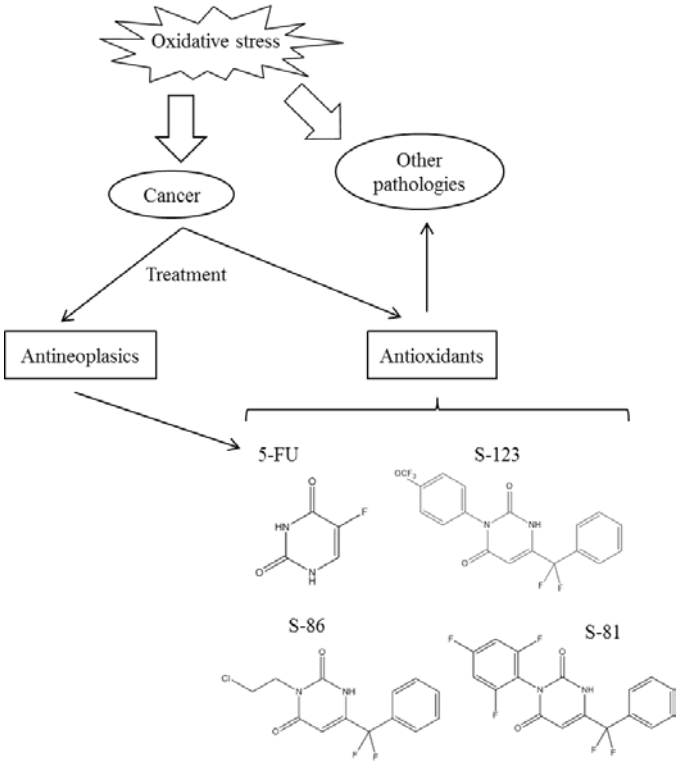
Graphical Abstract



Abstract.

The intake of antioxidants has increased in the last years in order to treat some pathologies associated with oxidative stress such as cancer, diabetes mellitus, atherosclerosis, myocardial infarction, acute pancreatitis, Parkinson's and Alzheimer's disease among others. In colorectal cancer, a widely antineoplastic drug used is the fluorinated uracil molecule 5-Fluorouracile (5-FU). The aim of this study is to assess the antioxidant capacity observed by the inhibition of lipid peroxidation by 5-FU and other fluorinated uracil derivates: 6-[Difluoro(phenyl)methyl]-3-(2,4,6-trifluorophenyl)pyrimidine-2,4(1H,3H)-dione (S-81), 3-[2-Chloroethyl]-6-[difluoro(phenyl)methyl]pyrimidine-2,4(1H,3H)-dione (S-86), 6-[Difluoro(phenyl)methyl]-3-(4-trifluoromethoxyphenyl)pyrimidine-2,4(1H,3H)-dione (S-123). The results showed a significant decrease in MDA production of 58.12% in S-86, 44.61% in S-123, 24.11% in 5-FU and 10.83% in S-81 in the sample with highest concentration (10µM). 5-FU also showed a Total Antioxidant Capacity of 0.68 Trolox Equivalent Antioxidant Capacity.

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Comparison of Base Distributions in Dengue, Zika and Other Flavivirus Envelope and NS5 Genes

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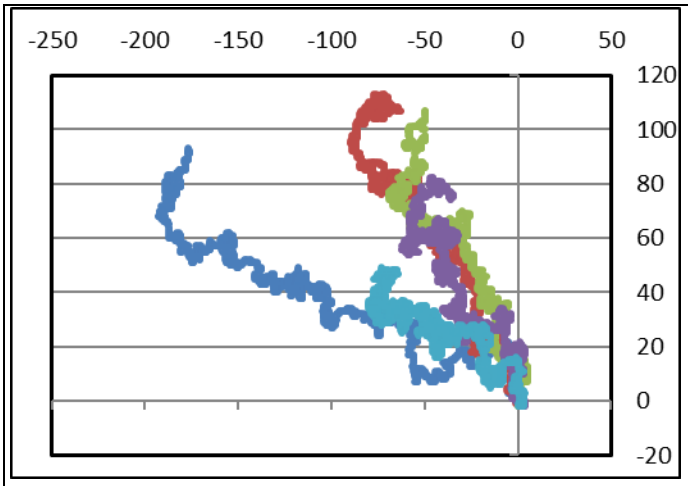

Abstract

Among the nucleotide sequences of flaviviruses, those of the Zika virus and Dengue type 2 are believed to share a high degree of similarity. Our study of the nucleotide sequences of the envelope and NS5 genes shows that the sequences of the Dengue type 2 are sharply different compared to other human infecting flaviviruses. This is emphatically seen in a 2D graphical representation and distinctly discriminated in terms of relevant RNA descriptors. In this report, we demonstrate this difference through various parameters and consider possible reasons for such variations that seem to have been largely neglected in the literature.

Introduction

The recent epidemic of the Zika virus in South and Central Americas have focused attention on the flavivirus group of viruses which also include all four types of Dengue virus (DENV1, DENV2, DENV3, DENV4), West Nile virus (WNV), Yellow fever virus (YFV) and Japanese encephalitis virus (JEV) besides Zika virus (ZIKV). All these viruses share strong homology at the protein level, and slightly weaker homology at the nucleotide sequence level [1]. Phylogenetic studies place the four DENV serotypes in one clade and ZIKV is found to be closest to DENV2. It is therefore widely held that ZIKV and DENV2 have the closest relationship among all the aforementioned flaviviruses [2].

Closer examination of the gene and protein sequences of these viruses reveals slightly divergent results. Taking one structural and one non-structural gene, viz., envelope (E) and NS5, as examples for this study, we have found sequence differences between DENV2 and other flaviviruses to be reasonably correct in the case of the protein sequences, although the DENV2 sequence has a fair excess of the lysine amino acids. However, the DENV2 nucleotide sequence of these genes have a widely different base distribution compared to the ZIKV as seen in a 2D graphical

representations. In this brief report, we enumerate the various analyses we have done and explore the reasons and ramifications of the differences.

Materials and Methods

The flaviviruses investigated here include 5 sequences selected for our analyses of the envelope (E) genes of DENV2, ZIKV, WNV, YFV and JEV and their corresponding NS5 sequences. DENV2 was particularly chosen because in the literature, it is cited as being phylogenetically closest to ZIKV [2]. The envelope and NS5 genes were selected primarily due to the fact that the envelope protein is responsible for host cell fusion and endocytosis, whereas the NS5 gene encoding the RNA dependent RNA polymerase is important in gene replication [2]; that these are the longer genes in the viral genome also helps in the analysis.

Base distribution and quantification were done using one of the 2D graphical representation methods [3] where an RNA sequence is represented in a 2D grid and for each base, a point is plotted by moving one unit in the negative x-direction if it is an adenine, in the y-direction if it is a guanine, in the x-direction for a cytosine and in the negative y-direction if it is a thymine. Plotting these points successively for each base in the sequence generates a graph that represents the base distribution in the sequence. Using the x, y values to get a weighted center of mass (c.m.), μ_x and μ_y, we can compute a graph radius, g_R, from the origin to the c.m. The g_R becomes representative of the overall spread of the graph and can be considered as the RNA sequence descriptor [4]. We show the base distribution of the Flavivirus genes in this 2D representation and compute the sequence descriptors, g_R, for comparison purposes. Comparison is also made between the gene sequences using CLUSTALW [5].

Results and Discussion

The distinction between the nucleotide sequences of DENV2 and the other flaviviruses is perspicuously seen in the 2D graphical representations (Fig. 1 and 2). A large excess of adenine and guanine exist in both the envelope and NS5 genes of DENV2 (Table 1). The Base Distribution Index or Sequence Descriptor, g_R, indicates the magnitude of the spread of the nucleotide sequence graphs. The average g_R values computed for the envelope and NS5 genes of ZIKV, YFV, JEV and WNV are 61.38 ± 11.97 and 181.35 ± 7.26, respectively, both of which are overshot by a large margin by DENV2 - 120.60 for envelope gene and 249.98 for NS5 gene. Such large variations indicate a markedly different codon bias profile of DENV2 on comparing with the other flaviviruses (see Fig. 3 for the graph of the codon bias in envelope genes, data not shown).

Interestingly, a BLAST pairwise analysis of the envelope genes shows only 61% identity between DENV2 and ZIKV, but that can be misleading since a similar analysis between two totally unrelated genes, DENV2 envelope gene (1485 nucleotides) and H5N1 neuraminidase (1410 nucleotides), shows 50% relative identity. This implies that such kind of analysis is not a reliable indicator of identity. Detailed study of the nucleotide sequences shows the 2D graphical representation to be a pointer to the true differences. This aspect seems to have been little explored in the literature.

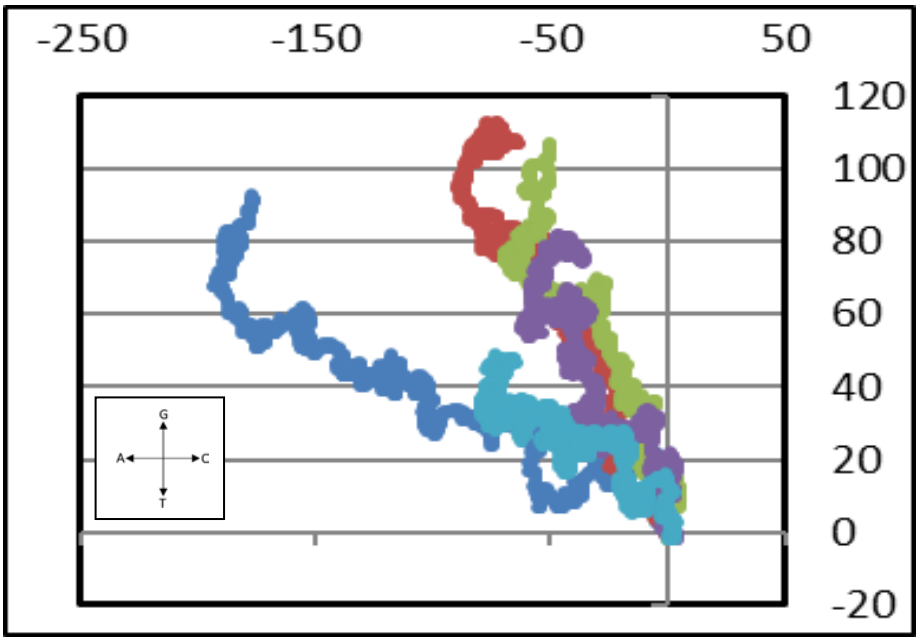

Fig. 1. Flavivirus envelope genes in a 2D graphical representation. Axes AGCT. Deep blue - DENV2, Light blue - JEV, Purple - WNV, Green – YFV, Red – ZIKV.

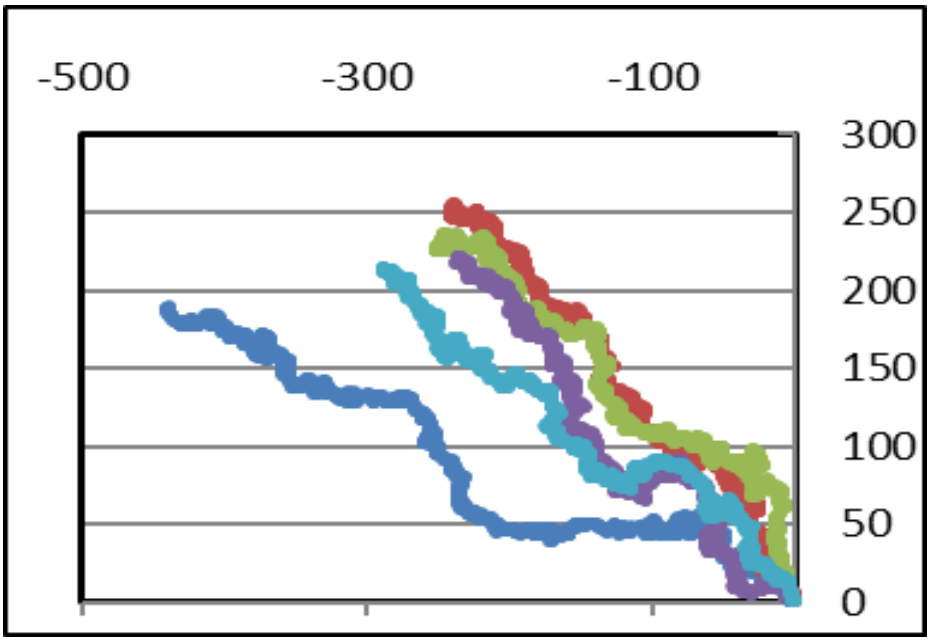

Fig. 3. Flavivirus NS5 genes in a 2D graphical representation. Axes AGCT. Deep blue - DENV2, Light blue - JEV, Purple - WNV, Green – YFV, Red – ZIKV.

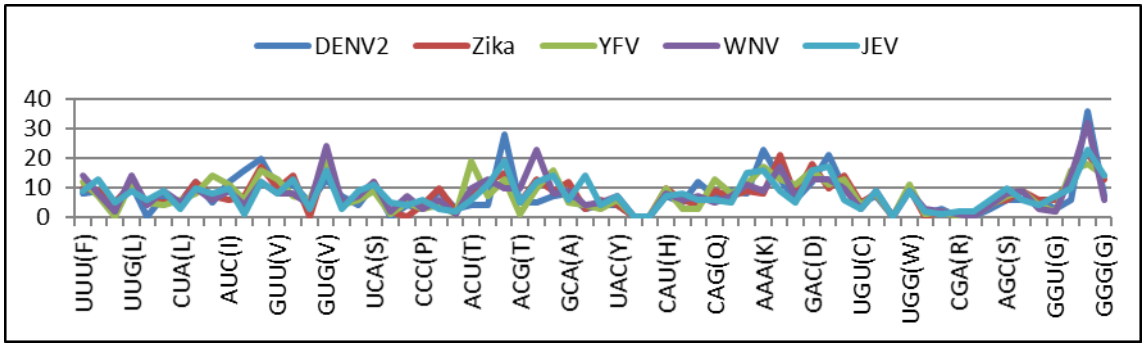

Fig. 2. Flavivirus envelope codon bias

Table 1. Nucleotide Composition and g_R Values

Virus	Envelope Gene					NS5 Gene				
	A	**C**	**G**	**T**	g_R	**A**	**C**	**G**	**T**	g_R
DENV2	494	291	382	317	120.5958	949	525	715	511	249.9799
ZIKV	400	325	432	337	78.43309	780	566	821	542	182.087
YFV	405	306	413	355	60.98772	802	563	794	556	181.9147
JEV	319	368	415	326	53.03495	171	135	167	110	189.5519
WNV	390	342	417	354	53.06449	770	595	816	534	171.8647

The protein sequences do not express as much divergence as is shown by the nucleotide sequences. The CLUSTALW pairwise alignment scores of protein and nucleotide sequences of envelope and NS5 are given in Table 2. Although, it is noted that the proteins of DENV2 constitute a slightly higher composition of lysine (K) and threonine (T) arising from the codon usage bias, the availability of synonymous codons largely maintains the close similarity between the protein sequences on translation.

Table 2. CLUSTALW Pairwise Alignment Score for Nucleotide and Protein Sequences

Virus	Nucleotide Sequences									
	Envelope Gene					NS5 Gene				
	DENV2	ZIKV	YFV	JEV	WNV	DENV2	ZIKV	YFV	JEV	WNV
DENV2	-	49.5	39.9	43.9	51.8	-	56.9	52.2	56.1	56.6
ZIKV	-	-	41.5	49.7	48.8	-	-	52.9	57.6	58.8
YFV	-	-	-	39.7	42.0	-	-	-	53.0	53.2
JEV	-	-	-	-	64.0	-	-	-	-	68.6
	Protein Sequences									
	Envelope Protein					NS5 Protein				
	DENV2	ZIKV	YFV	JEV	WNV	DENV2	ZIKV	YFV	JEV	WNV
DENV2	-	52.1	41.8	45.1	43.4	-	67.8	62.1	67.3	68.4
ZIKV	-	-	40.4	54.2	53.2	-	-	63.7	69.0	70.7
YFV	-	-	-	41.6	41.6	-	-	-	63.8	62.3
JEV	-	-	-	-	77.2	-	-	-	-	83.4

In spite of DENV2 being one of the more virulent pathogens and probably the most virulent among the four DENV serotypes, there is little reflection in literature of the acute base distribution difference between DENV2 and the other flaviviruses that has resulted in the codon usage profile of DENV2 being sharply different from the other flavivirus genes. One probable correlation of the disparity in the nucleotide sequences, observed in our analyses, that can be made is between DENV2 codon adaptation and the host tRNA pool [6]. Some models indicate that certain viral codon usage is optimized to match the tRNA pool in the host organism that enables faster translation elongation [7]; there are also speculations that high levels of gene expression result in tRNA starvation which slow down the replication and translation processes and result in modulation of gene expression [8,9]. These factors may combine to create a more efficient replication process for the DENV2 as compared to other flaviviruses.

Conclusions

So far, Zika and Dengue virus analyses have reported that DENV2 and ZIKV are quite close. We have found that the base distribution patterns of the two viruses are different, and we have shown these explicitly in the case of the envelope and NS5 gene sequences, through the 2D graphical representations. Quantitative estimates show that the graph spreads are about 30 to 50% larger for the DENV2 genes. We speculate that such disparity probably gives DENV2 an edge through its codon usage bias which uses the cellular tRNA pool for optimization of gene expression and regulation, making DENV2 a highly virulent pathogen.

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Some Comments on Mathematical Descriptors of Biomolecular Sequences and their Characteristics

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Abstract

The advent of techniques of graphical representation and mathematical characterization of biomolecular sequences has seen the growth of a genre of non-alignment methods for analyses of their similarities/dissimilarities. The new descriptors are important and convenient to provide a quantitative measure of the composition and distribution of the basic units, allow discrimination amongst members of a family of similar sequences with a low computational overhead and hold promise for discovery of new systematics. These opportunities led to a plethora of models for graphical representation and numerical characterisations, but the question is how far the various sequence descriptors derived by these different mathematical approaches encode non-redundant information. We briefly consider the issues that when comparative studies of biomolecular sequences are undertaken, it is important to consider which properties are being considered and choose models that allow for computational closure and non-redundancy. We believe graphical representation and numerical characterization models have a significant role to play in non-alignment similarity/dissimilarity analysis of bio-molecular sequences, but the issues have to be approached with an eye to specific properties being investigated.

The advent of techniques of graphical representation and mathematical characterization of biomolecular (DNA/RNA/protein) sequences [1] have seen the growth of a genre of methods for their characterization that predicts properties without need for multiple alignments. In a recent paper analyzing the Zika virus nucleotide sequences, we had used such techniques to characterize the Zika virus genome.

The new descriptors of biomolecular sequences are important and convenient from several aspects:

1. They provide a formal quantitative measure of the biomolecular sequences that capture the essence of basic units' composition and their distribution;
2. Such quantitative information allow for the possibility of discrimination amongst members of a family of similar sequences;
3. Their computation overhead is limited since there is no recourse to computational models required in multiple alignments;

4. They provide a novel approach to routine tasks of bio-sequence analyses which may lead to discovery of new systematics.

The original impetus for graphical representation arose from the work of Hamori et al [2] who mapped a DNA sequence into a three-dimensional grid with, effectively, the four nucleotides mapped along the four cardinal directions in the xy plane and the nucleotide number measured along the z-axis. Plotting each base by taking a step in the designated direction and connecting to the next base point by a short line resulted in a three-dimensional curve, when all points were plotted, representing the distribution of bases along the sequence. Since such graphical representation was practically difficult when done on a paper or a computer screen, some authors proposed two-dimensional graphical representations that captured the essence of such representations by dispensing with the elevation along the z-axis that separated each nucleotide from its neighbors [1,3-5]. This resulted in degeneracies in the representation of the paths taken when the movements had to alternate between two bases placed on the same axis. Quantitative measures were devised to distinguish between two closely related sequences: Gates [3] proposed the Manhattan distance where distance between two nucleotides in a DNA sequence were measured as a sum of the distances measured in terms of steps taken along the axes to reach from one base to the target base., Raychaudhury and Nandy [7] proposed a geometrical measure of moments of the graphs and a graph radius as DNA descriptors.

Zeroth order: $X = N_G - N_A \quad Y = N_C - N_T$

First order: $\mu_x = \Sigma\, x_i / N \quad \mu_y = \Sigma\, y_i / N \quad g_{\underline{R}} = \sqrt{(\mu_{\underline{x}}^2 + \mu_{\underline{y}}^2)}$

where N_i (I = A, C, G, Y) represents the base composition numbers, X, Y are the end points of the 2D curve, N is the total number of bases, μ_x, μ_y are weighted average co-ordinates and $g_{\underline{R}}$ is the graph radius which measures the base distribution in terms of the graph spread and thus represents a sequence descriptor.

Although such 2D graphical representations provided a reasonable view of the base distribution along a sequence, the degeneracy feature was a stumbling block that inspired many authors to rushin to fill the void [1]. Randic et al [7] resurrected the 3D model in a new form and also instituted a D/D matrix whose eigenvalues λ_i were supposed to represent the sequence descriptor. The issue of (a) designing a descriptor for a DNA sequence, (b) devising a means to discriminate between sequences and (c) avoiding degeneracy became a Holy Grail of the DNA descriptor world. By last count there may be over a hundred models devised to accomplish this task and new models are being proposed yet (see e.g., 8,9].

Given this plethora of models, and that all of them proposed descriptors computed either through geometrical methods or eigenvalues of underlying matrices, it is pertinent to ask how far the various sequence descriptors derived by different mathematical approaches encode redundant information [10]. The 2D-dynamic model of Bielinska-Waz et al [11], where the redundancies of the original Nandy 2D model [4] was to be removed by apportioning masses at each vertex, reproduced the exact same descriptor values at the 1st order moments level as the naïve 2D model [4] applied to the Zika virus genomes [12]. In a separate exercise where seven different representations of DNA sequences were analyzed for descriptor computations as applied to various globin and other genes [10], some were

found to be strongly correlated; those that were not must be representing some specific characteristics of the base composition and distribution, but it has not been clear what these are.

Thus, when comparative studies of biomolecular sequences are undertaken, it is important to consider which properties one is looking at and choose models that allow for fair computation, non-redundancy with other models and improvement over multiple alignment systems. In another paper elsewhere in this conference [13] we show that a commonly held belief in the similarity of Dengue type 2 virus and Zika virus genomes, supported by a BLAST analysis, the nucleotide sequences are indeed quite different as demonstrated in a 2D graphical representation. Non-alignment, graphical representation and numerical characterization models thus have a significant role to play in similarity/dissimilarity analysis of bio-molecular sequence analysis, but the issue has to be approached with an eye to specific properties being investigated.

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Inhibition of Secretory Aspartyl Protease of *Candida albicans* by metabolites of *Streptomyces chrestomyceticus* strain ADP4

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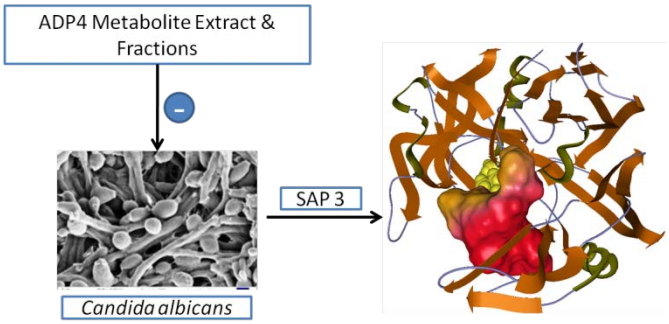
Graphical Abstract



Abstract

Candida albicans is a commensal but a significant opportunistic pathogen. Various pathogenic attributes and virulence factors are found to be responsible for devastating *Candida* infections. Secretory aspartic proteases (Saps) enable hyphae formation, adhesion and phenotypic switching; digestion of the host cell membranes and evading host immune response by degrading and inactivating the central human complement components. Therefore, an agent capable of inhibiting production of *C. albicans* Saps will be useful in the treatment of such infections. The partially purified fractions of *Streptomyces chrestomyceticus* strain ADP4 displayed strong anti-*Candida* activity, hence were investigated further for their ability to inhibit production of Saps. Strong inhibition of production of Saps was observed when tested against the ATCC strain of *C. albicans*. Docking studies of the GCMS-predicted molecules of the metabolite extract and of the various fractions with a Sap of *C. albicans* were performed using VLife MDS4.6. These studies revealed the significant affinity of GCMS-predicted molecules when compared with the standard Sap inhibitor, Pepstatin A.

Introduction

Despite several advancements in drug discovery and development, there is still an enormous need for antifungal drugs. Infectious diseases like Invasive Candidasis (IC) still remains associated with high rates of morbidity and mortality worldwide. *Candida* sp., an opportunistic human pathogen, is capable of causing a variety of infections ranging from mucosal to life–threatening systemic candidiasis especially among immune-compromised patients [Pfaller et al 2007; Chin et al 2016].

Candida adheres to mucosal surfaces of the host by interacting with specific ligands present on host cell surface through specific molecules referred as adhesins [Williams et al 2013]. The production of extracellular enzymes represents another virulence factor of *Candida* sp. It aids in the invasion of host tissues and destroys or de-range constituents of host cell membranes, leading to the membrane dysfunction and/or physical disruption [Ghannoum 2000; Naglik et al 2003]. Increasing resistance of *Candida* sp. towards antifungal drugs like azoles and echinocandins has further complicated the scenario and compels to research for new antifungal agents as well as new targets [White et al., 1998; Sardi et al., 2011].

Developing anti-*Candida* drugs with lowered cost, better efficacy and minimum side effects will enhance the potential of such drug. In recent years, there has been increasing research investigating the biosynthetic potential of *Streptomyces* sp. [Srivastava et al., 2014; Singh and Dubey, 2015]. In our previous studies, it was found that the metabolites from *S. chrestomyceticus* strain ADP4 had very good potential as anti-*Candida* agents [Srivastava and Dubey, 2016]. The metabolite extract was found to have anti-biofilm activity against the strains of *C. albicans*. Therefore, developing drugs targeting different virulence factors of *C. albicans* has been the main aim of this project. In light of these facts, the present study was designed to examine the inhibition of virulence factor like Sap of *C. albicans* by partially purified secondary metabolites of *S. chrestomyceticus* strain ADP4.

Materials and Methods

The metabolite extract was prepared as reported earlier [Srivastava and Dubey, 2016]. It was further purified by column chromatography on Silica Gel. Bioautography technique was used to screen all the fractions for their anti-*Candida* activity. The purity of active fractions was evaluated by Thin Layer Chromatography (TLC). Different visualizing agents like UV light, iodine vapor and anisaldehyde-sulphuric acid reagent were used for detection of the compounds. GCMS of metabolite extract and various fractions were done in order to assess the probable compounds.

The partially purified fractions were analyzed for their anti-*Candida* activities. Different concentrations of the partially purified fractions were used for determining the values for minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFCs) against a panel of *C. albicans* ATCC strains. The partially purified fractions were also examined for their ability to inhibit *C. albicans* virulence factor like Secretory aspartic proteases (Saps), which plays an important role in the establishment of *Candida* infection. Docking studies using the VLife MDS 4.6 tool was done in order to assess the mechanistic approach [Singla et al., 2017; Singla et al., 2017].

Results and Discussion

A total of seven partially purified fractions were analyzed for anti-*Candida* activity. Five of them showed the activity with MIC and MFC values of <500µg/mL against different ATCC strains of *C. albicans*. The molecules of fractions 1, 3 and 5 were found to inhibit production of Saps by the test strains of *C. albicans*. Docking studies of the probable molecules in the above fractions, identified by GCMS analysis, indicated significant affinity of these molecules with the amino acid residues of Sap 3 when compared with the co-crystallized ligand, pepstatin A.

Conclusion

The work reported here showed that the strain ADP4 produced anti-*Candida* compounds which inhibited production of Sap, widely regarded as an important virulence factors associated with Candidiasis.

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Criminalistics and its role in prevention

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Abstract. Addressing the study of the problem of the Prevention of Crime and Infractions of the Law from the Criminalistic point of view is the fundamental objective of this work; It is true that theoretically several concepts have been formulated that have explained the scope of prevention as a criminological category, using as synonyms of prevention, terms such as: avoidance, precaution, control, prediction of delinquency, etc .; nowadays it is synonymous with: suppression, precaution, prophylaxis. Some authors criticize the term prophylaxis because the expression contains a hygienic medical meaning due to the equality between "crime-disease" and "delinquent-sick", as well as the cure of the offender that was the end of Criminal Law in the beginning of Criminology. For others, these terms do not differ, while the concept of crime prevention is used to designate both the objective premises of the liquidation of the crime and the subjective factors of the fight against it. For us, the prophylaxis concept of crime only expresses a special activity of criminal prevention, considering prophylactic activity as an integral part of criminal prevention, so we understand that both terms can be used interchangeably.

Reference: Brito Febles, O. “La Profilaxis de los delitos como tarea de la Ciencia Criminalística”. Tesis de Doctorado. La Habana. Cuba. 1997.

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Standardization of the Safety Level of the Use of DMSO in Viability Assays in Bacterial Cells

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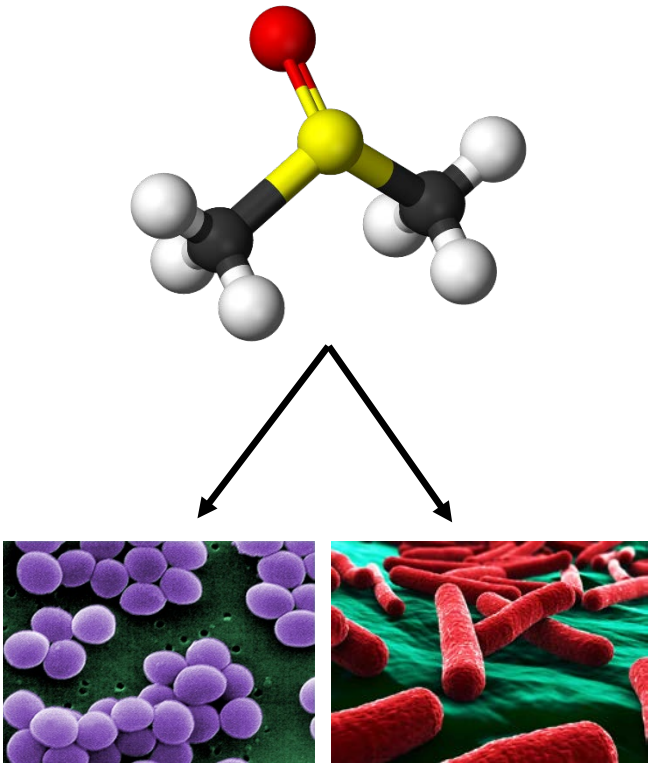
Graphical Abstract



Abstract.

The antibacterial potential of the most diverse medicinal plants has benefited humanity for centuries, and precisely because of this, the number of studies investigating the antimicrobial activity of essential oils and their components is increasing. However, the hydrophobic character of the essential oils has made the experiments difficult, requiring the use of organic solvents in the tests in order to avoid such complications. Among the most commonly used solvents are dimethylsulfoxide (DMSO/C_2H_6OS). To date, the literature has not yet determined a standardization of the usual concentration of DMSO suitable for bacterial experiments, so that its use does not check the efficacy of the tested phytoconstituent by interactions between the solvent and the exploited compound. In view of this reality, the present study intends to standardize the DMSO concentrations that do not interfere in the viability of the bacterial strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC

	25923, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 25922 and *Enterococcus faecalis* ATCC 29212. In order to reach this objective, the disc diffusion method in Müller Hinton Agar of a reagent impregnated on a filter paper disc was used, using different concentrations of DMSO to determine the minimum inhibitory concentration (MIC) of the five microorganisms listed. The experiment was carried out in triplicate in order to safeguard the effectiveness of the method tested. The incubation was done in an oven at 35º C, for a period of 24 hours. For the test method the conclusions of the tests performed were expressed by the arithmetic mean of the diameter of the inhibition halos formed around the disks. The test demonstrates that for the concentrations and microorganisms tested, DMSO provides safety in its use.

Introduction

In the scientific field, extracts and essential oils from plants are used as natural sources of new compounds to combat bacterial infections. However, the estimation of the antibacterial activities of many plant-derived compounds is hampered by the low solubility of these compounds in water. Solubilizers, such as surfactants and solvents, among them dimethylsulfoxide (DMSO), have been used to solve this problem, but it may be difficult to distinguish the contribution in the antimicrobial activity of the solubilizer from the compounds under investigation [1].

DMSO was originally synthesized by Zaytsev in 1866, and has since been extensively investigated for possible industrial and biological utility, and a considerable amount of literature has developed on its properties and uses [2]. It is an organosulfur of formula C_2H_6OS of molecular weight 78 g/mol, boiling point 189 °C and freezing temperature 18.5 °C; an amphipathic molecule composed of a polar domain characterized by sulfinyl and two non-polar methyl groups, which makes it capable of solubilizing polar and non-polar substances and transposing hydrophobic barriers (Figure 1) [3].

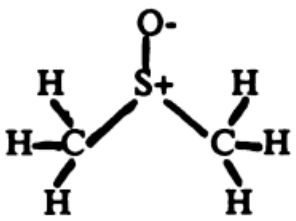


Figure 1: Polarized form of dimethylsulfoxide

These properties are important for pharmacological compounds that act as vehicles intracellularly as they allow non-water-soluble therapeutic and toxic agents to be generally soluble in DMSO [4].

An important biological action observed in DMSO is its ability to cross cell membranes; other pharmacological properties of DMSO include: immunomodulation; vasodilation; anti-platelet aggregation with protection against ischemic injury; diuretic, among others. The higher its concentration in water, the greater the penetrating capacity, the ideal range being between 70-90% of DMSO [5].

Thus, DMSO has been used as a commercial solvent since 1953, also possessing several other applications: therapeutic use, excipient for formulations in veterinary therapeutics, as a control group for testing natural products, for treating cells cultured in certain experiments and several in vitro studies. Despite the multiple applications of DMSO, its physiological and pharmacological characteristics as well as their effects are not fully understood, and further investigations into its pharmacological activities and which concentration of use is safe are required [6].

It has recently been found experimentally that DMSO has the ability to protect *Escherichia coli* from antimicrobial-mediated rapid death, interfering with the efficacy of the drugs tested, in this experiment DMSO inhibited death by ampicillin, kanamycin and two quinolones but had little effect on MIC. DMSO-mediated protection correlated with ROS reduction [7]. Another assay also reported a possible inhibition of microbial growth by interference of this solvent, thus, one can't rule out the possibility that DMSO interfered in the experiment, possibly causing a potentiating effect of the antimicrobial activity of tested compounds [8]. In view of such reports, caution is advised in using DMSO-dissolved antimicrobials for short-term death assays as well as accurate investigations to find out which DMSO concentration is most reliable for use in such experiments.

Materials and Methods

The microorganisms used for testing were Gram positive bacterial strains: *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212; and Gram negative strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Proteus mirabilis* ATCC 25922.

Disk diffusion tests with different concentrations of dimethylsulfoxide (DMSO) were carried out in order to identify concentrations of DMSO capable of carrying out an antibacterial activity in the five microorganisms. To test all concentrations, three petri dishes were used for each strain tested. The incubation was done in an oven at 35º C, for a period of 24 hours.

A suspension of the five microorganisms was performed, whose turbidity degree was 0.5 McFarland scale, corresponding to 1 x 10^8 CFU/mL, which was spread with the aid of a Drigalsky loop. After sowing on the plate, each disk was impregnated with different concentrations of the reactants and pressed against the plate in order to ensure complete contact with the agar surface, being applied individually and evenly distributed, so that the distance from the center of the edge of the board did not exceed 24 mm. The plates containing Müller-Hinton Agar were inverted and placed in an oven at 35 ºC for 24 hours after the application of the discs therein, and thereafter the halos were read.

The tests were performed and their results were expressed in mm by the arithmetic mean of the diameter of the inhibition halos formed around the discs during the disk diffusion test.

Results and Discussion

The disc diffusion method is performed to determine patterns of susceptibility to antibiotics according to the guidelines of the Institute of Clinical and Laboratory Standards (CLSI) [9]. It is an easy-to-perform and economical technique developed by the European Committee for Antimicrobial Susceptibility Testing (EUCAST), its standardized methods and clinical breakpoints have been adopted by clinical microbiology laboratories in Europe as well as in other parts of the globe [10].

From the evaluation of the results recorded in **Table 1**, it was verified that there was no inhibition halo formation at any of the concentrations tested, that is, there was no impediment to the growth of the investigated microorganisms. This demonstrates that the use of DMSO as a chemical solvent in experiments with the probed strains, in concentrations not exceeding 80%, according to the assay performed, is considered safe, not causing any inhibition of microbial growth.

Table 1: Verification of the antimicrobial activity of DMSO in different concentrations in the fusion disc method.

Microorganism	Substances / Halo (mm)								
	AMC	DMSO							
		80%	40%	20%	10%	5%	2,5%	1,75%	0,88%
S. aureus ATCC 25923	30	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E. faecalis ATCC 29212	22	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E. coli ATCC 25922	29	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
P. mirabilis ATCC 25933	22	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
P. aeruginosa ATCC 27853	33	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø

AMC: amikacin disc (30 μg), Ø: absence of inhibition halo of bacterial growth.

The reading of the results was done by measuring the diameter of the halos, in mm, formed around the discs containing the reagents, when greater than 6 mm it becomes visible indicating susceptibility of the microorganism to the substance tested [11].

The experiment was carried out by Filipe et al. [12], where the antimicrobial activity of the leaves of *Cydonia oblonga* miller was tested against several microorganisms, including E. coli and S. aureus. DMSO at 20% concentration was used in the assay and, corroborating our finding, the experiment did not detect any interference with the growth of these bacteria at the concentration used.

Macieira [13] conducted a research on the antimicrobial activity of marine cyanobacteria, where eight bacterial species were selected: four Gram-positive (*Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium* and *Bacillus subtilis*) and four Gram-negative strains (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* enteric). In this experiment DMSO was used as a negative control and also proved to be a safe solvent, which offered no interference with the results obtained in the assay.

Soares and Benitez [14] in their research on the antimicrobial activity of botanical extracts and their interaction with antibiotics against two strains of *Listeria* spp isolated from food and the standard strain ATCC 7644. The authors demonstrated that DMSO only showed toxic action for to *L. monocytogenes* in concentrations above 12.5%.

Conclusions

The results obtained by the study evidenced the need to standardize an appropriate concentration of DMSO for use in these experiments, considering that there is a discrepancy in the findings of several authors on the antimicrobial effect of the different concentrations of this solvent used. The interpretation of the effects of DMSO on the microorganisms with which it interacts is of great importance in view of its expanded use as a solvent in the most diverse therapeutic and pharmacological studies.

From our experiments we can state that despite recent discoveries involving the use of DMSO with interactions and / or changes in the efficacy of certain drugs, for the strains tested in the trial, namely: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 25922 and *Enterococcus faecalis* ATCC 29212, DMSO use was shown to be safe and innocuous at concentrations not greater than 80%.

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The imputability from the perspective of comparative law

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The topic of imputability has had a different approach in the different Codes of the world in accordance with the development of society and the well-known historical types of State and Law, each legal system possessing its legislative peculiarities, so that its assessment from the The perspective of comparative law offers us a more complete vision of it and the possibility of valuing different assessments through the prism of doctrinal and jurisprudence thinking. As Cobo del Rosal and Vives Antón point out: "Those who voluntarily place themselves in situations of unimputability to carry out the crime are already beginning their execution, and if that is the case, in the case of the actio liberae in causa dolosa, no there is a dissociation between the moment of action and the moment of imputability. " The basis of this idea falls on the statement of prominent jurists such as Von Lizst, Carrara and Maurach that indicate that knowingly placing oneself in a situation of unimpeachable means becoming an instrument of crime itself. In the same way that the imputable subjects use the unimputable to achieve their criminal purposes by becoming mediate authors, the former can also use themselves and commit crimes in the same way. The subject who is expressly placed in transitory mental disorder, is in fact catalyzing the illicit nature of their behavior. The free actions in their cause can be raised in the cases of drunkenness and sleep and they can not only be fraudulent but guilty, there are different criteria around their punishment being the most widespread which considers that these already exist at the beginning of execution of the conduct that is prosecuted. This does not mean that there are not many contrary opinions and arguments that the theory of actio liberae in causa can harm the principle of culpability and even legality, considering the need for the subject to be imputable also at the time of realization the fact. Thus, the impossibility of referring guilt to that moment has led to contradictions with dogmatic principles and various authors have pronounced in order to achieve a coordination between the above and the possibility of imposing a penalty. For its part, SAINZ CANTERO states: "The dominant doctrine is inclined in these cases to bring back the moment of the imputability to the one in which the cause was placed (the triggering action or omission), understanding that if in that time the subject of the action was imputable, we must have it as such, even if it were not, in the execution of the typical behavior ". However, in the opinion of BAJO FERNANDEZ: "The problem of coexistence between the theory of the actio liberae in causa and the dogmatic principles is only apparent." And to give grounds for this assertion, among other arguments, it refers to the fact that posed that there is a similar way of acting between the person who places a bomb and activating a certain mechanism makes it explode hours later and who "predisposes his own power of corporal action to act when the mind is in a state of unconsciousness". And continues: "Indeed this is so, but while the first assumption is explained because there is already a typical act of execution of the act to place the bomb, the second is not done with that circumstance, which requires an explanation of his punishment." Simply attending to reasons of material justice most of the legislations do not accept the exemption in such cases.

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Some historic considerations on democracy

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Democracy was born in the city-states of classical Greece, in the fifth century BC. It reached its most complete form in the city of Athens, in the time of Pericles. The characteristics of Greek democracy are those that come closest to the ideal of direct democracy, in which the group of citizens participates directly and continuously in making decisions about the affairs of the community. However, from an institutional perspective, it is a very simple and primitive construction.

In Athens the citizens met several times a year, it is estimated that at least 40, on the hill of Pnyx to discuss the affairs of the community. The agenda of discussions was established by the "Committee of 50", constituted by members of a "Committee of the 500", representatives, in turn, of the hundred demes that made up the city. The period of public office was very short (less than two months in the "Committee of 50", one year in the "Committee of 500") and the designation was made by lottery methods in the first case and rotation In a second. The discussion and deliberation among citizens formed the basis of this system of democratic participation. Decisions were made, normally, by way of consensus, and at the time of the apogee of the system in Athens a quorum of 6,000 participants was required for the decisions of the assembly to be valid. All this gave rise to a kind of "democracy without a State".

Direct democracy, as practiced in Athens, requires very special conditions of development, which have not occurred again in history. The citizen was a total figure, whose identity did not admit distinction between the public and private spheres: political life appeared as a natural extension of being itself. The interests of citizens were harmonious, a phenomenon typical of a homogeneous society that, moreover, had a small size, which favored direct relations between all. In classical Greece the existence of a wide stratum of slaves was a fundamental condition for the functioning of direct democracy. Thus, citizens were able to meet frequently to decide directly on laws and policy measures.

As Giovanni Sartori points out, after the decline of Greek democracy, the word democracy practically disappeared for a period of 2,000 years. They spoke rather of public res. In Rome, for example, the idea of mixed government was introduced, which represented diverse interests or groups that constituted the community. The system quickly adopted oligarchical features (government of a few), in which the formal commitment of popular participation resulted in a very limited capacity for control.

The expansion and consolidation of Christianity in the Western world displaced political reflection towards the universe of theology: the issue of political participation ceased to be a concern for more than a millennium. In the Middle Ages it reappeared in a different form that, at the time, had little to do with democracy. In several European countries, monarchs, urged by economic needs, called assemblies to deal with matters of State, fundamentally associated with the lifting of taxes and

warmongering companies. The members of these assemblies very loosely represented the estates that made up the kingdom: the nobility, the clergy and the bourgeoisie.

From there arose the idea of responsibility of the monarch before some of his subjects; This was the beginning of what is now known as Parliament. In England, in the fourteenth century, Parliament forced the king to sacrifice ministers to grant subsidies and then to present account statements; in France, Spain and Scandinavia similar phenomena happened. However, with the consolidation of the absolutist monarchies, the parliaments stopped being convened from the seventeenth and eighteenth centuries; England was the exception. Even so, the idea of political representation (effective or not) was beginning to penetrate Western political thought. Its origin was far from democratic, but it provided a solution to the problem of participation in complex political communities of large size.

At the end of the Middle Ages and during the Renaissance great transformations began to take shape, which little by little would return to make political participation an important topic of reflection and a popular demand that centuries later would become more universal. In the social, economic and political spheres there were changes that would have repercussions in the world of values.

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Considerations on contraventions and environmental education

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The system of abiotic, biotic and socioeconomic elements with which man interacts, while adapting to it, transforming it and using it to satisfy its needs, constitutes the environment. This system has been seriously damaged in recent times by industrial, nuclear and household waste and the use of fossil fuels, refrigerant gases, aerosols and other toxic gases that pollute the atmosphere and causes more and more damage to the ozone layer. After becoming aware of these serious effects on environmental ecosystems, the protection of the environment has become the fundamental premise that attempts to sustain life on earth, protected by the application of all the legislation that exists in this matter; but this alone is not enough, because if we lack the education and knowledge that we need to have to achieve this goal from childhood, if we lack environmental culture and if man does not see himself, in all aspects of life, inserted As regards the environment, the disastrous consequences will be inevitable. To reduce the frequencies of behaviors that affect natural resources, the Cuban State implemented a contraventional system with regard to the environment that allows, on the one hand, to educate citizens and on the other to sanction them if they incur in the figures described.

The analysis of the legislation that regulate the environment regarding contraventions and establish a relationship between them and the necessary environmental education is the objective of this work. A long time ago they knew behaviors that were given a criminal treatment without having a serious connotation in society. These acts were subject to minor penalties because they were less important than crimes or crimes because of the little injury they caused. The first term with which they were known in our country was "criminal misdemeanors" thanks to the Spanish Penal Code of 1870 that recognized them and for them imposed fines and administrative corrections.

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Employment of hyphenated approach for metabolomics fingerprinting of phenolics from *Torilis leptophylla* roots

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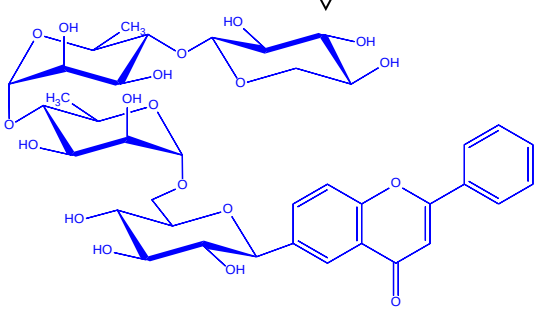
Graphical Abstract

Ultrasonic Assisted Extraction

Isolation & Purification
CC Sephadex LH-20

HPLC-DAD-ESI-MS profiling



Abstract

Torilis leptophylla synonymously called bristle fruit hedge parsley is widely appraised for its folkloric use to combat liver and gastrointestinal disorders. In order to have a complete picture of its phytoconstituents, an *in extenso* HPLC-MS analysis was carried out that led to the identification of 11 phenolic compounds in the roots of *Torilis leptophylla* including a *C*-linked glycoside reported here for the first time as Flavone-6-*C*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside. The present study paves a way for establishing the identity of metabolites, metabolomic fingerprinting and provides authentic basis for the better use of *Torilis leptophylla* in *in vivo* applications.

Introduction

Torilis species (Apiaceae), popular worldwide as hedge parsleys, comprises 11-13 species of aroma containing plants possessing diverse phytoconstituents and pronounced biological activities. Only three species of genus Torilis has been reported from various domains of Pakistan [1]. *Torilis leptophylla* is an annual herb that is traditionally a well known medication to relieve hepatic, gastric and intestinal disorders. Due of its potential to act as a disinfectant, the plant is highly efficacious against some pathogens [2]. Furthermore *Torilis leptophylla* leaves are taken as vegetable while stem and branches serve as fodder [3].

The comprehensive profile of *Torilis leptophylla* roots has not been documented earlier. In order to discern the full medicinal prospects of *Torilis leptophylla* roots, it is required to have a complete picture of its phytoconstituents therefore, the present study aimed at characterization, isolation and metabolomic fingerprinting of phenolics using HPLC-DAD-ESI-MS analysis.

Materials and Methods

Extraction

Torilis leptophylla roots extracts were prepared by infusion technique. Plant material (100 grams) was added to 300 mL of methanol: water (70: 30). Contents were irradiated at 35 KHz frequency and 220 volts by using Elma Ultrasonic LC-30H instrument for 15 minutes and stored at room temperature. After 24 hours infusions were filtered and the whole process was repeated thrice. Combined supernatants were evaporated to dryness *in vacuo* using Heidolph 4000-efficient rotary evaporator. As a consequence, crude extract having syrupy consistency was obtained.

Acid Hydrolysis

5 gm *Torilis leptophylla* roots were added to 2M HCl and methanol in (1:1) and contents were refluxed on a boiling water bath for 3 hours. Extract was cooled, filtered and filtrate was extracted thrice, each time with 25 mL of ethyl acetate to separate the pool of aglycones and sugars. Aqueous and ethyl acetate layers were separated and evaporated to dryness on a rotary evaporator. Aglycones (Agly) remained in ethyl acetate layer whereas sugars (Hyd) moved to the aqueous layer.

Spots of standard sugars and Hyd were applied on the TLC plate, developed, sprayed and heated in an oven at 105 ^{0}C for 5-10 min. until the brownish spots appeared. The sugars present in the sample were identified by comparison of their R_f values with those of the standard sugars [4]. The sugars were identified by using precoated silica gel $60F_{254}$ (Merck) as a stationary phase.

Column chromatography

Column chromatography was carried out by using Sephadex LH-20, a dextran gel that swells in water resulting in shrinkage of pore volume. Exploring the size exclusion chromatographic technique, slurry of sephadex made in MeOH : H_2O (70:30) was introduced into column having dimensions 17 × 1.5 cm, plugged with glass wool. 1.5 mL of herbal extract was introduced on the top of sephadex. Elution

was carried out by the same solvent and monitored by UV. Separation took place on the basis of molecular sizes. Larger molecules being eluted first followed by smaller sized molecules. Different fractions were collected which were later subjected to HPLC-DAD-ESI-MS analysis.

HPLC-DAD-ESI-MS analysis

Parameters

The flavonoids composition of *Torilisleptophylla* was determined by injecting 5 μL of extract in Agilent Technology 1200 LC instrument's stainless steel column (4.6 X 150mm) packed with 5 μmthickness of Agilent eclipse extra dense bonding (XDB) reverse phase C18 silica. Elution was carried out by using binary solvent system mobile phase (deionized water labeled as solvent A and HPLC grade acetonitrile labeled as solvent B). 0.1% formic acid was added in both the solvents. Sixty minutes gradient elution program was followed as 10% of acetonitrile at 0 minutes, 10% of acetonitrile at 15 minutes, 40% of acetonitrile at 40 minutes, 80% of acetonitrile at 50 minutes and 10% of acetonitrile at 60 minutes. Flow of mobile phase was maintained at a rate of 0.5 mL/min. Diode array detector was adjusted to detect three different wavelengths (254 nm, 320 nm and 370 nm). On the other hand mass spectrometer was equipped with electrospray ionization mode (ESI), Ion trap analyzer and photomultiplier tube as detector. Spectra were recorded in negative ionization mode between mass range of 50-2200 *a.m.u.*

Samples preparation

Clear concentrated hydroalcoholic extracts were subjected to HPLC-DAD-ESI-MS analysis to explore the phenolic compounds.

Results and Discussion

HPLC-DAD-ESI-MS Analysis

HPLC Chromatogram of the isolated fraction of hydro-alcoholic extract of TL is shown in fig.1. This pure fraction was obtained from column chromatography using sephadex as a stationary phase and MeOH : H_2O (70:30) as mobile phase.

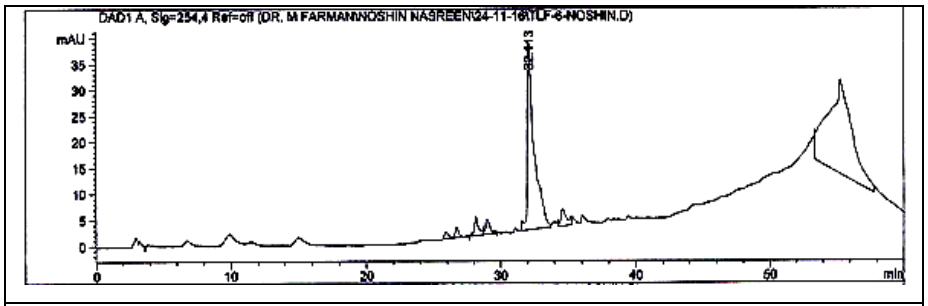
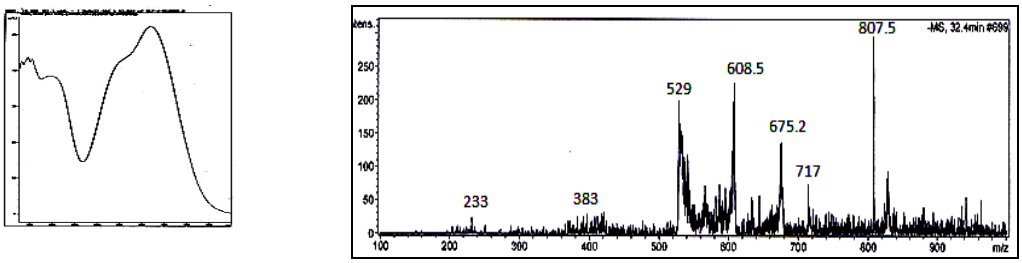


Fig.1 HPLC chromatogram of isolated fraction TL-F6

Compound eluted at retention time of 32.4 min when the composition of mobile phase was 28% acetonitrile in deionized water. More proportion of water revealed more hydrophilic nature of the eluted compound. *i.e.*, tetraglycosides. DAD response showed characteristic band pattern of flavones where band II corresponding to ring A appeared at 240 nm. The intensity of band I was higher as compared to band II. Hypsochromic shift of 10 nm from normal value indicated glycosylation on ring

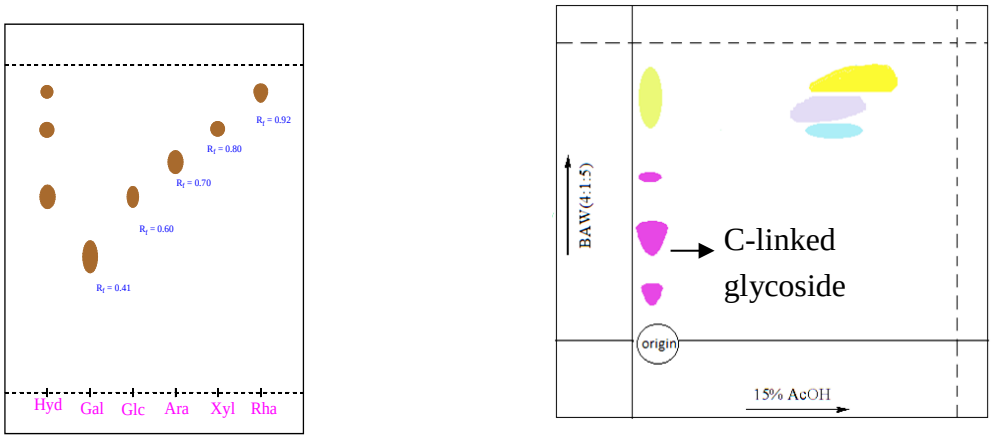
A. Typical value of absorption of band I at 330 nm indicated no substitution on B ring.The shape, intensities and λ_{max} values of the two bands were closely related to flavone-6-*C*-tetraglycoside [5]. Spectral data for compound TL-F6 is shown in fig. 2.



(a) (b)

Fig. 2 Spectral data for compound TL-F6 a)DAD response b) ESI-MS spectrum of compound TL-F6

The ESI-MS spectrum of the compound TL-F6 recorded in the –ve ionization mode showed deprotonated molecular ion at m/z 807. Sequential losses of 132, 146, 146 and 149 mass units probably justified the consecutive attachment of Sugars. The pictorial representation of TLC and PC analyses of sugars and aglycones obtained from acid hydrolysis is shown in fig. 3.



Stationary phase= Silica gel 60F_{254}(Merck) impregnated with Sodium dihydrogen phosphate

Mobile phase = Acetone :Water (9:1)

Spraying reagent =Aniline hydrogen phthalate

Stationary phase = Cellulose

Mobile phase

1D = BAW (4:1:5)

2D = 15% AcOH

Fig. 3 TLC and PC analyses of Hyd and AGLY obtained from acid hydrolysis

The TLC and PC analyses of the hydrolysate provided extra information in probing the structure of glycoside. Nature of sugars was determined from mass losses however the identity was established from co-chromatography of hydrolysate. Loss of pentose, hexose and deoxyhexose sugars were assigned to xylose, glucose and rhamnose respectively.

The PC analysis provided supplementary information regarding the *C*-linked nature of glycosides. Loss of 149 mass units and additional 13 mass units with aglycone fragment disclosed the *C*-linked nature of aglycone.

Linkages between the sugars were determined on the basis of literature cited. In nature [1→4] linkage of xylose to any other sugar, [1→4] linkage of rhamnose to glucose and [1→4] linkage between two rhamnose moieties is known. The structure was determined keeping in view the DAD response, the differences in m/z, sequential losses of sugars and deductions from TLC and PC analyses.

Deprotonated molecular ion appeared at m/z 807 $[M-H]^-$and it determined m/z 808 as the molecular mass of the compound. The fragment at m/z 717 indicated $^{0,2}X$ cleavage of xylose *i.e.,* $[M-H-{}^{0,2}X_{xyl}]^-$[6].The peak corresponding to m/z 675 indicated the cleavage of xylose moiety *i.e.,* $[M-H-xyl]^-$. Signal at m/z 529 resulted due to sequential cleavage of xylose and rhamnose from the deprotonated molecular ion *i.e.*,$[M-H-xyl-rham]^-$. Fragment corresponding to m/z 383 indicated losses of xylose and two rhamnose moieties from the compound *i.e.,* $[M-H-xyl-rham-rham]^-$. Appearance of peak at m/z 283 suggested the sequential losses of one xylose, two rhamnose and a *C*-linked glucose moiety from the compound *i.e.,* [M-H-xyl-rham-rham-glc(*C*-linked)]$^-$. The justification of major fragments formed in ESI-MS further ensured that the compound was Flavone-6-*C*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside. Spectral data for the characterization of compounds in *Torilis leptophylla* roots by HPLC-DAD-ESI-MS analysis is shown in table 1.

Table 1: Spectral data for the characterization of compounds in *Torilis leptophylla* roots by HPLC-DAD-ESI-MS analysis

Compounds Codes	**DAD λ_{max}value**	**Pseudo molecular Ion**	**Fragment ions**	**Identified compounds**
TLF-6	240,330	$[M-H]^-$	807,717,675,608, 529,383,233	Flavone-6-*C*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside
TLF-7	230,325	$[M-H]^-$	693,531,427,369	Flavone-7-*O*-*β*-D-glucopyranosyl-(1→6)- *β*-D-glucopyranosyl-(1→4)- *β*-D-xylopyranoside
TLF-8	220, 240sh, 326	$[M-2H+ACN]^-$	887,643,319	Ampelopsin-7-*O*-*α*-L-3″-propanoylrhamnopyranosyl-(1→6)-*β*-D-3″-caffeylglucopyranoside

TLF-9	230,320	$[M-H]^-$	563,503,329,269, 239	Apigenin-7-*O*-*β*-D-xylopyranosyl-(1→4)-*β*-D-glucopyranoside
TLF-10	268, 342	$[M-H+H_2O]^-$	889,737,685,563, 269	Apigenin-7-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→4)-*β*-D-xylopyranoside
TLF-11	240, 340	$[M-H]^-$	563,473,269	Apigenin-4′-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-xylopyranoside
TLF-12	248, 350	$[M-H+H_2O]^-$	595,477,449,269	Apigenin-7-*O*-rutinoside
TLF-13	270, 334	$[M-H+CH_3OH+CH_2O]^-$	329,267	3-Methyl-7-deoxy galangin
TLF-14	265,350	[M-H+Acetyl] $^-$.	327,285	7-acetyl luteolin
TLF-15	248, 350	$[M-H+2H_2O+CH_3OH]^-$	337,269	Apigenin
TLF-16	252, 335	$[M-H+ACN]^-$.	262,221	Flavone

Conclusions

The detailed study on metabolomic finger printing of *Torilis leptophylla* roots was successfully conducted and an innovative and upto date phytoconstituents profile of the genus was developed. The research proved that *Torilis leptophylla* is a vast fountain of secondary metabolites that can be utilized in a better way for future drug development.

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A brief account of the search for peptide vaccines

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Abstract: An increasing trend of viral diseases have led to search for faster response systems for preventives and therapeutics. While traditional vaccinations have led to spectacular successes against smallpox and polio viruses, the need for rapid response to new viral epidemics, like in the case of Ebola and Zika viruses, has called for new ways of tackling such infections. The fact that viral diseases are notoriously difficult to control, due primarily to their nature of fast mutational changes leading to growth of new strains and development of strains resistant to prevailing medications, adds an extra layer of complexity to the task. Peptide vaccines provide one means of responding to the epidemics. Since they are developed based on the viral sequences and focused on the antigenic sites, peptide vaccines are amenable to rational design paradigms. The first such vaccine for the canine parvovirus was developed in the 1990s and many peptide vaccines against human viral diseases are under different stages of phase trials. While production of such vaccines can be fast and cost-effective, and there are several issues still to be resolved, peptide vaccines are expected to play a major role in the future. In this very bried review we recount current status and some of the different approaches to design of peptide vaccines.

Keywords: viral epidemics, peptide vaccines, Zika virus vaccine, coronavirus, dengue virus, papillomavirus

An increasing trend of viral diseases have led to search for faster response systems for preventives and therapeutics [1]. For some viruses the prevalent technique of vaccinations have led to spectacular successes such as in eradicatng smallpox and almost complete eradication of polio from the list of the most dreaded scourges in human history. The premise of vaccine technology is to expose the body to an attenuated form of the virus to enable the immune system to recognize the invading pathogen so that it is prepared to overcome the live viruses when infections do occur. In general, however, viral diseases are notoriously difficult to control due primarily to their nature of fast mutational changes.

The problem with traditional vaccines is two-fold. For one, the entire process of creating the vaccines through egg-cell technology is time consuming and costly. From lab to market, costs for development of drugs and vaccines are estimated to be upwards of $1.8 billion for drugs [2,3], and between $200 to 500 million for vaccines [4] and take up to 10 years; vaccine research and product development is lengthy, complex, and loaded with binary outcome risks. Secondly, by the time such a vaccine or drug

is put to use, mutational changes in the viral sequence may creating resistant strains that render the drug or vaccine futile; the fate of Relenza, an influenza drug, is a case in point. Influenza vaccines used for the last two years in the USA have turned out to be ineffective due to sequence changes in the virus.

These experiences have led to search for newer means to tackle the viral infectivities. One approach is to do in silico analysis to search for relevant molecules to inhibit viral endocytosis to reduce the lead time for drug discovery. An alternative approach is to elicit the body's own immunological response through targeted peptide vaccines. This method (Fig.1) is comparatively fast, less costly and more efficient in production of the vaccines [5]. As of November 2017, there were over 500 peptide vaccines in various phase trials listed in NIH, USA, ClinicalTrials.gov website testifying to the intense search for a breakthrough in this line of research. This method of rational design of peptide vaccines still has some issues such as vaccine delivery, protein folding and adjuvant use to be solved but holds great promise for rapid response in development and production. We briefly mention here some of the studies that have been done in this respect.

The first report of peptide vaccine was against the canine parvovirus [6], which was subsequently licensed. Studies of *in silico* identification of suitable peptides were carried out by Brossart *et al.* [7] from human cell-surface associated mucin encoded by MUC-1, gene as well as by Ludewig *et al.* [8] who observed anti-tumour and anti-viral immune responses upon administering peptide antigen vaccine against lymphocytic choriomeningitis virus. Liao *et al.* [9] validated that a peptide vaccine based on human papillomavirus protein E5 epitopes used in conjunction with a CpG adjuvant resulted in strong cell-mediated immunity and anti-tumour behaviour in mouse model; CpG is a short single-stranded DNA molecule. Oany et al [10] searched through 56 strains of human coronavirus spike protein for the peptides with best epitope potential and selected a 9-mer peptide for a T-cell vaccine and a 7-mer peptide for B-cell vaccine design. Islam et al [11] determined a conserved high-scoring epitope in the chikingunya virus using alignment techniques and predicted a B-cell vaccine target. Chakraborty et al [12] identified conserved segments of the envelope protein of all four types of dengue virus through sequence alignment, determined the best with good surface exposure and selected those with high antigenicity.

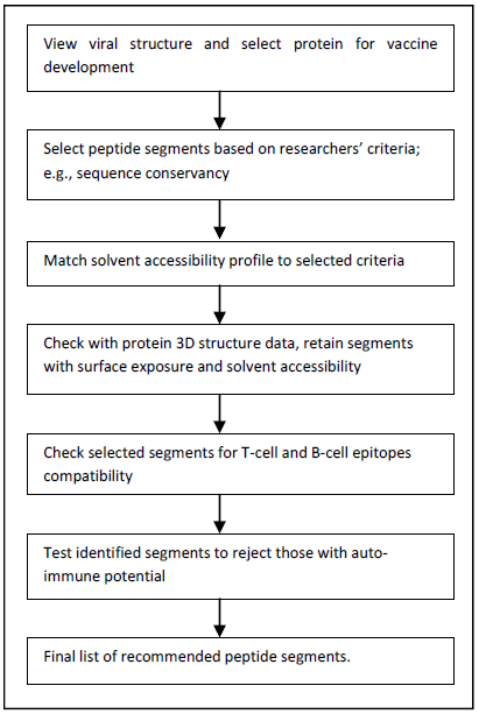

Fig. 1. Work flow chart of peptide selection process. *(Reproduced from Nandy, A and Basak, SC, Int. J. Mol. Sci.* ***2016****, 17, 666)*

The issue with these approaches is that those that have used current strains to determine peptides with strong epitope potential may fail with genetic drift and shift over even short time spans. To avoid such problems, we used graphical representation and numerical characterisation method for protein sequences to identify the best peptide vaccine candidates where a sufficiently large number of sequences of the relevant surface protein of the virion were scanned using such techniques to identify segments that were highly conserved over reasonably long time spans. These were then matched against the protein's Average Solvent Accessibility (ASA) profile to identify segments that were surface exposed, which were then verified using 3D crystal structure data wherever available. Those peptides that met these criteria were then matched against the target population's HLA profile to determine the best immunological response potential. The chosen segments were tested for autoimmune threats and a final list of candidate peptides for vaccines presented.

This approach enabled us to analyse several viruses which have caused epidemics acrtoss time and geography. Our first attempt was with avian (H5N1) influenza neuraminidase [13], which was later repeated for the H7N9 hemagglutinin protein [14]. A similar exercise for rotavirus [15] yielded four possible vaccine candidates. Application to several human papillomavirus envelope protein [16] showed that there could be peptide vaccines that can inhibit several papillomaviral types at a time. In a more recent example, the technique has been applied to comparatively sparse data of the Zika virus [17] that have caused severe disruptions in the Americas in 2015-16.

However, as mentioned earlier. there are still several problems to be overcome before peptide vaccines become a reality. Although there are several advantages of peptide vaccines over traditional types, and many are in advanced phase trial stages, no such vaccine has yet been licensed for human use. Issues such as adjuvants, stability, long term storage and the like need to be standardised. But when such problems are overcome, peptide vaccines hold the key for a cost-effective, fast response to deadly epidemics like the Ebola crisis or Zika virus rampage [18] in recent years.

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In silico molecular GRIP docking studies of antioxidant potentials of black tea compounds

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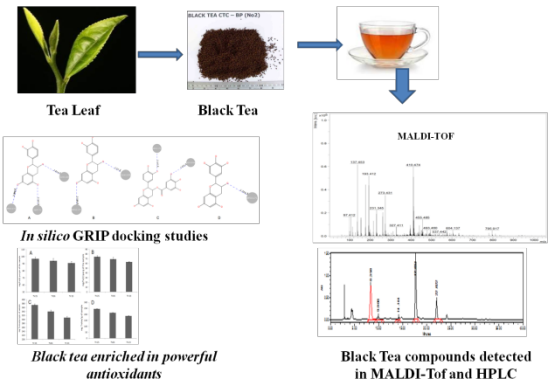
Graphical Abstract



Abstract.

Black tea is a very widely accepted and popular beverage that has attracted the research limelight and has exhibited multifaceted health effects due to the presence of wide range of pharmacologically active molecules. This paper aims to explore the antioxidant potentials of black tea catechins by *in silico* molecular GRIP docking studies. Basing on the result of dock score epicatechin gallate, catechin, epicatechin, epigallocatechin gallate all exhibited significant antioxidant potentials against the targeted enzymes.

Introduction

Tea (*Camellia sinensis*) belonging to family *Theaceae* is one of the most widely consumed beverages of the world and have proven to have multidimensional health potentials due to the actions of several pharmacologically active molecules in it (Sen and Bera, 2013). Black tea is very much preferred in Indian context due to the flavoring contents and astringency. Black tea is rich in several pharmacologically active molecules the catechins, benzotropolone compounds the theaflavins, methyl xanthenes viz. caffeine, theobromine, theophylline etc (Sen and Bera, 2013; Bhandari et al., 2015). *In silico* molecular GRIP docking studies aided in understanding the antioxidant potential of tea catechin molecules at mechanistic level. Rapid escalations in drug development costs, labor intensive screening of innumerable new chemical entities greatly limits the drug development process. More protein target molecules became available with the emergence of proteomics, genomics, bioinformatics, NMR and crystallography. Computational tools like *in silico* modeling is just suitable for the purpose of identification and analysis of the active sites and potential drug molecules binding to such sites (Singla, 2014; Meruva et al., 2014; Jadhav et al.,). Bioinformatic software tools offers a fast and frugal screening of active phytomedicinal compounds thereby diminishing labor, cost and time (Meruva et al., 2014). The aim of protein-

ligand docking is to calculate the binding energy of the protein-ligand reaction complex at given atomic co-ordinates. The key parameters for flexible docking include energy functions, protein catalytic sites and active residues (Meruva et al., 2014). For rapid, accurate protein-peptide and protein-ligand docking, GRIP™ by V Life software is a novel methodology available for rigid as well as flexible docking purposes. It makes use of a set of ligands with its conformers to be docked into the receptor cavity. This software helps to search for the active sites, consists of pre-computation of grids and tries to maximize favorable interaction and minimize unfavorable and repulsive interaction by proving the best possible orientation. GRIP scoring function allows for fast and precise capturing of ligand-receptor interactions in the active sites of proteins. In GRIP docking, unique conformers of a set of ligands are considered as input and offers the advantages of wide range of parameterizations, both ligand guided as well as cavity guided docking options, considers hydrogen bonding, repulsions and dispersion interactions with manual, automated and batch mode operations (Jadhav et al.; De et al., 2017).

Materials and Methods

The Proteins used for GRIP Docking include Copper-zinc superoxide dismutase (4B3E), glutathione peroxidase (3KIJ) and erythrocyte catalase (1DGB) of Homo sapiens were used for the current study. Their PDB structures were taken from RCSB. V life MDS 4.3 is very robust software with inclusion of all the necessary simulation modules. The 2D-structures of catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallo catechin (EGC) and epigallocatechin gallate (EGCG) the major catechin available in black tea were drawn in the 2D drawing application (2D Draw app) of MDS 4.3, followed by its conversion into 3D form by using default conversion procedure. Their energy minimization was done by using Merck Molecular Force Field (MMFF). MMFF is a class II force field designed to be a transferable force field for pharmaceutical compounds that accurately treats conformational energetics and non-bonded interactions. Molecular docking energy evaluations are usually carried out with the help of scoring function like dock score, PLP score, potential mean force (PMF) score, steric and electrostatic score. PLP score or Piecewise Linear Potential scoring function calculates both the shape and hydrogen bond complementarity of poses to the active site. The PLP score is a pair wise additive scoring function. The PLP function is incorporated by the MDS V Life Science software in the GRIP docking method which calculates the ligand-receptor binding affinity in terms of the PLP score. The PLP score is designed to enable flexible docking of ligands to perform a full conformational and positional search within a rigid binding site. These molecules were docked into the active site of 4B3E (copper-zinc superoxide dismutase), 3KIJ (crystal structure of human peroxidase) and 1DGB (catalase) that can be obtained as co-crystallized with bicarbonate ion or NADPH or by the use of cavities. The parameters fixed for docking simulation were: number of placements is 100, rotation angle at 10o, exhaustive method, ligand-wise results-10, scoring function-PLP score. By rotation angle, ligand would be rotated inside the receptor cavity to generate different poses of ligand inside the receptor cavity. By placements, the method will check all the 100 possible placements into the active site pocket and will result the best placements out of 100. After docking simulation, the best docked conformer of test molecules and reference ligands were then checked for their interactions with targeted proteins like hydrogen bonding, hydrophobic, pi-staking/aromatic, charge and van der Waal's interactions (Singla and Bhat, 2010; Singla et al., 2013; Malleshappa and Patel,

2013; Igoli et al., 2014; Igoli et al., 2014; Singla et al., 2012).

Results and Discussion

Catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) have undergone binding interactions with different amino acid residues by van der waals interactions, hydrophobic interactions, charge interactions with superoxide dismutase, glutathione peroxidase and catalase. All the mentioned catechins exhibited negative dock scores showing their strong binding affinities with the copper zinc superoxide dismutase. The scoring functions of Catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate were -48.81, -48.08, -61.67, -52.27, and -60.24 respectively. EGCG exhibited the highest negative dock score and can as considered as most potent antioxidant though all catechins showed the activities. The potencies of catechin and epicatechin were found to be almost equivalent in terms of docking score. Considering the binding interactions of the above mentioned black tea catechins with glutathione peroxidase the dock score of the Catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate were determined to be -60.23, -58.63, -61.19, -57.88 and -60.14 respectively. The potent catechin in terms of dock score was ECG. In this case catechin and EGCG showed to be equipotent in terms of dock score. Considering the case with catalase, ECG was found to be the most potent with a dock score of about -89.64 followed by EGCG with a dock score of -86.79; catechin and epicatechin were found to be equipotent with dock scores of -68.58 and -68.01 respectively followed by EGC (-66.66). From the results of *in silico* GRIP docking studies it is clear that the different catechins of black tea possess significant antioxidant potentials. Oxidative stress is the root cause of several chronic and degenerative diseases. Intake of natural antioxidants that are available in dietary sources and beverages can serve as an effective adjuvant therapy. However epicatechin gallate with highest affinity towards all proteins was found to be most active in comparison to other catecins. Here it is to be mentioned that the homeostatic condition of the body is disturbed due to oxidative stress and the situation is counteracted due to elevated superoxide dismutase (SOD) level. But in due course SOD level depletes and if potent natural antioxidants like EGCG etc present in black tea be supplemented at this stage it can effectively combat the crisis.

Conclusions

In silico molecular GRIP docking studies was helpful in the identification of the potent black tea catechins with antioxidant activities at different levels of potencies.

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FRAMA 1.0: Framework for Moving Average Calculation in Operators in Data Analysis

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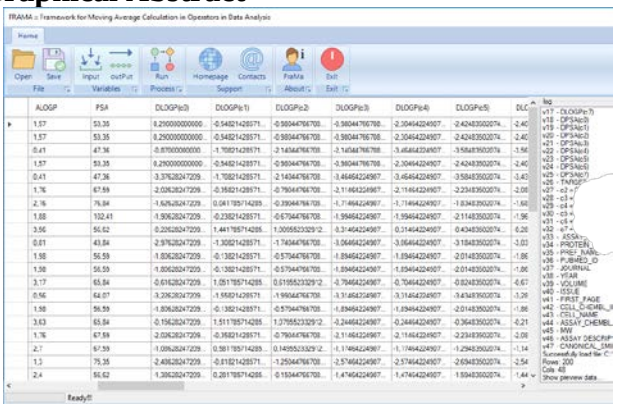
Graphical Abstract



Abstract. Moving Average (MA) operators are used in Box-Jenkins's ARIMA models in time series analysis (1). We can used MA operators of structural descriptors are useful to quantify multiple conditions or parameters in complex datasets in Omics, Medicinal Chemistry, Nanotechnology, *etc.* (2-7). Speck-Planche and Cordeiro have also used this kind of models in multiple problems (8-11). In this work, we develop a desktop application that allows applying mathematical and statistical calculations in batches, on input and output variables selected by the user. From the obtained result a percentage sample of data is taken with a random contrast on which Machine Learning algorithms are applied

Introduction

In principle, we can calculate numerical parameters to quantify the structure of chemical compounds, peptides, and/or proteins. We can also use them as input variables for Machine Learning (ML) algorithms in order to predict the biological properties of these drugs, peptides, or proteins (13-29). On the other hand, Perturbation Theory (PT) models allow us to predict the solutions to a query problem (q) based on a previous known solution for a similar problem or problem of reference (r). In a recent works, we outlined a new type of ML method called PTML (PT + ML) based on both kind of models with applications in drug discovery and proteome research (25, 30). The PTML method uses different kind of PT operators to predict the properties of one system based on the properties of a system of reference. For instance, Moving Average (MA) operators used in Box-Jenkins's ARIMA models in time series analysis (31). We have used MA operators of structural descriptors are useful to quantify multiple conditions or parameters in complex datasets in Omics, Medicinal Chemistry,

Nanotechnology, *etc.* (32-37). Speck-Planche and Cordeiro have also used this kind of models in multiple problems (38-41).

Discussion

González-Díaz *et al.* introduced a general-purpose PTML modeling technique useful to quantify the effect of perturbations in complex bio-molecular systems including DPINS and other networks (48, 49). Using PTML the model we can predict the values of the scoring function $f(\varepsilon_{ij})_{new}$for the DPI. The PTML model start using as input with the expected value of biological activity $f(\varepsilon_{ij})_{expt}$for one compound assayed in the conditions $\mathbf{c}_j$and add the values of the PT operators $\Delta D_k(m_i, c_j)$. The expected value $f(\varepsilon_{ij})_{expt} = \langle\varepsilon_{ij}\rangle$is the average value of the biological activity parameter ε_{ij} for all cases in ChEMBL dataset with the same c_0 = Activity parameter ε_{ij}(Units).These PT operators added $\Delta D_k(m_i, c_j) = D_k(m_j) - \langle D_k(c_j)\rangle$are intended to account for the changes (perturbations) in the system with respect to the expected values. Specifically, perturbations on thevalue of the molecular descriptors of the drug $D_k(m_j)$ with respect to the expected value$\langle D_k(c_j)\rangle$for a drug measured under the conditions of the experiment $\mathbf{c}_j$. These PT operators resemble the Box-Jenkins MA operators (25, 30). We use both Linear Discriminant Analysis (LDA) and Artificial Neural Network (ANN) algorithms to seek alternative linear and non-linear models (50). At follow, we depict the compact and developed forms of a PTML linearmodel:

$$f(\varepsilon_{ij})_{new} = a_0 \cdot f(\varepsilon_{ij})_{expt} + \sum_{k=1}^{kmax}\sum_{j=0}^{jmax} a_{jk} \cdot \Delta D_k\left(m_i, c_j\right) + e_0 \quad (1)$$

$$f(\varepsilon_{ij})_{new} = a_0 \cdot f(\varepsilon_{ij})_{expt} + \sum_{k=1}^{kmax}\sum_{j=0}^{jmax} a_{jk} \cdot \left(D_k(m_i)_{new} - \left\langle D_k(c_j)\right\rangle_{ref}\right) + e_0 \quad (2)$$

Results and Discussion

FRAMA, is a desktop application that supports different file formats, allows perform data preprocessing tasks on the selection of input and output variables, and its sub classification as grouping variables and continuous variables, where operations, operators and obtaining parametric values are applied, such as Mergin Data, Shannon Entropy, Z-Score, Moving Average, Euclidian Distance, among others. From the results obtained, a sample is selected for the application of Machine Learning algorithms on a sample of data

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Cocoa polyphenols (*Theobroma cacao*) as natural Amazonian antioxidant in sausage fresh.

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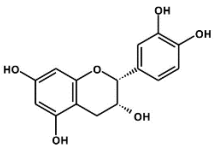
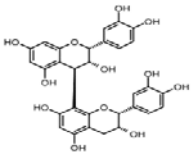
Graphical Abstract

Cocoa (*Theobroma Cacao)*

Polyphenols (*Theobroma cacao*) are used as natural



Epicatechin



Procianidin

Sausage prepared

ANALYSIS

Microbiological **Shelf life**
Organoleptic test

Abstract

Ecuador is considered one of the richest countries regarding biodiversity and its Amazon region guests several flora and fauna species. Cacao (*Theobroma Cacao*) is a tropical fruit with high commercial and biological importance due to the presence of polyphenols with antioxidant activity. In the present work cocoa polyphenols (*Theobroma cacao*) are used as natural Amazonian antioxidant in sausage fresh. Experimental units of sausage samples with different percentages (0, 2, 4, 6%) of cocoa polyphenols in the formulation with an experimental size of 5 kg were made; once the sausage was prepared, samples of 100 g were taken to perform sensory analysis and 100 g of samples destined for microbiological analysis. The microbiological analysis was performed on each sample of the formulated product, analyzes on the newly elaborated samples (1 day of elaboration) and an analysis with samples was developed after an estimated time (30 days) to assess the differences and active action of the antioxidant as a natural Amazonian preservative were performed. 15 people evaluated sensory characteristics to the sausage samples through a tasting test. As main results the natural antioxidants use allows prolonging the product shelf life, which results in an increase in color stability, since it prevents the transition from myoglobin to metamyoglobin, as well as maintaining its organoleptic conditions unalterable, slowing down oxidative phenomena such as product rancidity or increasing resistance to bacterial growth, since antioxidants of polyphenolic nature have antimicrobial activity.

Keywords: Polyphenols, antioxidant, functional foods, fresh sausage.

Introduction

For years, various strategies have been developed to prevent oxidative deterioration in products of meat origin through the use of antioxidants (Rostamzad *et al*., 2011). Most of these strategies have focused on limiting oxygen access to meat components susceptible to oxidation phenomena such as lipids and proteins. At the same time, new storage methods have been developed, such as vacuum packaging or packaging in a modified atmosphere in order to prevent the appearance of oxidation phenomena in the final product (Armenteros *et al*., 2012).

One way to reduce the occurrence of oxidation phenomena in meat and / or meat products is the use of antioxidants. The term antioxidant is generally attributed to any substance that is present at low concentrations, with respect to those of an oxidizable substrate and retards or prevents the oxidation of that substrate (Halliwell and Gutteridge, 1990). When antioxidant reacting with the free radical, it gives an electron oxidizing in turn and becoming a weak radical, with little or no toxic effects. In recent years it has been shown that a diet rich in plant polyphenols can improve health and decrease the incidence of cardiovascular diseases (Quiñones *et al*., 2012). In the present work cocoa polyphenols (*Theobroma cacao*) are used as natural Amazonian antioxidant in sausage fresh.

Materials and methods

Location and duration of the experiment

The present research was carried out in the Agroindustry's Laboratory, located in the Amazon State University, Km. 2 1/2 via Puyo to Tena (Paso Lateral), province of Pastaza, between coordinates 0º 59 '-1 "S and at a length of 77º 49' 0" W, it is found in the Amazonian Region of Ecuador in the west of the province of Pastaza, at about 924 m.a.s.l. Temperature 18 to 24 ºC.

Experimental units

Experimental units were formed for each sample of chorizo with different percentages of *Theobroma cacao* polyphenols in the formulation (0, 2, 4, 6%) and an experimental size of 5 kg of prepared dough. Once the sausage was prepared, samples of 100 g of each replicate sample were taken to perform sensory analysis and 100g of samples destined for microbiological analysis.

Microbiological analysis.

The microbiological analysis was performed on each sample of formulated product, analyzes were performed on the newly elaborated samples (1 day of elaboration) and an analysis with samples was developed after an estimated time (30 days) to assess the differences and active action of the antioxidant as a natural Amazonian preservative.

Sensory analysis and shelf life.

Through a tasting test, 15 people evaluated sensory sausage samples. An evaluation the product to know the shelf life in 30 days was made.

Results and discussion

Sensorial analysis

40% of the evaluated peoples that correspond to 6 persons, likes sausage samples without any addition of natural antioxidant; 9 from 15 people like the product with 2% natural antioxidant addition, 7 people who represent 47% have similarity in the sausage with 4% natural antioxidant incorporated, finally, 6 people equivalent to 40% like it and they like the product with 6% antioxidants. It should be noted that 4 people (27%) like the product a lot when they sensually find it optimal when 4% natural antioxidant is added (table 1).

Table 1. Percentage (%) of antioxidant in sausage samples.

Level of liking	0%	2%	4%	6%
I like very much	2	0	4	2
I like it	6	9	7	6
I do not like or dislike	5	6	3	4
I do not like	2	0	1	2
I dislike a lot	0	0	0	1
Total	15	15	15	15

Natural antioxidant and antimicrobial systems are set to become an important component in food preservation methodology. Wojciak et al. (2016) studied the effect of alternative natural preservatives (*Sinapis alba* L.-M, *Rosmarinus officinalis* L.-R, *Juniperus communis* L.-J) in combination with acid whey after the ripening period (21d) and over a prolonged storage period of sausage. An antioxidant activity of extracts exercise was performed. The antimicrobial, oxidative stability and sensory properties of these natural preservatives were compared to curing-control. Significantly lower rancid odor and rancid flavor were observed for R and M compared with the control sample. Incorporation of acid whey with rosemary extract will give the product a threefold effect: high quality (sensory acceptance), healthy benefit (elimination of nitrite and nitrate from meat products) and safety (improved microbiological and oxidative stability).

Marangoni and Moura (2011) determined sensory prolife of four samples of Italian salami using a methodology based on the Quantitative Descriptive Analysis. They select twelve individuals as judges and properly trained and used the following criteria: discriminating power, reproducibility, and individual consensus. The salami with coriander essential oil had lower rancid taste and rancid odor, whereas the control showed high values of these sensory attributes. Regarding brightness treated with coriander essential oil showed the best result.

By other hand, the addition of okra flour to an emulsified meat product (Frankfurter type sausage) was evaluated (Kitagawa *et al*. 2010) based on the physical, chemical, technological, and sensory characteristics of the final product. The results showed that the sausages containing okra flours A and B, as well as the control sausage, were accepted by the sensory panel. Moreover, there were no significant differences ($p \leq 0.05$) in the physical (color, objective texture, and emulsion stability) and chemical (pH and proximate composition) measurements of the sausages with and without the okra flour.

Microbiological analysis

The following table shows the significant reduction of microbial load in the stored sausages and that they contain polyphenols percentages incorporated in their formulation, while in the control product, since there is no protective agent, a microbiological growth can be observed (table 2). The use of natural antioxidants allows to prolong the useful life of the product which is in an increase of the stability of the color, since it avoids the transition from myoglobin to metamyoglobin, as well as maintains its

organoleptic conditions unalterable slowing down oxidative phenomena as the rancidity of the product or increasing resistance to bacterial growth, since antioxidants of polyphenolic nature have antimicrobial activity (Naveena *et al.*, 2008).

Consumers are becoming more aware of the toxicological implications of artificial additives in foods. Natural antioxidants, in addition to reducing the deleterious effects of lipid oxidation, are currently extremely highly valued. Santi *et al.* (2015) investigated the effect of addition of sun mushroom (*Agaricus blazei* Murrill) powder on the oxidative and microbiological stability of pork sausage during the shelf life. The results of the proximal composition and microbiological analysis for coagulase positive *Staphylococcus*, coliforms at 35 ºC and 45 ºC, *Salmonella sp* and sulfite-reducing Clostridium were consistent with those required by Brazilian legislation. The color of the products was of a decreased redness at the end of the storage period, on the 35th day, the TBARS values for the sausage with 4.0% powder was 0.509±0.12 mg MDA/kg sample and for the control was 1.131±0.12 mg MDA/kg sample. The sun mushroom powder had no effect on microbiological stability. It is concluded that sun mushroom was effective in terms of the oxidative stability of pork sausage when added in powdered form at concentrations of 1.0%, 2.0% and 4.0%.

Table 2. Total of microbial load in the stored sausages

General data			Indicators							
			Fresh samples				Sample with 30 days of preparation			
Type of sample	Code	Sample	Residual total coliforms	Mesophil bacteria count	Total coliforms	*E. coli*	Residual total coliforms	Mesophil bacteria count	Total coliforms	*E. coli*
Sausages	001	0%	<3	$2x10^4$	579	NA	<3	$2,2x10^4$	655	Nd
Sausages	002	2%	<3	$2,6x10^4$	723	NA	<3	$2,2x10^4$	567	Nd
Sausages	003	4%	<3	$2,2x10^4$	564	NA	<3	$1,8x10^4$	344	Nd
Sausages	004	6%	<3	$2,8x10^4$	490	NA	<3	$2,1x10^4$	302	Nd

Maximum Permissible Limits			
Total coliforms	Mesophil bacteria count	Total coliform	*E. coli*
0,3 – 1 < 1/g	M ufc/g	<2 NMP/100 ml	<0 NMP/100 ml Absence

Note:
ufc/g: colony forming unit per grams.
NMP/100ml: Most probable number of coliforms/100 milliliters of sample.
NA: Not Applicable

Conclusions

Cocoa polyphenols (*Theobroma cacao)* presents good qualities as an antioxidant in the use of sausages, allowing a better conservation and providing organoleptically unique qualities. Chorizo with 2% presents the best sensory characteristics and most welcome in the respondents. Meat products made with the addition of natural antioxidants from the Amazon provide, in addition to their basic nutritional properties, providing consumers with food that allows them to obtain better quality, health and life expectancy.

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Optimizing queries via search server ElasticSearch: a study applied to large volumes of genomic data

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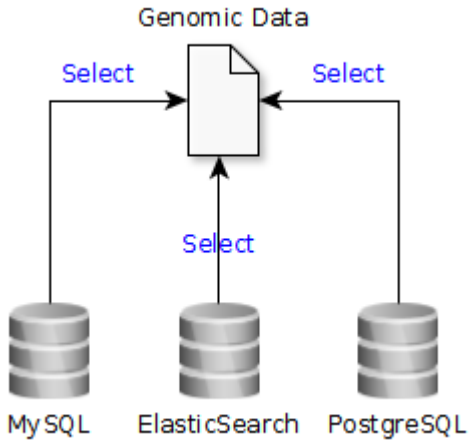
Graphical Abstract



Abstract

This work aims to use the ElasticSearch server to optimize searches on genomic data made publicly available by the UCI Machine Learning Repository. As a case study, the results obtained were compared with the MySQL and PostgreSQL relational databases. With the proposal presented, a gain of more than 90% was achieved through the use of ElasticSearch technology.

Introduction

ElasticSearch[1] is an open source search server started by Shay Banon project published in 2010. Its main concepts of use include: index, document, document type, nodes, cluster, shard, and replic [Kuc and Rogozinski 2013]. In this technology the records do not use the usual normalization of tables because the tool structure is designed to have superior search performance. Databases like NoSQL and MongoDB also operate in a very similar way.

When it is necessary to analyze large volumes of data, Bioinformatics acts as a multidisciplinary field that integrates knowledge from different areas. Its applicability goes from the analysis of biological data to the construction of tools and methodologies that allow the use of the computer for tasks usually laboratory. An important fact in this issue was the advent of the Human Genome[2] Project (HGP) and the subsequent availability of the data obtained for the entire scientific community [Pennisi 2001]. With this, the search for results in viable processing time has become a great challenge among bioinformatics, especially with regard to genomic data[Alencar 2010].

1 https://www.elastic.co/products/elasticsearch

2 Genome is the name given to the DNA set of all the chromosomes of an ovum or sperm, being constituted of 3.4 billion bases

Materials and Methods

The hardware composes a structure of a computer by: 1 processor with 8 cores 2.2 GHz, RAM of 6 GB and hard disk of 100 GB/SSD. The queries were performed on the same database, replicated in each of the following technologies: MySQL, PostgreSQL and ElasticSearch. In order to organize the applied methodology, the term "query" refers to a "select" in MySQL/PostgreSQL and a "search" request in ElasticSearch. Table 1 shows the results of the experiments for the "splice.data" file for the Molecular Biology (Splice-junction Gene Sequences) Data Set, available from the UCI Machine Learning Repository <https://archive.ics.uci.edu/ml/datasets>. The final, preprocessed file resulted in 1.4 million records, inserted through scripts in each database. The query was performed in the "NameGene" column, a field of type varchar (50).

Table 1. Results for the different technologies

Technology	Index method	Search time (s)
MySQL	*Full-Text Index*	0,697
PostgreSQL	*Full-Text Index*	1,125
ElasticSearch	*Default*	0,058

Table 1 shows that the technology with the highest performance and speed is ElasticSearch. This is mainly due to its more accurate caching system for repeated searches. For example, when performing a query that has already been done previously, the tool already maps the records, organized in the form of documents, which guarantees speed for searching for values in databases.

Conclusions

According to the experiments, it is clear that ElasticSearch achieves very good results in the response time of a query. A performance gain of 91.7% and 94.9% was observed using ElasticSearch technology compared to the MySQL and PostgreSQL relational databases, respectively. As a future work, we intend to investigate how to adapt the searches to the ElasticSearch database with the use of LIKE, commonly used in queries with autocomplete, but not contemplated until the last version tested.

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Data compression with Python: application of different algorithms with the use of threads in genome files

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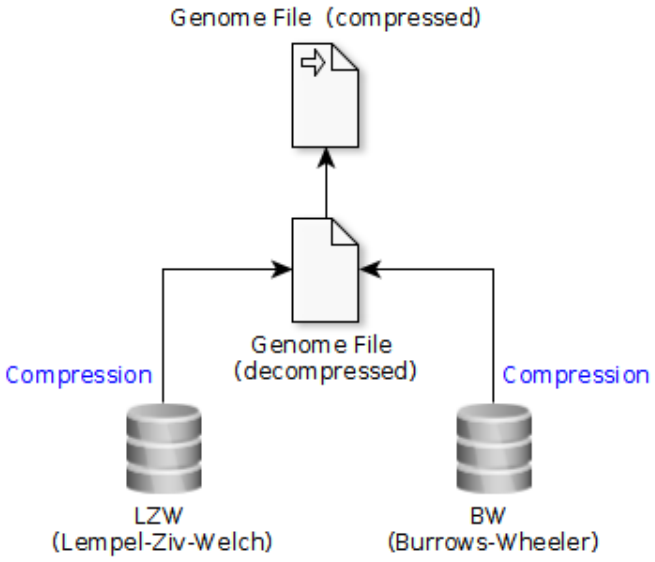
Graphical Abstract



Abstract

This work proposes and evaluates an implementation of different algorithms of data compression using the Python programming language allied to the use of threads. As a case study it was used genomic data available from the NCBI (National Center for Biotechnology Information) public database. The results show a compression ratio of approximately 40% in the size of the files after the application of the LZW (Lempel-Ziv-Welch) algorithm, thus presenting considerable superiority over the BW (Burrows-Wheeler) algorithm.

Introduction

Data compression is an important resource used to deal with the increasing size of files. Many genome analysis projects are currently being developed, especially with the advent of the Human Genome Project [Verli et. to 2014]. With the large amount of information generated through genetic sequencing as well as the need for its processing and analysis, new technologies and algorithms are often developed. Basically, the usual compression methods are divided into two groups: (i) algorithms that apply the concept of reversible transformation to a block of text, thus forming a new block of smaller size, but with the same characters [Burrows 1994]; (ii) algorithms based on sequential compression that make use of a dictionary to replace a sequence with a pointer that signals an earlier event of the same sequence [Welch 1984].

This work proposes and evaluates an implementation of different algorithms of data compression using the Python programming language allied to the use of threads. As a case study it was used genomic data available from the NCBI (National Center for Biotechnology Information) public database. The results show a compression ratio of approximately 40% in the size of the files after the application of the LZW (Lempel-Ziv-Welch) algorithm, thus presenting considerable superiority over the BW (Burrows-Wheeler) algorithm.

Materials and Methods

For the experimental environment it was used a simplified structure composed of a computer with: 3.07GHz processor (12 cores), 12 GB RAM and 500 GB hard disk. The analyzes of the BW and LZW algorithms were performed considering the rate and time of data compression. The term "compression ratio" was used to divide the size of the compressed data by the size of the initial data. To compute the compression time, only the main part of each algorithm is considered, ignoring parts such as variable declaration and inclusion of libraries. Table 1 shows the results of the experiments for the file "ref_ASM45574v1_gnomon_scaffolds.txt" with a total size of 122 MB, referring to an excerpt from the genome identified by "*Aligator sinensis*", belonging to the family *Alligatoridae*.

Table 1. Results for the different compression methods

Method	Compression ratio (%)	Compression time (s)
BW	0	-
LZW	39.98	29.72

Table 1 shows that the BW algorithm did not perform compression for the 122 MB file used, causing execution locking. The method with the best compression rate is LZW, this is due to the fact that the original file to be compressed has many identical or similar stretches. In the experimental tests performed the use of the BW algorithm was limited to sequence files with a size of approximately 10 MB.

Conclusions

The main contribution of this work was to present an algorithm option for data compression based on the Python programming language. By default, the algorithms were not designed to work in parallel, however, with the use of the Python Threading library this was achieved. With the experimental environment implemented, it was possible to analyze the performance of both the compression rate and the compression time for each algorithm. As future works, we intend to extend the range of algorithms to be studied as well as the application and analysis of decompression metrics with emphasis on public genomic data.

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Applications of Structure Based Drug Design Approaches towards Design and Development of Calcium Channel Blockers

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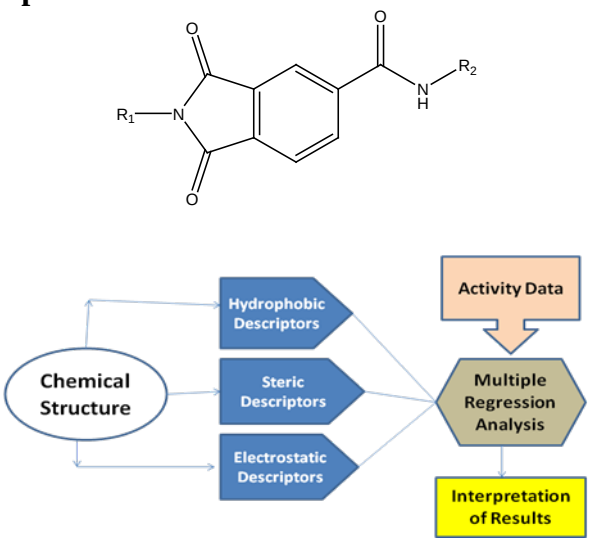
Graphical Abstract



Abstract.

Structure-Based Drug Discovery approaches facilitate the efforts towards drug discovery and in this communication these are applied towards development of Calcium Channel Blockers (CCBs). Quantitative structure-activity relationship (QSAR) studies were carried out on a series of 1,3-dioxoisoindoline-5-carboxamide derivatives and the contributing descriptors were identified as hydrophobicity, electronic constant (σ_{R1}) and some indicator parameter (I). The data set designed based on QSAR conclusions was screened against Voltage gated CCB receptor and the binding profile was predicted.

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Molecular docking studies of benzothiazinone derivatives in the search for new tuberculostatic agents

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Abstract: Despite the efforts to reduce the rate of infection, the resurgence of Tuberculosis, accompanied by strains of *Mycobacterium tuberculosis*, calls attention to the search for new drugs that can bypass resistance mechanisms. Among the strategies for the development of antitubercular lead compounds, benzothiazinones (BTZ) are included, with highly selective mechanism of action of DprE1 flavoenzyme. We studied four compounds in the antituberculosis activity (IC_{50} values between 0.029 - 0.060 μg/ml). These derivatives were subjected to energy minimization and Molecular Docking calculations in the software Molegro Virtual Docker, from which the binding free energy calculations showed that the suggested compounds had better binding affinity with DprE1 when compared to PBTZ169, a crystallized inhibitor of DprE1. Our results showed that the compounds showed similar crucial binding interactions between the compounds, which may determine that there was molecular rearrangement within the active site itself. Thus, our studies evidenced the probable interactions that favor the activity of the derivatives, as well as other interactions of the compounds with other residues not reported in the literature, and these may act as lead compounds in the development of new antituberculosis drugs.

Keywords: Tuberculosis, benzothiazinones, Docking

1. Introduction

The Tuberculosis (TB) is a contagious bacterial infection caused by a group of bacteria known as Mycobacterium, which comprises the bacillus Mycobacterium tuberculosis (Mtb). This is an optional intracellular pathogen that has developed resistance to first- and second-line anti-tuberculosis drugs. Antibiotic resistance and multidrug resistant TB strain are a serious problem because of the negative effects on the design of treatment strategies. Therefore, new classes of anti-mycobacterial compounds are required [1].

In recent years, a number of significant efforts have been made worldwide to treat tuberculosis [2]. Despite all current efforts in the development of anti-tuberculosis drugs, the fact is that only two new anti-TB drugs have been approved after a period of approximately 40 years. In addition,

these drugs are only recommended for MDR-TB (multi-drug resistant TB) while other treatment options are not available [3-4].

The 1,3-benzothiazin-4-ones, also known as benzothiazinones (BTZ), are a new class of potent antimycobacterial agents responsible for blocking the synthesis of D-Arabinofuranose, a component of arabinogalactan and arabinomannan, with a mechanism of action highly selective in the cell wall of mycobacteria [5]. BTZ043, as well as its derivative PBTZ169, is a nitroaromatic compound that acts on the subunit of the enzyme DprE1, an essential flavoprotein for the cell membrane of *Micobacterium tuberculosis* [6-8]. BTZ043, which contains activity similar to clinical isolates for M. tuberculosis [6], showed nanomolar activity [5], making it a promising candidate against tuberculosis.

Therefore, in this work we analyzed four benzothiazinones derivatives recently published by Zhang et al. (2017), which have already undergone synthesis, elucidation and in vitro tests and have shown promising activity against *Mycobacterium tuberculosis*. Soon after, we evaluated its inhibition in the DprE1 enzyme of the derivatives through Molecular Docking.

We also observed that some of the 4-benzothiazinones and PBTZ169 derivatives with the receptor amino acid residues were similar, highlighting them as critical interactions for the activity: ketone oxygen with residue Lys134, steric interaction of Fluorine with Lys367 , and the different portions of each molecule interacted with the Cys187 residue, which may determine that there was molecular rearrangement within the active site itself (Fig. 1) and explain how these residues are essential for the activity.

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Table 1. Energy Values of ligand and enzyme DprE1 interaction calculated from Molegro Virtual Docker program.

Compound	MolDock score
PBTZ169	–70.0994
10a	–72.5120
10e	–59.0168
10h	–64.1054
11a	–58.0330

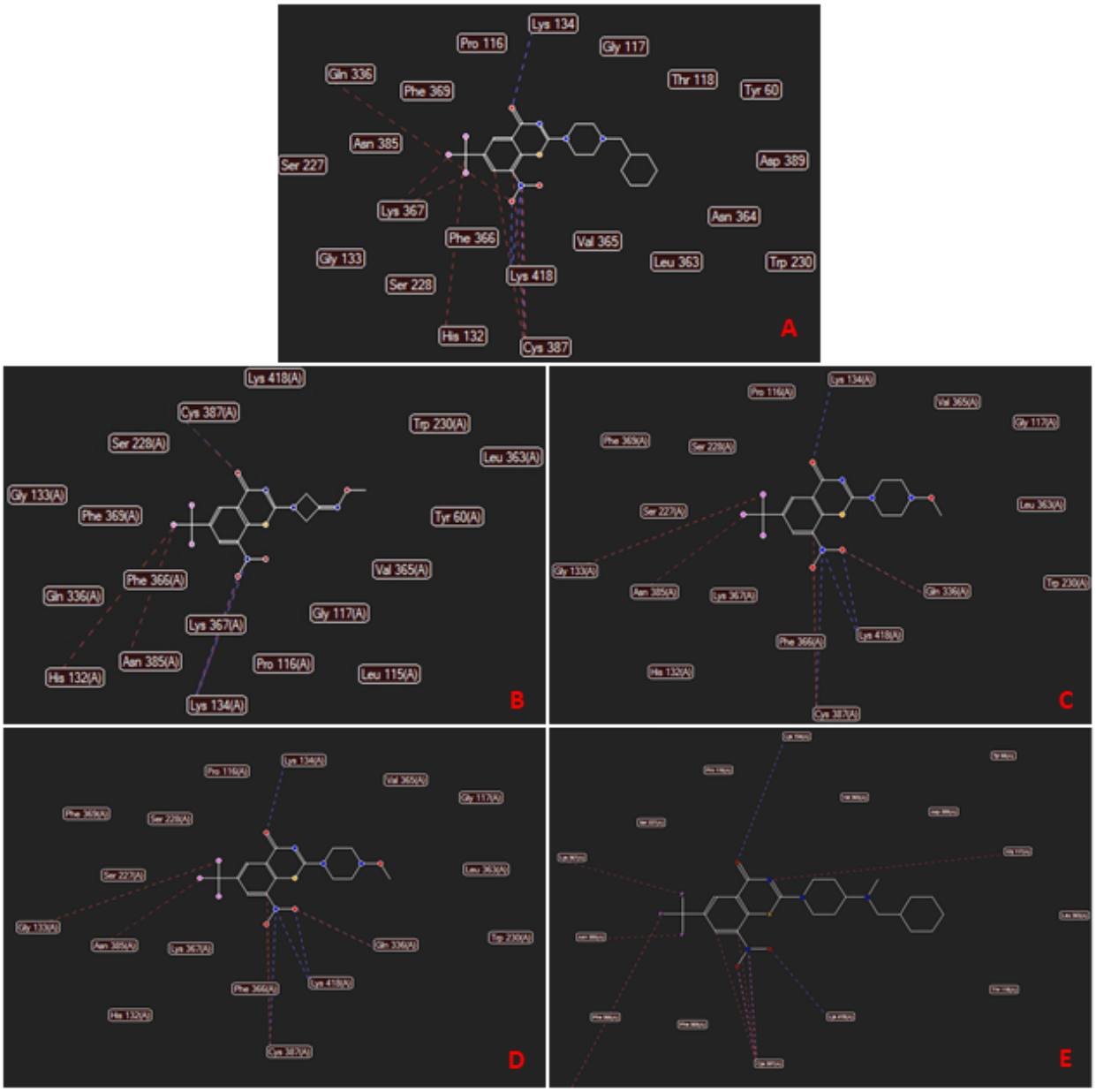

Figure 1. Hydrogen and steric bonds between the compounds and DprE1. a) PBTZ169, b) 10A, c) 10E, d) 10H and e) 11A.

For compound 10a steric interactions of Fluor with Asn385 and His132 were observed; hydrogen bonds at the residues of Cys387, Lys134 and Lys367 through the carbonyl portion of the heterocyclic and the portion attached to the nitro portion of the benzene ring. For compounds 10e hydrogen bonds were also observed at the residues Cys387 and Lys418 with the nitrobenzene group, the residue Lys418 with the oxygen bound to nitrobenzene and hydrogen interactions of the residue Lys134 with the oxygen of the carbonyl of the heterocyclic.

The steric interactions were observed at residues Gly133 and Asn385 on fluorine bound to the benzene ring, at the residue Cys387 to benzene and at Gln336 at the oxygen of nitrobenzene.

For compound 10h, hydrogen bonds were observed at residues Lys418 and Cys387 to nitrobenzene and from Lys134 the carbonyl portion of the heterocyclic. Steric interactions were observed at the residues of Lys387 with the trifluoromethylbenzene moiety, from Cys387 with nitrobenzene oxygen and directly with the benzene ring and from Gln338 with nitrobenzene oxygen.

As a final analysis, for compounds 11a hydrogen interactions were observed in the Lys418 residues with the oxygen of the nitrobenzene moiety; the second interaction of the Cys387 residue with the nitrogen and oxygen of the nitrobenzene moiety and also the residue Lys134 with the oxygen of the carbonyl moiety of the heterocyclic. Steric interactions were observed at the Gly133, Asn385 and Lys367 residues with the trifluoromethylbenzene moiety, interactions of the two-carbon Cys387s with respect to the benzene group and interactions of the Gly117 residue with the nitrogen of the heterocyclic moiety.

Recently, studies by Shaikh et al. (2016) has shown that its benzothiazinone derivatives have similar binding modes reported in this study, from which the hydrogen bonding acts as a support and guides the position of the compound in the active site, favoring the other steric interactions with the receptor. In addition, the interactions of the benzene ring, correlating the pi-pi interaction, favored the activity of compound 11a in the active site of DprE1.

Materials and Methods

The three-dimensional structures were drawn using HyperChem 8.0.3 software [9] and energy-minimized employing the MM+ force field. Posteriorly, we performed a new geometry optimization based on the semi-empirical AM1 method on the Spartan program [10]. The optimized structures were subjected to conformational analysis using a random search method.

The four benzothiazinones and the PBTZ169 ligands were submitted to docking with enzyme DprE1 (ID PDB 4NCR) [11]. The enzymes were imported from the Protein Data Bank (PDB) in the Molegro Virtual Docker (MVD) 6.0 program. The Moldock score algorithm was used as a score function in predicting the best interaction between ligand and receptor.

Conclusions

In this study were analyzed by means of Molecular docking 4 new benzothiazinone derivatives in the enzyme DprE1, of which they had better energy values when compared with the standard compound. As well, these compounds obtained stability of binding to the amino acid residues of the active site. Therefore, the study emerged as an aid in the continuous search for new drug candidates that may have greater antituberculose activity in the enzyme DprE1, blocking the mycobacterial formation.

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Virtual screening of a cyclics imides to evaluate potential new multi-target agents against species of Leishmania

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Abstract: Leishmaniasis is a neglected disease that does not have adequate treatment. To try to solve this problem, we have tested a database with 33 cyclic imides and evaluated their potential anti-Leishmanial activity (*L. donovani*) through ligand-based and structure based virtual screening. A diverse set selected from CHEMBL databanks of 818 structures (*L. donovani*) with tested antileishmanial activity against promastigotes forms, were classified according pIC50 values in order to generate and validate Random Forest model that show higher statistical indices values. The structure of four different *L. donovani* enzymes were downloaded from PDB databank and imides structures were submitted to molecular docking. *In silico* study allowed us to suggest that the cyclic imide 5_{27} can be tested as a potential multitarget molecule for leishmanial treatment, presenting activity against four strategic enzymes to treatment with probability of activity of 60%.

Keywords: Cyclic imides, Virtual Screening, Molecular Docking, *Leishmania donovani*, antileishmanial activity.

Mol2Net YouTube channel*: http://bit.do/mol2net-tube*
YouTube link: *please, paste here the link to your personal YouTube video, if any.*

1. Introduction

Despite the great development of modern medicinal chemistry there are some microbial diseases that remain without adequate chemotherapeutic agents, either due to problems of toxicity or resistance, among them is Leishmaniasis. Leishmaniasis is a complex of infectious diseases caused by parasites of the family Trypanossomatidae and genus *Leishmania*[1, 2]. It affects around 12 million people around the world, there are reported cases in 98 countries spread across 5 continents, mainly in poor countries, making the disease be classified as a neglected disease by the World Health Organization[2-4]. In this context, strategies to obtain new, more active and less toxic drugs should be stimulated. Sources of natural products combined with synthetic and chemoinformatic methodologies are strategies used to obtain molecules that are most likely to be effective against a specific disease. Computer-Aided Drug Design (CADD) has become an indispensable tool to the pharmaceutical industry and academia in the last years and has been employed during various stages of the drug designs process. Initially, this method focuses on reducing the overall number of possible ligands; in the later stages, during lead-optimization, the emphasis shifts to reducing experimental costs and the duration of time required to make a discovery. Applied ligand-based virtual screening using Volsurf and Molegro descriptors and a random forest algorithm (a method of machine learning) were included in the structure-based virtual screening[5-7].

A group of compounds with various biological activities are cyclic imides, which present a large class of compounds obtained by organic synthesis including several subclasses, among them maleimides, succinimides, glutarimides, phthalimides and naphthalimides, as well as their respective derivatives[8]. Because they are electronically neutral and of a hydrophobic nature, they easily cross cell membranes, leading to the important pharmacological effects of these imides, such as anti-inflammatory, antitumor, antimicrobial activities among others, which may be related to the size and characteristics of the groups present in the imidic ring, which may alter the steric characteristics of the molecules and altering their activity[9-13].

Taking into account the great medicinal importance of cyclic imides and their derivatives, as well as the excellent results found and published to date, the planning of 33 cyclic imides (Figure 1) was carried out and a virtual chemical screening was carried out to select molecules with higher probability to show the desired effect against selected *Leishmania* targets.

2. Results and Discussion

The Volsurf (v 1.0.7) program generated 128 descriptors that, together with the dependent variables (binary classification) that described whether the compounds were active (A) or inactive (I), were used as input data in the Knime program (v. 3.4.0) to generate the RF model. For all compounds that comprised the training data sets, the generation of all 128 descriptors by

Volsurf+ was rapid, taking approximately 25 minutes using a computer with an i7 processor, running at 2.6 GHz, and equipped with 8 GB of RAM.

Table 1 summarizes the statistical indices of the RF model for the training, cross-validation, and test sets for compounds tested against forms Promastigotes of *L. donovani*. For the training set, the learning machine program gave the same hit rates for the inactive compounds and active compounds, which were 100%. However, for the cross-validation and test sets, the RF model was better, in the study, at predicting the inactive compounds; the specificity (true positive rate) was lower for the cross-validation and test sets (71.06% and 79.31%, respectively) than the sensitivity (true negative rate), which was measured to be 91.19% and 91.43%, respectively (Table 1). The ROC plot that was generated for the test set, which plotted the true positive (active) rate against the false positive rates had an area under the curve (AUC) value of approximately 0.91, which is significantly higher than 0.5. The Matthews Correlation Coefficient (MCC) values for training, cross-validation, and test sets were 1.000, 0.645 and 0.716, respectively. Because an MCC value of 1 represents a perfect prediction, 0 represents random prediction, and -1 represents total disagreement between prediction and observation, the RF model shows significant MCC values.

We evaluated the potential of 1-7 imides as antileishmanial leads using the RF model and docking on selected Leishmania enzyme targets. The results indicate that these compounds could show activity. The compounds showed good performance against TOPI and OASS.

Five structures were indicated as potentially active (compounds 4 and 5 were indicated as potentially inactive) using the RF mode. Docking results gave similar values for all compounds. Therefore, we evaluated a databank of 33 cyclic imides to obtain a qualitative structure-activity relationship using a combined approach of virtual screening, structure based and ligand-based, in order to select compounds with potential higher antileishmanial activity.

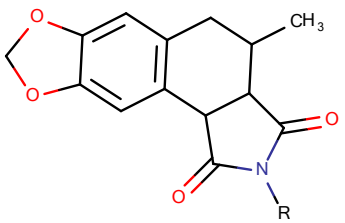
A computational chemistry multitarget model to predict the results of experimental tests for Leishmania with significant success has been reported in the literature[21], so we used our *in silico* results of the cyclic imides to select structures that presented lower energy binding (7 compounds) from each enzyme. Looking for multitarget compounds, we selected imides with activity against three or more enzymes. The compounds 27 and 32 presents activity against all enzymes: TOPI, NMT, Cyp and OASS. The compounds 11, 16, 18, 26 and 33 show low Moldock score energies against three different enzymes. From these structures, we selected only the compounds that were classified as active in the RF model. Therefore, our methodology was to apply two screening approaches simultaneously: ligand-based screening, using the RF model generated using Volsurf descriptors from the dataset of 818 compounds and structure-based screening using four enzymes. The ligand-based approach of those seven compounds shows six of them as active (probability over 50%)

corroborating with docking studies. Only the compound 11 present percentages close to the active line, with values of 48%.

The analysis of activity of selected imides demonstrates an affinity of this group of compounds with the enzyme NMT, where all presented a good interaction. Six compounds presented a good interaction with OASS, five compounds with TOPI and five with Cyp.

Table 1. Summary of training, internal cross-validation, test results, and corresponding match results, which were obtained using the RF algorithm on the total set of 818 compounds tested against forms Promastigotes of *L. donovani* (655 were in the training set and 163 in the test set).

	Training			**Validation**		**Test**		
	Samples	**Match**	**%Match**	**Match**	**%Match**	**Samples**	**Match**	**%Match**
Active	293	293	100	235	71.06	58	46	79.31
Inactive	525	525	100	420	91.19	105	96	91.43
Overall	818	818	100	655	83.97	163	142	87.12



1 33

1: R= 4-F,3-NO_2Ph
2: R= 4-Br,3-NO_2Ph
3: R= 4-Cl,3-NO_2Ph
4: R= 4-COOHPh
5: R= SO_2NH_2Ph
6: R= CH_2Ph
7: R= Ph
8: R= 4-CH_3Ph
9: R= 4-CH_2CH_3Ph
10: R= 4-$CH(CH_3)_2$Ph
11: R= 4-PhPh
12: R= 3-CH_3Ph
13: R= 3-CH_2CH_3Ph
14: R= 3-ClPh
15: R= 3-BrPh
16: R= 3-NO_2Ph
17: R= 3-COOHPh
18: R= 3-PhPh
19: R= 3-OHPh
20: R= 4-OHPh
21: R= 4-NO_2Ph
22: R=CH_2-4-CH_3Ph
23: R= CH_2-4-OHPh
24: R= CH_2-4-ClPh
25: R= CH_2-4-BrPh
26: R= CH_2-4-COOHPh
27: R= CH_2-4-NO_2Ph
28: R= CH_2-3-CH_3Ph
29: R= CH_2-3-OHPh
30: R= CH_2-3-ClPh
31: CH_2-3-BrPh
32: CH_2-3-CH_2CH_3Ph
33: CH_2-3-$CH(CH_3)_2$Ph

Figure 1. Cyclic imides tested

3. Materials and Methods

Dataset

From the ChEMBL database, we selected a diverse set of 818 structures (https://www.ebi.ac.uk/chembl/), which had been screened (in vitro) to inhibit the promastigote *L. donovani* The compounds were classified using values of -logIC_{50} (mol/L) = pIC_{50}, which led us to assign 293 actives (pIC_{50} ≥ 5.0) and 525

inactives ($pIC_{50} \leq 5.0$). We used a border in the pIC_{50} values looking for better prediction results. In this case, IC_{50} represented the concentration required for 50% inhibition of promastigote *L. donovani*. The compounds with pIC_{50} values between 4.7 and 5.0 were excluded to minimize the border effect and improve the discriminant power of the generated models. Our databank includes compounds 1-33. For all structures, SMILES codes were used as input data to Marvin 17.18.0.1784, 2017, ChemAxon (http://www.chemaxon.com). We used Standardizer software [JChem 17.18.0.1784, 2017; ChemAxon (http://www.chemaxon.com)] to canonize structures, add hydrogens, perform aromatic form conversions, clean the molecular graph in three dimensions, and save compounds in sdf format[14,15].

Volsurf Descriptors

Three-dimensional structures (3D) were used as input data in the Volsurf+ program v. 1.0.7 and were subjected to molecular interaction fields (MIFs) to generate descriptors using the following probes: N1 (amide nitrogen-hydrogen bond donor probe), O (carbonyl oxygen-hydrogen bond acceptor probe), OH_2 (water probe), and DRY (hydrophobic probe)[16]. Additional non-MIF-derived descriptors were generated to create a total of 128 descriptors. Volsurf descriptors have been previously used to predict antileishmanial activity of natural products on enzymes and predict activity of some molecules[17-18].

Models

Knime 3.4.0 software (KNIME 3.4.0 the Konstanz Information Miner Copyright, 2003-2017, (www.knime.org)[19] was used to perform all of the following analyses. The descriptors and class variables were imported from the Volsurf+ program, v. 1.0.7, and the data were divided using the "Partitioning" node with the "stratified sample" option to create a training set and a test set, encompassing 80% and 20% of the compounds, respectively. Although the compounds were selected randomly, the same proportion of active and inactive samples was maintained in both sets. For internal validation, we employed cross-validation using 10 randomly selected, stratified groups, and the distributions according to activity class variables were found to be maintained in all validation groups and in the training set. Descriptors were selected, and a model was generated using the training set and the Random Forest (RF) algorithm[20], using the WEKA nodes[21]. The parameters selected for RF included the following settings: number of trees to build = 1900, seed for random number generator = 1909501934341. The internal and external performances of the selected models were analyzed for sensitivity (true positive rate, i.e., active rate), specificity (true negative rate, i.e., inactive rate), and accuracy (overall predictability). In addition, the sensitivity and specificity of the Receiver Operating Characteristic (ROC) curve were found to describe the true performance with more clarity and accuracy. The plotted ROC curve shows the

true positive (active) rate either versus the false positive rates or versus sensitivity (1: specificity). In a two-class classification, when a variable that is being investigated cannot be distinguished between the two groups (i.e., when there is no difference between the two distributions), the area under the ROC curve equals 0.5, which is to say that the ROC curve will coincide with the diagonal. When there is a perfect separation of values between two groups (i.e., no overlapping of distributions), the area under the ROC curve equals 1, which is to say that the ROC curve will reach the upper left corner of the plot[22].

Docking

The structure of *L. donovani* enzymes Topoisomerase I (TOPI)[23], N-myristoyltransferase (NMT)[24], cyclophilin (Cyp)[24] and O-acetylserine sulfhydrylase (OASS)[25] downloaded from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). 1-33 structures were submitted to molecular docking using the Molegro Virtual Docker, v. 6.0.1 (MVD). All of the water molecules were deleted from the enzyme structure, and the enzyme and compound structures were prepared using the same default parameter settings in the same software package. The docking procedure was performed using a GRID of 15 Å in radius and 0.30 Å in resolution to cover the ligand-binding site of the enzyme's structures. The Moldock score algorithm was used as the score function[26]. For all enzymes the binding site was the same as the ligand present in the pdb file.

4. Conclusions

We have conducted a comparative ligand- and structure-based approach using Molegro Virtual Docking and machine learning RF to determine the antileishmanial potential of seven cyclic imides synthesized. In silico study allowed us to suggest that the cyclic imide 27 can be tested as a potential multitarget molecule for leishmanial treatment, presenting activity against four strategic enzymes to treatment with probability of activity of 60%.

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Author Contributions

JAL, CL, NC built database; JAL performed all calculus; and JAL, MTS and LS wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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The KP Algorithm for the analysis of the optimal flow of information

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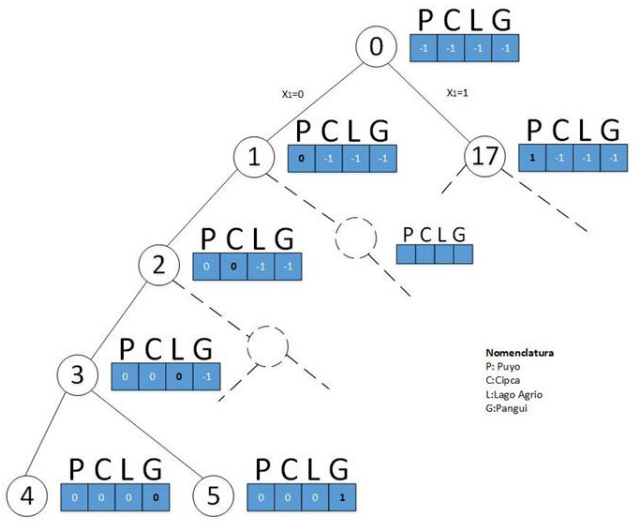
Graphical Abstract



Abstract.

The purpose of the present research is to propose a solution to optimize the flow of institutional academic information, given that, for the economic moment in Ecuador, government budgetary allocations do not cover the investment deficit in the renovation, acquisition and technological updating, generating inconveniences in the flow of university information, in compliance with the Law Reforming the Law of Creation of the Amazon State University, which allows to extend the academic offer to different sectors of the region.

The solution proposed is applied to the Academic Information System, where most of the records related to the generation of institutional evidences of university accreditation, internal control of academic processes, research, community ties and management are stored. The optimization analysis of the information flow is based on the application of the algorithm KP.

Introduction

Ecuador currently has as academic guidelines the compliance with quality standards that allow it to have a categorization within the field of Higher Education, consequently, computer systems of academic, institutional and administrative management, provide quality information and form the fundamental pillar in processes for obtaining reports for internal and external evaluation, in which the records on requested and applied indicators can be compared on institutions and careers, respectively.

The deployment of computer systems and their databases can have a marked impact on the extraction of records, because not all institutions have the technological and economic resources to interconnect their data centers or maintain a direct communication or high availability , and in some cases it is necessary to take into account the geographic distance, physical accessibility and availability of Internet services or interconnectivity in the headquarters and research centers, which directly influence the type of distribution of records or database.

Conclusions

The adaptation of the KP algorithm (Knapsack Problem) for the estimation of the optimization of the flow of the information allows to establish the database distribution model applying, using the federated storage engine, which positively affects the availability of information with the inclusion of the registers of the venues and research center, proven in the generation of reports and the continuity and autonomy of the same, as they do not have a centralized access dependent on Internet connectivity.

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Molecular docking study of triterpenoid azadirachtin A on acetylcholinesterase of Drosophila melanogaster (Diptera: Drosophilidae)

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Abstract: Organic molecules of botanical origin can offer a source of compounds of pest management that are more environmentally acceptable and an efficient alternative to replace persistent synthetic insecticides. The molecular docking study using Molegro Virtual Docker software identified that the triterpenoid azadirachtin A showed stable conformations, with lower energy in the ligand-receptor complex of the compounds analyzed in this study, thus having a high affinity for the active site of the enzyme acetylcholinesterase, from a variety of interactions, which can determine its insecticidal potential against the species Drosophila melanogaster.

Keywords: Drosophila, Docking, Triterpenoid

1. Introduction

Pest control has mainly depended on insecticides. Organophosphates, carbamates, pyrethroids and neonicotinoids show the development of insects resistant to various insecticides. To evaluate insecticide toxicities, Drosophila melanogaster is an interesting model (ARAIN et al., 2017).

Organic molecules of botanical origin can offer a source of pest control compounds that are more environmentally acceptable and an efficient alternative to replace persistent synthetic insecticides. The increasing interest in the potential of secondary metabolites in pest control favors the search for new sources of biologically active natural products with low mammalian toxicity, low persistence in the environment, and biodegradability (CESPEDES et al., 2013). Bio-insecticides are safer than synthetic pesticides due to rapid degradation in the environment and low toxicity to vertebrates (DERE et al., 2015).

Therefore, one of the alternatives is the use of botanical insecticides, Azadirachtin A, is a triterpenoid belonging to the class

limonoids, which is present mainly in the seeds of the neem tree (*Azadirachta indica*) (MORGAN 2009) and is one of the most biologically natural insecticides active (BOULAHBEL et al., 2015). Recently this compound was evaluated as a significant biopesticide and used for increasingly in pest control programs (BAJWA and AHMAD, 2012).

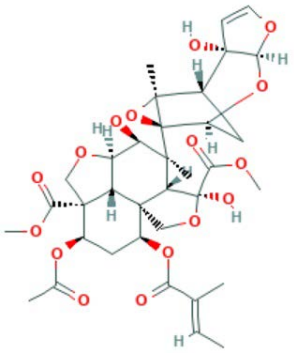
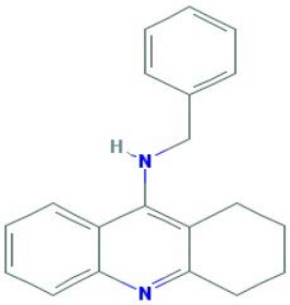
Therefore, the objective of this work was to verify the interactions between triterpenoid molecules azadirachtin A, the active principle of a synthetic insecticide carbofuran and the PDB ligand in the active site of the enzyme acetylcholinesterase of the species Drosophila melanogaster, helping to understand the determining characteristics of the interaction ligand-receptor.

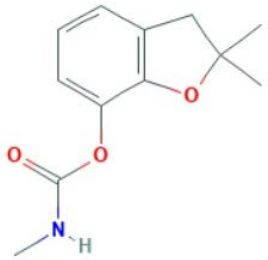
2. Results and Discussion

In the molecular docking study it was possible to verify that the activity of the selected triterpenoid Azadirachtin A, shows a greater affinity with the acetylcholinesterase enzyme than the commercial insecticide carbofuran and 9-n-phenylmethylamino-tacrine (PDB ligand).

In the Table 1, we can observe that the triterpenoid Azadirachtin A presented the lowest binding energy value, in relation to 9-n-phenylmethylamino-tacrine (PDB ligand) and to the active principle, carbofuran. This demonstrates that Azadirachtin A presented more stable conformations, thus, as the greater number of interactions with the amino acid residues in the enzyme acetylcholinesterase. Analyzes of the interactions identified by the amino acid residue of the enzyme with the ligands under study were also performed.

Table 1: Results of the best energy poses of the Docking Molecular study of the ligands tested and the crystallographic ligand in the active site of the enzyme acetylcholinesterase.

Name	Ligand	Moldock Score	HBond
Azadirachtin ($C_{35}H_{44}O_{16}$)		-206.492	-11.3839
9-n-phenylmethylamino-tacrine (PDB ligand)		-129.853	0

Carbofuran (2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate.		-100.335	-5

For the triterpenoid molecule azadirachtin A, the lower energy pose identified that the compound had several interactions with the amino acid residues of the active site of the enzyme acetylcholinesterase. Amino acids Tyr 162, Thr 154, Gly 155, Met 153, Gly 151, Gly 150, Phe 330, Tyr 370, Try 71, Phe 371, His 480, Asp 482 and Trp 83 perform steric interactions with the oxygen atoms of the groups esters and ether in the molecule. Interactions of hydrogen bonds with Trp 83 residues with the ether group were also identified as well as interactions with residues Asp 482, His 480, Leu 479, Tyr 71, Tyr 370, Thr 154 also with the oxygen atoms of the esters . And interactions of Van der waals with amino acid residues Phe 152, Tyr 324, Trp 321, Glu 80, Gly 79, Ans 84, Gly 79, lle 82, Gly 149, Leu 159, Tyr 148 and Glu 485. These interactions may be observed in Figure 1. No electrostatic interactions were identified.

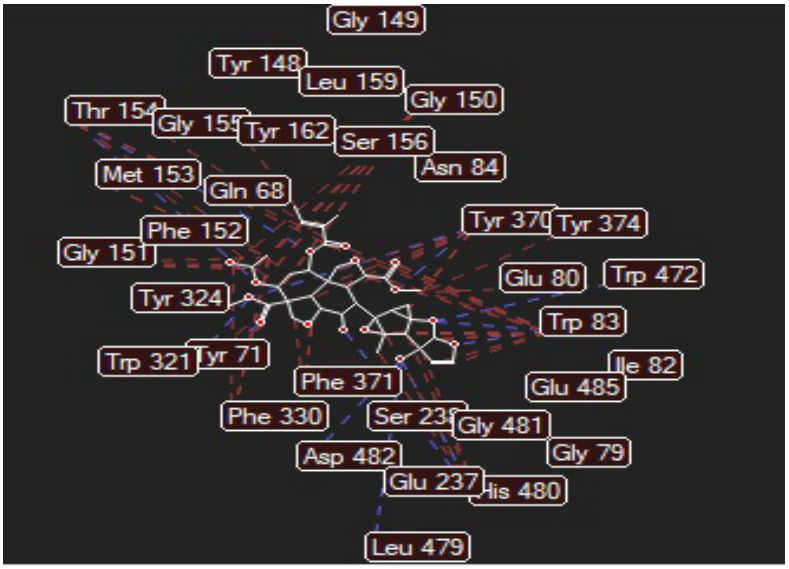


Figure 1: Representation of triterpenoid (azadirachtin A) at the active site of the acetylcholinesterase enzyme in molecular docking. The interactions of the hydrogen bonds are observed in the dotted lines in blue and the red ones represent the steric interactions, as well as interactions of van der waals were identified.

In the molecule of the active principle, carbofuran (**Figure 2**), hydrogen bonding interactions were identified with amino acid residues His 480 with carbonyl and Glu 237 with the nitrogen atom. And the steric interaction between the ether oxygen and the Trp residue 83. As well as Wan der Waals binding interactions were observed with residues Gly 79, Trp 472, Leu 479, Try 370, Gly 481, Tyr 162, Gly 149, Gly 150, Ser 238, lle 484. In this molecule no electrostatic interactions were identified.

In the 9-n-phenylmethylamino-tacrine compound (PDB ligand) (Figure 3) steric interactions with the benzene ring were identified with residues Tyr 71 and Phe 330, as well as interactions of the ether group with Trp 83 and Gly 150 on carbon 3 of cyclohexane. And interactions of Van der waals with amino acid residues Trp 321, Gly 151, Phe

371, Ser 238, His 480, Try 162, Gly 149, Glu 237, lle 484, Gly 79, Gly 481, Leu 479, Trp 472, Glu 80 and Thr 154. In this molecule no electrostatic interactions were identified, nor were hydrogen bonds observed.

In the analysis of the results it was possible to identify that the steric interaction with the amino acid residue Tryptophan 83 was observed in the three compounds. Steric interactions with the amino acid residues Glycine 150, Tyrosine 71, Phenylalanine 330 were observed only in the triterpenoid (azadirachtin A) and in the PDB ligand.

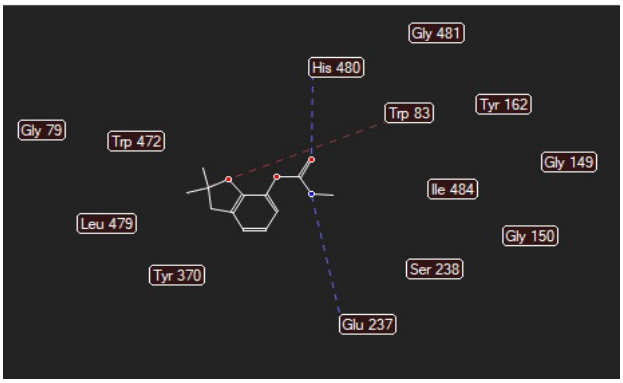


Figure 2: Representation of the active principle carbofuran in the active site of the enzyme acetylcholinesterase in molecular docking. The hydrogen bond interactions are observed on the dotted lines in blue, while the steric interactions are identified on the dotted lines in red.

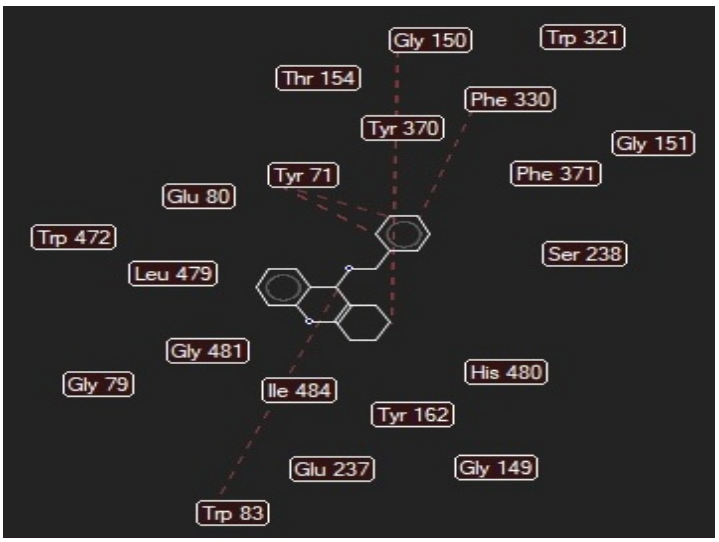


Figure 3: Representation of 9-n-phenylmethylamino-tacrine (PDB ligand) at the active site of the acetylcholinesterase enzyme in molecular docking. The steric interactions are identified on the dotted lines in red.

3. Method

The compounds selected for this work were: a triterpenoid (Azadirachtin A) that was isolated from the species *Azadirachta indica* (Meliaceae) published by (Morgan 2009), an active principle of a synthetic insecticide (carbofuran) and the ligand PDB (9-n -phenylmethylamino-tacrine). These molecules were designed using Hyperchem v. 8.03. Then, they were subjected to geometry optimization, conformational analysis and energy minimization, initially using the MM molecular mechanics method (HOCQUET & LANGGARD, 1998) and then the semi-empirical quantum method AM1 (Austin Model 1) (DEWAR et al., 1985).

Protein Data Bank (PDB)

The acetylcholinesterase enzyme was selected because of its importance in the nervous system of insects, because in order for nerve impulses to be transmitted through synapses, it is necessary for a neurotransmitter, acetylcholine (ACh), to transmit these impulses from one neuron to another, until it reaches the cell to be excited. After this excitation is performed the acetylcholine needs to return to the inside of the neuron where the nerve cell returns to the resting state and can be excited again. This return is accomplished by the enzyme acetylcholinesterase that breaks Acetylcholine into choline + acetate, which within the neuron rejoins acetylcholine for a new transmission.

The active principles of insecticides, such as Carbofuran, which belong to the chemical group of organophosphates and carbamates and act by binding to the enzyme acetylcholinesterase inhibiting its action, resulting in an accumulation of acetylcholine in the synapse causing hyperexcitability due to continuous transmission and uncontrolled nervous impulses including tremors, seizures, collapse of the central nervous system and death (MATIAS, 2016).

According to Cespedes et al. (2013), global agricultural systems consistently use pesticides of synthetic origin, such as carbamates and organophosphates. These active pesticide targets target acetylcholinesterase and have resulted in a generation of new insect strains resistant to the original pesticides. The development of resistance is related to the modification of receptors involved in the mechanisms and targets of action of a given molecule.

Docking

Initially, the crystallized structure of the acetylcholinesterase protein was obtained in the PDB (Protein Data Bank) under the code 1DX4, being this protein of origin of the species Drosophila melanogaster. The resolution of the crystallographic structure deposited in the PDB is 2.7Å. Anchoring of the molecules was performed using a 15Å GRID in the radius and 0.30Å resolution at the enzyme binding site with the structures.

Molecular docking calculations were performed in the Molegro Virtual Docker v.6.0.1 software and the Algorithm Molde Score algorithm. The water molecule and cofactors were removed from the protein to aid in understanding the ligand-receptor interaction. A template was created on the enzyme using the 9-n-phenylmethylamino-tacrine linker, which was obtained along with the pdb file. The Moldock score algorithm was used as a score function to predict the best interaction between ligand and receptor. Next, a docking wizard was created, in which the enzyme molecules and the ligands were inserted, to analyze the stability of the system through the interactions identified with the active site of the enzyme, taking as reference the value of MolDock Score energy.

4. Conclusion

In conclusion, the molecular coupling study using the Molegro Virtual Docker software identified that the triterpenoid azadirachtin A showed more stable conformations, with a lower energy in the ligand-receptor complex of the compounds analyzed in this study, thus having a high affinity for the active site of the enzyme acetylcholinesterase, from a variety of interactions, which may determine its insecticidal potential against the species Drosophila melanogaster. In relation to the active principle of carbofuran, this showed a lower binding affinity with the amino acid residues of the enzyme, although it is widely used as a commercial insecticide (Furadan). Therefore, docking studies have proved to be a useful tool capable of identifying electronic affinity and helping to understand the ligand-receptor interaction.

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A Combined Approach of Ligand-based and Structure-based Virtual Screening to Select Structures with Potential Antichagasic Activity from SISTEMATX Sesquiterpene Lactones Database.

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Abstract. Chagas disease is an endemic disease caused by *Trypanosoma cruzi*, which affects more than eight million people, mostly in the Americas. A search for new treatments is necessary to control and eliminate this disease. Sesquiterpene lactones (SLs) are an interesting group of secondary metabolites characteristic of Asteraceae that have presented a wide range of biological activities. From the ChEMBL database, we selected a diverse set of 4,452, 1,635 and 1,322 structures with tested activity against the three T. cruzi parasitic forms, amastigote, trypomastigotes and epimastigote, respectively, to create random forest (RF) models with an accuracy of greater than 74 % for cross-validation and test sets. Afterwards, a ligand-based virtual screen of the entire SLs of Asteraceae database stored in SistematX (1,306 structures) was performed. In addition, a structure-based virtual screen was also performed for the same set of SLs using molecular docking for *T. cruzi* cruzain. Finally, using an approach combining ligand-based and structure-based virtual screening along with the equations proposed in this study to normalize the probability scores, we verified potentially active compounds and established a possible mechanism of action.

Keywords: Asteraceae; Chagas' disease; Ligand-based virtual screening; Structure-based virtual screening; Sesquiterpene lactones; Machine learning.

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1. Introduction

Chagas' disease is an endemic disease caused by Trypanosoma cruzi, which affects more than seven million people, mostly in the Americas [1]. The search for new treatments is necessary for the control and elimination of this disease. Natural products have been an invaluable source of inspiration for the development of therapeutic agents [2,3]. Sesquiterpene lactones (SLs) are one of those interesting small molecules for the search of new chemotherapies against infectious diseases [4,5].

Using a combined approach of ligand-based and structure-based virtual screening (VS) with the entire SLs databank stored in SistematX (http://sistematx.ufpb.br), we verified potentially active compounds against Trypanosoma cruzi and established a possible mechanism of action.

2. Results and Discussion

Ligand-based VS

The training set hit-rate values for the three RF models are quite close to or exactly 100%; nevertheless, for cross-validation and test sets values range from 64.6% to 91.1%, with epimastigote and trypomastigote models serving as better predictors of inactive molecules than the amastigote model. The specificity of the epimastigote model is better than the other two models, as the percentage of true negative compounds predicted in the test set (91.1%) was higher than the cross-validation set (85.6%). The amastigote model is the most sensitive of the three, presenting a true positive prediction rate of 76.7% and 79.1% for the cross-validation and test sets, respectively. In turn, the models for the two other parasitic forms were approximately 10% less sensitive to the values reached in the amastigote model.

Using this machine learning algorithm, a virtual screen was performed on a set comprising 1,306 molecules obtained from SistematX. For amastigotes, 34 SLs were predicted to be antichagasic compounds, with probability values ranging from 0.50 to 0.58. Some common structural features are observed among the structures with higher probability values, SLs **1–2** (Figure 1). are acetylated molecules germacranolides contained an epoxide moiety in their structures.

Otherwise, 17 SLs were predicted to be anti-T. cruzi compounds for the trypomastigote parasitic form, with probability values ranging from 0.50 to 0.64. Desacetyl-isotenulin (**3**, Figure 1) was the structure with the highest probability value. The structures of the active molecules are similar (guaianolides). Finally, the epimastigote model was less selective than the other two models, as 420 active molecules were predicted, with probability values ranging from 0.50 to 0.82. As in the amastigote model, structural similarity was observed between SLs with higher probability values (**5-6**).

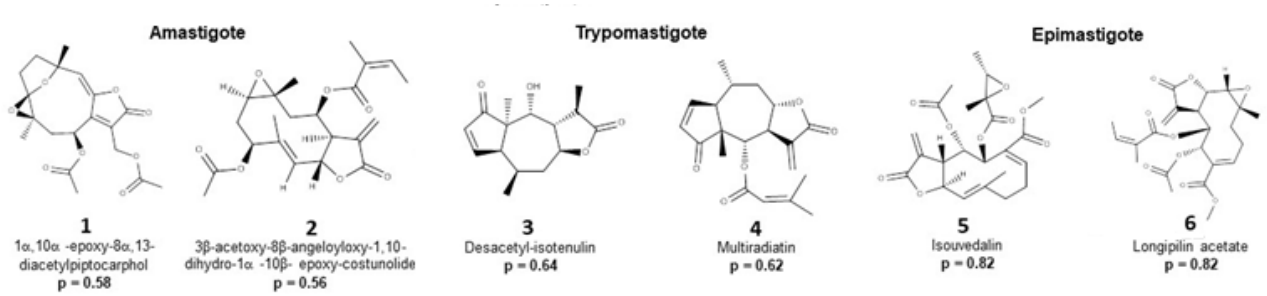

Figure 1. Potentially active sesquiterpene lactones identified using a ligand-based virtual screening; *p*= active probability value.

Structure-based VS

Initially, molecular docking was validated by redocking of the original ligand for *T. cruzi* cruzain. This score is listed in Table 1 with their respective RMSD value.

Table 1. The docking energy (kJ/mol) of two of the best-ranked SLs from the structure-based approach for cruzain. Ligand = energy (kJ/mol) for the PDB ligand and the RMSD values obtained from the redocking procedure.

T. cruzi protein	SL (KJ/mol)	Ligand (KJ/mol)	Redocking RMSD
Cruzain	**7** (-91.4) **8** (-84.2)	(-80.0)	0.79

After, a virtual screen of 1,306 SLs was performed. Based on the binding energy values, all tested molecules were ranked using the following probability calculation (p_s, Equation 1):

Equation 1:

$$p_s = \frac{E_i}{E_{min}} \quad \text{if} \quad E_i < E_{ligand}$$

where p_s = structure-based probability; E_i = docking energy of compound *i*, and *i* ranges from 1 to 1306 (SLs dataset); E_{min} = the lowest energy value of the dataset; E_{ligand} = the ligand energy from protein crystallography.

For 753 SLs, values greater than 0.5 and binding energy values less than the ligand were observed. The structures **7** and **8** (Figure 2A), two guaianolide SLs extracted from *Lactuca georgica*, presented the highest active probability values in structure-based VS. Figure 2B shows the conformations of both SLs in the active site of Cruzain, as well as the hydrogen-bonding (H-bond) interactions of compound **7** (Figure 5B) with residues Cys 25, Trp 26 and Trp 184. Molecule **8** also participated in H-bond interactions with Cys 25 and Trp 184. In both SLs, an H-bond was observed between the carbonyl moiety of carbon-2 with Trp 184.

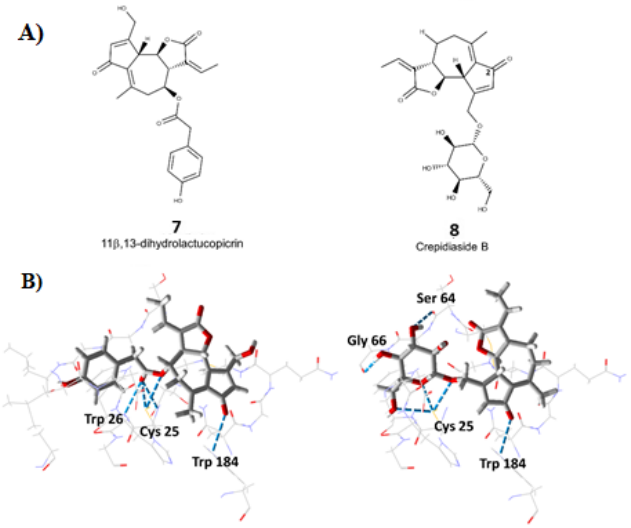

Figure 2. A) Structure of crepidiaside B (**7**) and 11β, 13-dihydrolactucopicrin (**8**). B) Docking conformations SL **7** and **8** in the pocket of *T. cruzi* Cruzain (PDB ID: 4XUI). The blue dotted line represents H-bond interactions between SLs **7** and **8** with Cruzain residues (black labels).

Ligand - based and Structure based VS combined approach.

Using the equation 3, an approach combining structure-based and ligand-based virtual screening was performed to verify potentially active molecules as well as their possible mechanism of action, facilitating the identification of potential multitarget compounds.

Equation 3:

$$p_c = \frac{p_s + (1 + TN)\ x\ p}{2 + TN}$$

where pc = combined probability p_s = structure based probability; TN = true negative rate; p = ligand-based probability

Table 2 summarizes the results for the best-ranked SLs obtained using the combined approach. Some structures that previously displayed a high active probability value in the ligand-based virtual screen appear to be interesting potential structures for each *T. cruzi* parasitic form.

Table 2. The best-ranked structures for each parasitic form obtained using an approach combining ligand-based and structure-based virtual screening.; p = active probability value in ligand-based VS; p_s = active probability value in structure-based VS. p_c = combined probability value

Cruzain				
Parasitic form	**Structure**	***p***	***p*$_s$**	***p*$_c$**
Amastigote	**1**	0.58	0.83	0.67
Trypomastigote	**4**	0.62	0.64	0.63
Epimastigote	**9**	0.73	0.91	0.79

Structure **1** and **4,** have the highest p_c values for amastigote and trypomastigote parasitic form, these two compounds also presented high probability scores in Ligand-based VS. Structure **9** (Figure 3), emerges as an interesting structure that acts in cruzain of epimastigotes, since that have good results in the two VS methodologies as well as in the combined-approach.

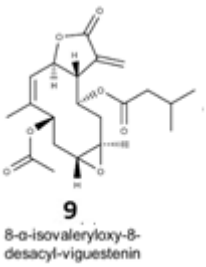

Figure 3. Structure of 8-α-isovaleryloxy-8-desacyl-viguestenin

3. Materials and Methods

From the ChemBL database were obtained 4,452, 1,635 and 1,322 structures with activity against the three parasitic forms of *T. cruzi*, amastigotes, trypomastigotes and epimastigotes, respectively (https://www.ebi.ac.uk/chembl/). The compounds were classified using values of pIC_{50} (-log IC_{50}), which led us to divide them into active ($pIC_{50} \geq 5$) and inactive ($pIC_{50} < 5$) structures.

For all structures including 1,306 SLs obtained from SistematX database, SMILES codes were used as input data in Marvin; ChemAxon (version 16.11.28 (2016), a calculation module developed by ChemAxon, http://www.chemaxon.com/). We used Standardizer software (Jchem, version 16.11.28 (2016), a calculation module developed by ChemAxon, http://www.chemaxon.com/); ChemAxon to canonize structures, add hydrogens, perform aromatic form conversions, and clean the molecular graph in three dimensions. After were calculated 128 3D-molecular descriptors in Volsurf+ software. Obtained results were imported to Knime 3.1.0 software (www.knime.org). All variables were submitted to autoscaling and after were partitioned to generate two groups, a training group composed by the 80% of the whole molecules set and a test group composed by the remaining 20%. Using a Random Forest algorithm, three models were performed. Models were evaluated through cross validation and a test set (20%). After a Ligand-based VS of the 1,306 SLs were performed in these models (Figure 4).

The structure of *T. cruzi* protein, Cruzain (PDB ID: 4XUI) in complex with the respective inhibitor (PDB ID: 2VC), were downloaded from the Protein Data Bank—PDB. The docking procedure was performed using MOLEGRO virtual docker 6.0, using a GRID with a radius of 15 Å and a resolution of 0.30 Å to cover the ligand-binding site in the structure of cruzain (Figure 4).

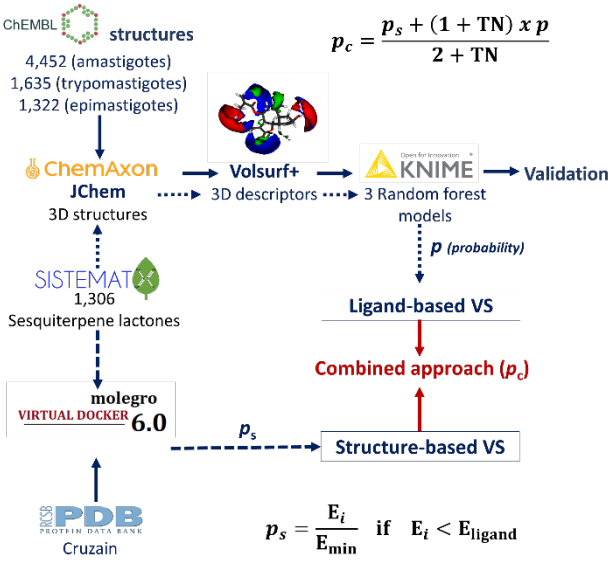

Figure 4. General scheme of the methodologies used to select potentially antichgasic compound through of a combined approach.

4. Conclusions.

In the present study, potential antichagasic SLs for the three parasitic forms and some structural features were determined from RF models of *T. cruzi*. In addition, a structure-based virtual screen using PDB structure of *T. cruzi* cruzain for the entire SL set allowed the selection of potential inhibitors of this enzyme. Finally, using a combined approach of structure-based and ligand-based VS enabled the identification of promising multitarget antichagasic SLs.

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Author Contributions
LS built database; CHA performed all calculus; and CHA and MTS wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest
The authors declare no conflict of interest.

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Application of Self-Organizing Maps generated from Molecular Descriptors of Flavonoid in the Chemotaxonomy of the Asteraceae Family

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Abstract: The Asteraceae family belongs to the Asterales order, it consists of approximately 1,600 genera and 24,000 species, divided into 12 subfamilies and 44 tribes, is one of the largest families of angiosperms in the world. Asteraceae is remarkable the presence of flavonoids, these have the necessary requirements to be used successfully in chemotaxonomy because are found in abundance in the Asteraceae, presents structural diversity, are stable structures and relatively easy to identify, therefore can be used as taxonomic markers. The aim of this study is to classify Asteraceae tribes based on the number of occurrences of flavonoids from our in-house databank (available at www.sistematx.ufpb.br) using descriptors calculated by DRAGON 7.0 software. The 2371 botanical occurrences with respective 74 molecular fragment descriptors were used as input data in SOM Toolbox 2.0 (Matlab) to generate Self-Organizing Maps (SOMs), classifying four tribes: tribes Anthemideae (A), Gnaphalieae (G), Tageteae (T) and Senecioneae (S). Some descriptors show higher contribution to differentiate the flavonoids: RFD, nCIC and NNRS. Since these SOM are built based on physicochemical properties, so it is possible to use this tool in the search for flavonoids with potential biological activities with the respective taxonomic information.

Keywords: Asteraceae, flavonoids, chemotaxonomy, databank, descriptors, Self-Organizing Maps

1. Introduction

The Asteraceae family (Compositeae) is one of the largest families of angiosperms in the world [1]. Some 1,600 genera and 24,000 species of this family have been described botanically and several revisions regarding its chemistry and biology were published [2]. The latest classification recognizes 12 subfamilies and 44 tribes are usually represented by herbaceous plants and small shrubs, rarely by trees [2,3].

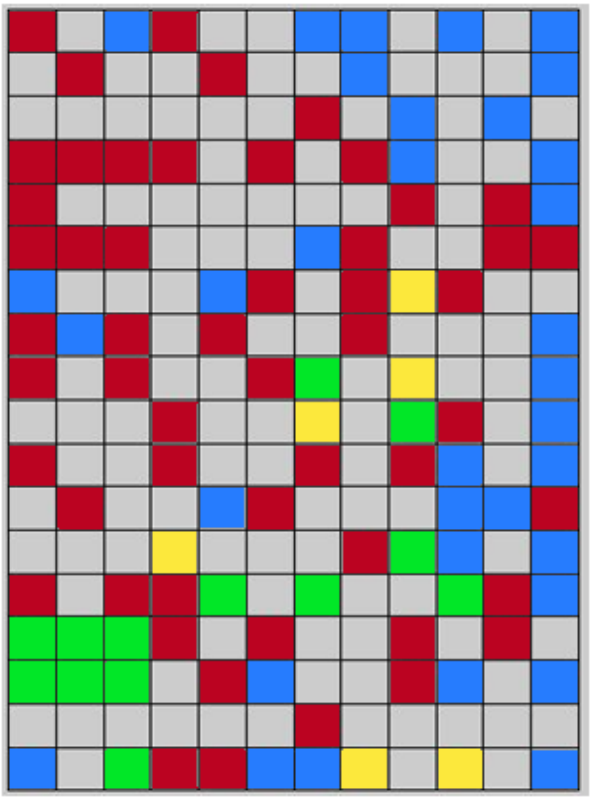
In Chemistry of Natural Products, secondary metabolites are important chemical markers, have a restricted distribution and specific botanical sources [4,5]. Among them stand out the flavonoids, contain a basic structure consists of 15 carbon atoms arranged into three rings (C6-C3-C6), have the necessary requirements to be used successfully in chemotaxonomy because this class are stable structures and relatively easy to identify, presents structural diversity, are found in abundance in the Asteraceae family [6], they can be used as taxonomic markers at lower hierarchical levels [7].

In this study, the Asteraceae tribes were classified based on the number of occurrences of flavonoids from our in-house databank using descriptors calculated by DRAGON 7.0 software [8]. With the Matlab software [9], chemical patterns were recognized and analyzed from unsupervised artificial neural networks, along with the SOM (Self Organizing Map) to create the maps.

2. Results and Discussion

From the botanical occurrence data collected, were generated 74 molecular descriptors for each molecule, through software DRAGON, then one can calculate the self-organizing matrix for each molecule, dividing the data into groups according to similarity. **Figure 1** shows the Self-Organizing Maps obtained with the flavonoids descriptors of the tribes Anthemideae, Gnaphalieae, Tageteae and Senecioneae. Flavonoid hit rates belonging to the A, G, S and T tribes were 91%, 80%, 79% and 73%, respectively.

Figure 2 shows The SOM cluster similar compounds regarding molecular fragments that were labeled according to the botanical occurrence in these four tribes.



Fiure 1. Self-Organizing maps obtained with the flavonoids of the tribes Anthemideae (red), Gnaphalieae (blue), Tageteae (green) and Senecioneae (yelow).

In the matrix we could observe the separation between the tribes Anthemideae, Gnaphalieae, Tageteae and Senecioneae. The tribe Anthemideae is represented by the lighter areas of the map, that is, show low values for the descriptors in general. The tribe Gnaphalieae is represented by darker areas of the map presenting high values generated descriptors. The tribe Tageteae is represented by darker area the left and the tribe Senecioneae, to present few molecules, it is more difficult to be seen in the matrix. The number of rings (or independent cycles) in a graph is commonly known as the cyclomatic number. The tribes Gnaphalieae present lower values for the descriptor **nCIC** (number of rings in a molecule), while the tribes Anthemideae, Tageteae and Senecioneae presents high values. The tribe Gnaphalieae present high values for the descriptor **NNRS** (normalized number of ring systems), while the tribes Anthemideae, Tageteae and Senecioneae present lower values. The tribe Gnaphalieae present lower values for the descriptor **RFD** (ring fusion density), while the tribes Tageteae and

Senecioneae, mainly Anthemideae, present high values.

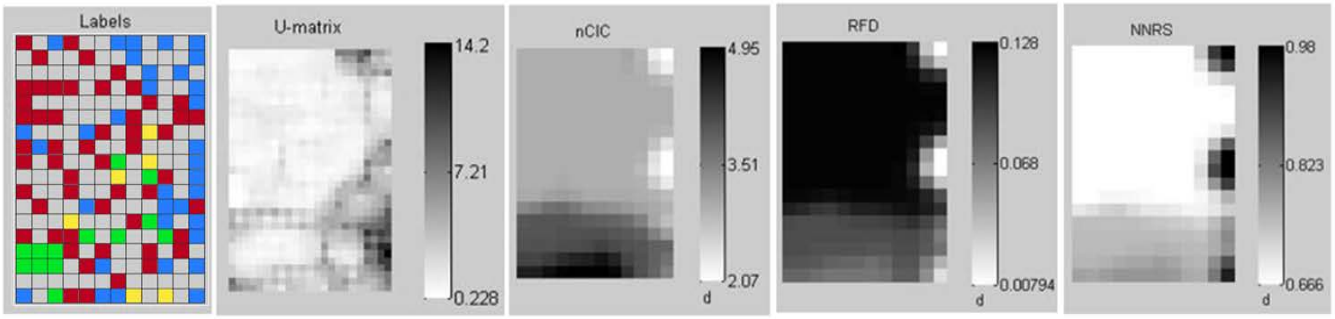


Figure 2. Map, matrix and descriptors generated: nCIC, NNRS and RFD.

3. Materials and Methods

2371 occurrences of flavonoids, which were extracted from 567 species, 47 genera and 5 tribes of the Asteraceae family, were registered in two dimensions using ChemAxon, then were used as input data in the Dragon software to generate 74 molecular descriptors. Then the descriptors were used as input data in Matlab, using the SOM Toolbox 2.0 for formation of Self Organizing Maps (SOMs), separating the tribes Anthemideae (A), Gnaphalieae (G), Tageteae (T), Senecioneae (S) and Carduoideae (CR).

4. Conclusions

The Self-Organising Map obtained separated the four tribes of the Asteraceae when using molecular descriptors. . Since these SOM are built based on physicochemical properties, so it is possible to use this tool in the search for flavonoids with potential biological activities with the respective taxonomic information.

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NMR analysis of a new canthin-6-one alkaloid from *Simaba bahiensis* (Simaroubacae)

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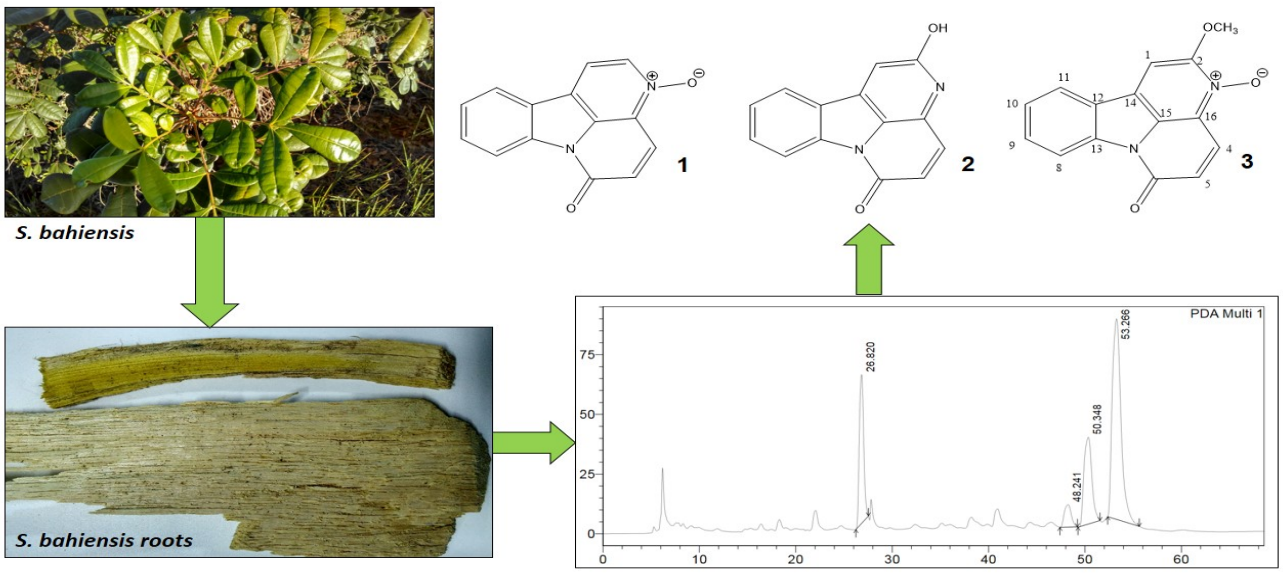
Graphical Abstract



Abstract.

Alkaloids of the canthi-6-one type have been reported in differents natural sources. These alkaloids have showed a wide range of pharmacological properties including cytotoxic, antibacterial, antifungal, antiparasict, antiviral, anti-inflammatory and beside it, some of them show excellent photophysical properties that gives an interesting use as fluorescent dye probe in florescent cellular microscopy. Because it, new studies have been carrying out in an attempt to identify new alkaloids with pharmacological properties. Some researchers have been trying to synthesize new derivatives or identify new compounds in natural sources. Among the >60 canthin-6-one alkaloids already reported in natural sources, more the half are present in plants of the family Simaroubaceae. In the *Simaba* genus, up to the present, only eighteen alkaloids was described in seven species, but other plants of the genus that occur in the brazilian's flora haven't been studied yet. In this work we describe the first phytochemical study of the specie *Simaba bahiensis* (Simaroubaceae), collected at the city of Camaçari, Bahia State, Brazil. Also, it's related complete structural determination using differents NMR experiments and HRMS of a new canthin-6-one alkaloid in addition to two others already known

Introduction

Canthi-6-one alkaloids occur in plants from families Rutaceae, Simaroubareace, Amaranthaceae, Caryophyllaceae and Zygophylaceae [1-3]. More than 60 members of this type of alkaloid were isolated from natural sources since first report in 1952 [4]. These alkaloids have shown a wide range of pharmacological properties including cytotoxic, antibacterial, antifungal, antiparasict, antiviral, anti-inflammatory and beside it, some of them show interesting photophysical properties that gives an interesting use the fluorescent dye probe in florescent cellular microscopy. New studies have been carring out in an attempt to identify new alkaloids with pharmacological properties by synthesis or isolation from different natural sources [2]. In family Simaroubaceae more than 30 canthi-6-one alkaloids have already been described with different substitution patterns and it shows that the investigation of new alkaloids in this family it's an important way to search new chemical entities [2, 3]. In this work we describe the first phytochemical study of *Simaba bahiensis* (Simaroubaceae), collected at the city of Camaçari, Bahia State, Brazil. Also, it's related complete structural determination using differents NMR experiments and HRMS of a new canthin-6-one alkaloid and two others already known.

Materials and Methods

Roots of *S. bahiensis* was collected in Arembepe, Bahia State, Brazil (S lat: -12.800833 long: -38.214444 WGS84 alt. 10m). Professor MSc Maria Lenise Silva Guedes performed the botanical identification and a voucher was deposited at Herbário Alexandre Leal Costa (ALCB) in Biology Institute of Federal University of Bahia with identification 1235018a.

The yellower parts of *S. bahiensis* roots (17,57g), called roots deposits (RD) was manually separated and extracted with MeOH (750 mL) at ultrasound for 10 minutes. The methanol RD extract (0,77g) was produced by evaporation of alcoholic solution. The separation of RD extract was performed by preparative HPLC using: C_{18} column 250 mm; 21.0 mm; 5 μm; 100 °A, gradient elution with solvents A (H_2O) and B (methanol), 0–35min 80 to 50% A, 35-70 min 50%A, flow 8,0 mL.min^{-1}, diode array detector (DAD), injection volume was 100 μL, the total injection volume was 1,5 mL. Three fractions were collected, evaporated and then analyzed by NMR and HRMS.

Results and Discussion

The figure 1 shows the chromatogram of HPLC separation and the collected peaks. NMR ^{1}H spectrum, of collected peak $R_{t(min)}$ = 53.266, shows signals related to aromatic systems and one singlet that corresponds to methoxyl group. The ^{1}H pattern observed for this substance indicates the existence of a canthi-6-one alkaloid substituted by a methoxyl. Four hydrogens (δ_H 8.62, 7.97, 7.69 and 7.50) of ring A of the canthi-6-ones were assigned considering multiplicity and chemical shifts. Hydrogens at positions 4 and 5 (δH = 7.74 and 6.93, J = 9.8 Hz) are present, the latter signal being a singlet for a hydrogen at ring C, which could be at position 1 or 2, another position would be the methoxyl group.

The irradiation of hydrogen δ_H 7.30, by NOEDIFF experiment, resulted in an increase of the doublet signal δ_H 7.97. The signals at δ_H 7.50 and δ_H 7,30 were increased when δ_H 7.97 was irradiated, indicating the proximity between the H-11 position in ring A and the hydrogen δ_H 7,30 present in the C-ring. NOEDIFF experiments suggest that the bound of the methoxyl group in this substance occurs in position 2. Irradiation of the hydrogens of the methoxyl group in δ_H 4.20 increased the signal of the H-4 δ_H 7.75. All NOE experiments was compared with previous reported data [5].

These NMR data led to 2-methoxy-canthi-6-one alkaloid, but HRMS shows ion $[M + H]^+$ = 267.0733 m/z with molecular formula $C_{15}H_{10}N_2O_3$, and diverged 16 m/z from the proposed alkaloid 2-

methoxy-canthi-6-one. According to the NMR and MS data, the only possibility that justify this mass difference is the presence of an N-O^- group at the structure.

The IR analysis confirms the N-O^- bond in the intense absorption at 1303 cm^{-1}. It is a first report of spectroscopic data that shows the presence of alkaloid 2-methoxy-canthi-6-one N-oxide (**3**). NOESY, HMBC and HMQC were performed and the results are described at table 1 and figures 2 and 3. Peaks that corresponds to compounds **1** and **2** were analyzed by NMR and HRMS all data were compared to previous described data [6, 7].

Other canthi-6-on alkaloids have been reported in the literature, among the structures most have substitutions at ring A, structural varieties with substitutions at the position 2 on ring C are uncommon. Although the substitution pattern is easily identified by ^{1}H NMR and NOE experiments can allow unambiguous determination of the substitution pattern in ring A and C [5], the complementary spectroscopic techniques as IR and HRMS allow the determination of the presence of N-oxide groups.

Conclusions

The first phytochemical study of *S. bahiensis* were possible identify 3 canthi-6-one alkaloids and the new one 2-methoxy-canthi-6-one N-oxide, previous studies shows the occurrence of alkaloids with N-oxide but without substitution at position 2.

Substitution pattern is easy to identify by ^{1}H NMR, but is necessary use different experiments to correctly determine the position of substituents at rings A and C, since studies of structure relationships have determined that different substitution patterns in these rings influence the pharmacological activity of these substances.

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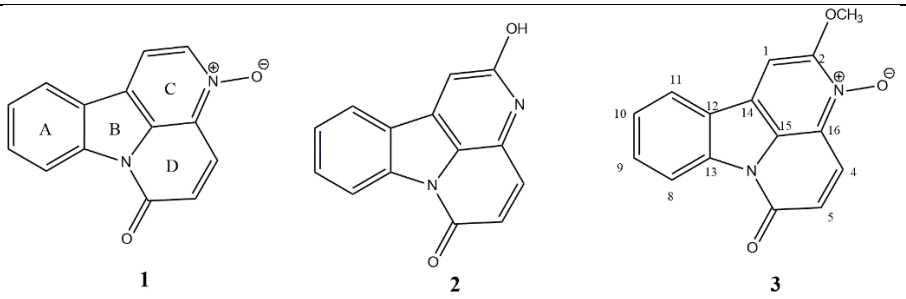

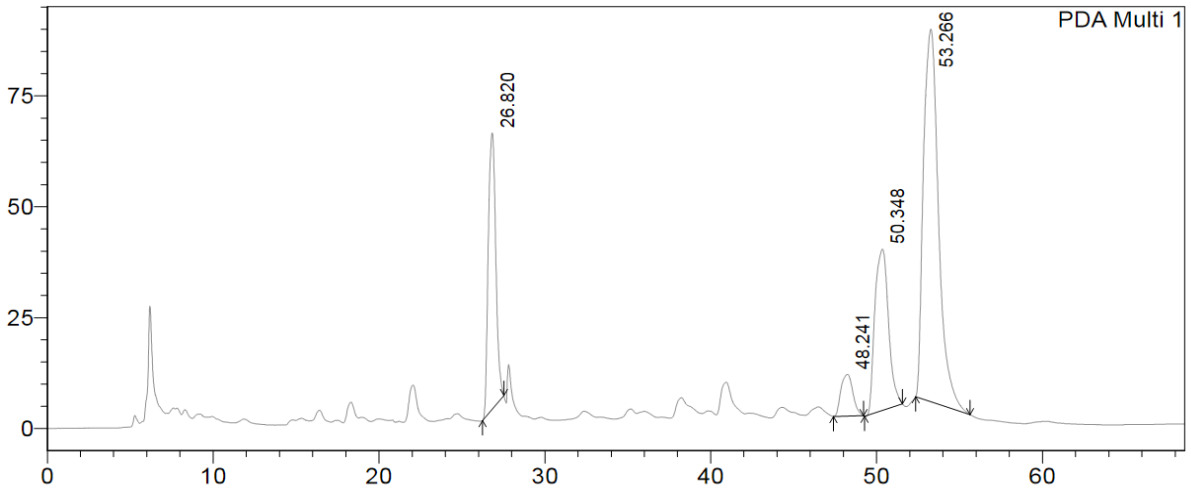

Figure 1: Chromatogram (254 nm) of RD methanol extract obtained by preparative scale. Compound **1** ($\mathbf{R_{t(min)}}$ = 48.241) compound **2** ($\mathbf{R_{t(min)}}$ = 50.348) and compound **3** ($\mathbf{R_{t(min)}}$ = 53.266).

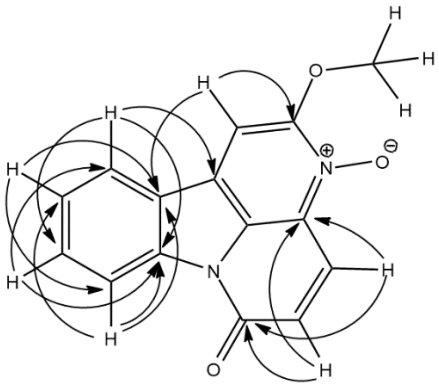

Figure 2: HMBC (400MHz) observed correlations (arrows) of compound **3**.

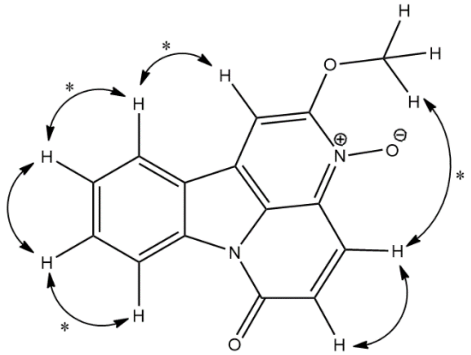

Figure 3: NOESY (400MHz) and (*) NOEDIFF (500MHz) observed correlations (arrows) of compound **3**.

Table 1: NMR data 2-methoxy-N-oxide-canthi-6-one.

	^{1}H δ(ppm), J(Hz)	^{13}C δ(ppm)
1	7,29 (1H, *s*)	114,13 [a]
2	----	157,64 *[b]
4	7,74 (1H, *d*, *J* = 9,8)	125,48 [a]
5	6,93 (1H, *d*, *J* = 9,8)	127,85 [a]
6	---	157,78 *
8	8,62 (1H, *dl*, *J* = 8,0)	117,39 [a]
9	7,69 (1H, *ddd*, *J* = 8,8; 8,0 ;1,2)	132,23 [a]
10	7,50 (1H, *ddd*, *J* = 8,8;7,6 ;1,2)	126,41 [a]
11	7,97 (1H, *dl*, *J* = 7,6)	123,92 [a]
12	---	123,33 [b]
13	---	142,15 [b]
14	---	135,73 [b]
15	---	---
16	---	122,84 [b]
-OCH_3	4,20 (3H, *s*)	64,60 [a]

a = values supported by HMQC b = values supported by HMBC ; * = values may be changed

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Identification of diterpenos flexibiliene from *Stillingia loranthaceae*, using LC-MS^2 and Molecular Networking

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Abstract: The genus Stillingia (Euphorbiaceae) is represented by 30 species distributed in the America and islands of the Pacific. In Brazil, seven species are distributed between Caatinga and Atlantic Forest, four of which are predominantly Caatinga. Only four species of Stillingia were studied chemically. Diterpenes with rare flexibilane skeletons have been reported from the roots of S. sanguinolenta. These compounds demonstrated interesting pharmacological activities. The use of hyphenated techniques, such as LC-MS2, coupled with bioinformatics techniques such as Molecular Networking, are able to rapidly identify substances from complex biological extracts. Thus, the objective of the study was the identification of flexibilene diterpenes, using LC-MS2 and Molecular Networking, of root bark of S. loranthaceae. The botanical identification was carried out in the Herbarium Alexandre Leal Costa at the Biology Institute of UFBA. The hexane extract (HE) from the root bark was analyzed by LC-MS2, and the data were used to generate a molecular network in GNPS site. It was possible to observe a cluster represent this diterpene skeleton in the molecular network. This data associated to MS/MS fragmentation approach suggested the presence of several new flexibilene diterpenes and known compounds (tonantzitlolone A-C) already identified from other Stillingia species.

Keywords: Flexibilene diterpene, Molecular Networking, LC-MS/MS and *Stillingia loranthaceae*.

1. Introduction

Recently was identified of *S. sanguinolenta* the substances tonantzitlolones A and B have considered rare diterpenes with cyclic flexibilene backbone[1]. These compounds showed interesting cytotoxic activities against human cancer kidney and breast cell lines[1]. The unusual backbone and interesting pharmacological activities have been raising the number of studies of this genus[2]. Recents studies show synthesis of these diterpenes and pharmacological activities of antiviral[3], cytotoxic[4] activities and inhibition of the enzyme kinesin 5[5-6].

Studies using hyphenated techniques, such as LC-MS / MS, allow analysis of the chemical profile of complex matrices without procedures to isolate their substances[7]. Another advantage of these techniques is the high sensitivity that allows the analysis of substances with low concentrations in complex matrices such as natural products and it may be improve the identification processes of substances that were difficult to identify and isolate[8].

The development of new analytical techniques with new bioinformatics approaches, such as Molecular Networking, that have emerged as a tool capable of visualizing the chemical spaces present in the samples analyzed by MS / MS[9] experiments. The use of this tool, makes the analysis of several spectra generated in an LC-MS / MS analysis quicker and easier to visualize[10].

This work suggested the presence of several new flexibilene diterpenes and known compounds (tonantzitlolone A-C) already identified from other Stillingia species.

2. Results and Discussion

The hexane extract was subjected to chromatographic analysis using C18 column and ACN: H2O (0.1% formic acid) as eluents. The generated data were analyzed using the GNPS site platform to obtain Molecular Networking.

Based on the cosine similarity, the MS2 spectra of the substances were grouped into clusters. The generated molecular networking had 318 nodes (148 after blank removal). The intensities of the lines between the nodes were related to the cosine values, indicating how much greater the thickness the greater the degree of similarity between the nodes.

It was observed the presence of a cluster in the Molecular Networking of the extract of the root bark referring to the flexibilenos diterpenes. Data from the literature show characteristic losses of the diterpene skeleton generating a common ion to skeletons with m / z 333 (Fig. 1)[3]. This ion can be observed in the spectra of MS2 of the nodes present in this cluster indicating if they are compounds with the skeletal flexibilene (Fig 2). Three nodes had precursor ions m/z 447 [M- H_2O + H], m/z 505 [M- H_2O + H] and m/z 463 [M- H_2O + H] tonantzitlolone substances A, B and C, respectively. After injection of the extract into the LC-HRMS it was possible to verify the molecular

formulas of the ions and to suggest that they be these substances (Fig. 3). In addition to these substances, it is possible to observe the presence of other substances with *m/z* not yet reported in the literature, these being novel compounds.

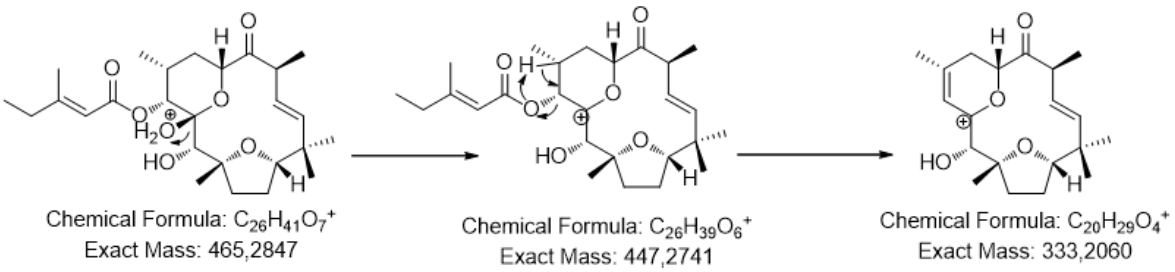

Figure 1. Proposed fragmentation mechanism for tonantzitlolones A.

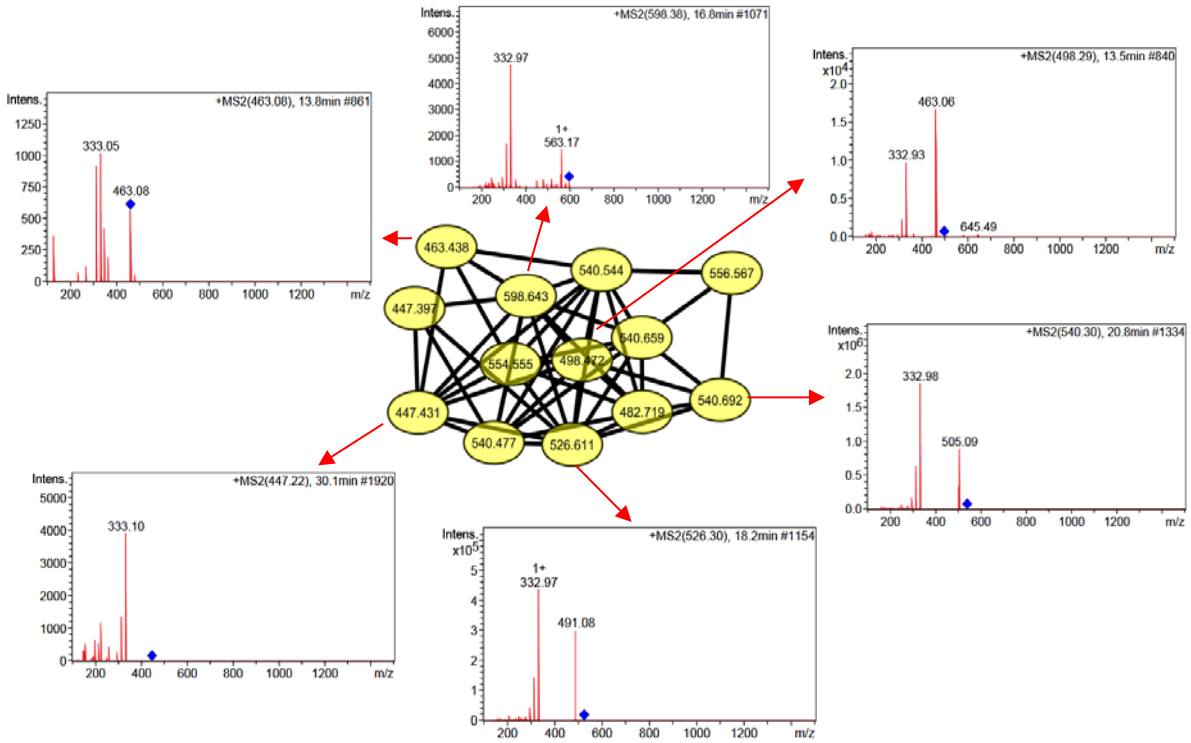

Figure 2. Cluster of flexibilenes diterpenes and MS/MS spectra of some nodes.

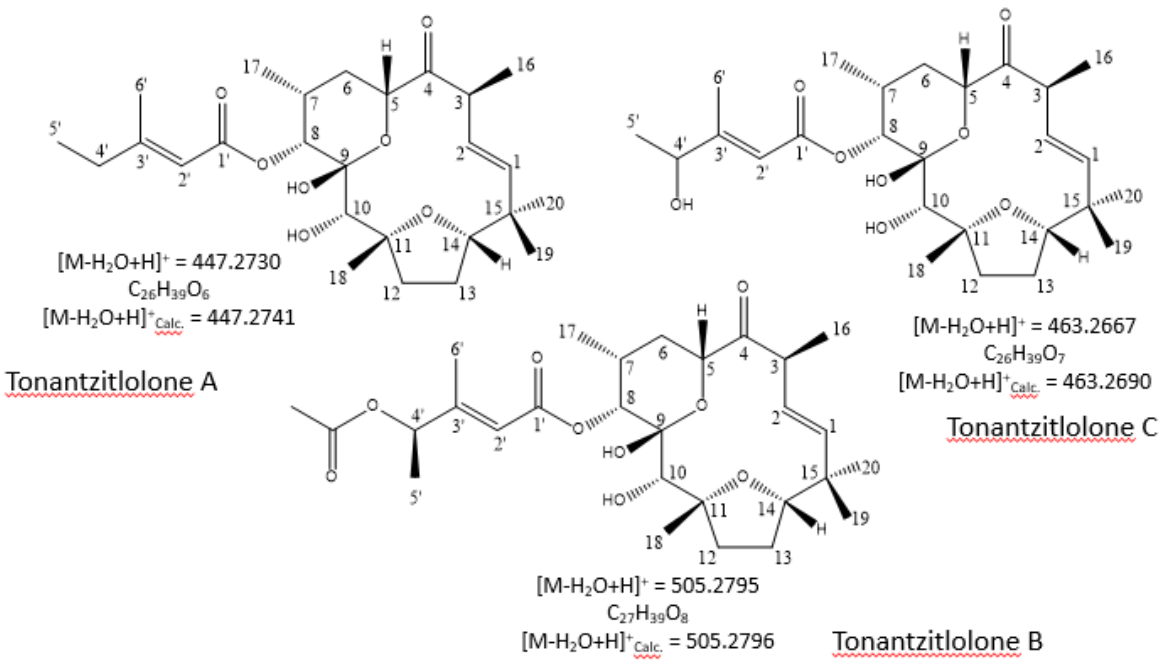

Figure 3. Diterpenes identified in *Stillingia loranthaceae*.

3. Materials and Methods

Material Vegetal

The specimens were collected in Morro do Chapéu, Bahia, Brazil in march 2016. The vouchers specimens were identified by Prof. M. L. S. G., and deposited with the Herbarium Alexandre Leal Costa(ALCB), Institute of Biology, Federal University of Bahia with the registration number 123491.

Analysis of the hexane extract from the root bark by HPLC-IT-MS / MS

1.0 mg of the extract was dissolved in 1.0 mL of ACN and filtered using 0.45 µm PVDF filter. HPLC-IT-MS / MS analysis was performed using a UFLC (Shimadzu) containing two solvent pumps LC-20AD, auto sampler SIL-20AHT, system controller CBM-20A coupled with an Ion-Trap mass spectrometer (AmaZon X).

HPLC experiments were performed using a C18 (Kromasil - 250 mm x 4.6 mm x 5 µm) column with the gradient elution: solvent A = H_2O and formic acid (0.1% v/v); Solvent B = ACN; Elution profile = 0.0 - 70.0 min (85% B); 70.0-80.0 min (85-100% B); 80.0 - 100.0 min (100-100% B); 100.0 - 110.0 min (100-85% B); 110.0-130.0 min (85-85% B), injection volume of 20 µL and flow of 0.6 mL/min. The parameters of the Ion-Trap analysis were: capillary 4.5 kV, ESI in positive mode, end plate offset 500 V, nebulizer 10 psi, dry gas (N2) with flow rate of 6 mL/min and temperature of 250 ºC. CID fragmentation was performed in auto MS/MS mode using advanced resolution mode for MS and MS/MS mode. The spectra (m/z 50-1000) were recorded every 2 sec.

Molecular Networking

The data obtained by HPLC-IT-MS / MS were subjected to a conversion to the mzXML

format using the MSconvert program. This file was submitted to the GNPS platform - GNP (http://gnps.ucsd.edu), where it was submitted to an analysis and generated a Molecular Networking. The Cytoscape 2.8.3 program was used to visualize the generated data[11].

The algorithm used in GNPS compared all possible pairs of MS / MS spectra vectors, considering mass tolerance for fragment peaks (0.5 Da), parental mass tolerance (1.0 Da), minimum number of peaks corresponding to spectral alignment (6) with a minimum cosine score of 0.6. The higher the cosine score between two spectra, the more similar the MS / MS spectra are, and the more similar the molecules[9].

After organizing the spectra based on the similarity of fragmentations, the data were analyzed in Cytoscape. In order to avoid erroneous interpretations of contaminants or analysis noise, the blank injections (mobile phase) were introduced into the spectra network as a group of distinct samples and removed from the network.

4. Conclusions

From the analysis of the data of the hexanic extract of S. loranthaceae root bark, by LC-MS2, Molecular Networking and MS/MS fragmentation approach suggested the presence of several new flexibilene diterpenes and known compounds (tonantzitlolone A-C) already identified from other Stillingia species. Therefore, this extract is promising for the isolation and identification of new diterpenes flexibilenes.

Acknowledgments

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Protein model built through molecular modeling by homology of a potential target of anti-leishmania drugs

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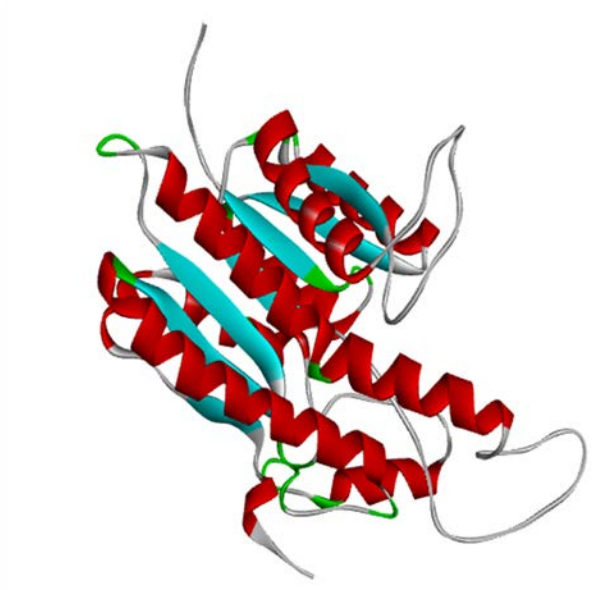
Graphical Abstract



Abstract

Molecular modeling by homology is a methodology widely used for the construction of protein structures that have not yet been crystallized. The constructed models can be used for the identification of inhibitors, representing a great method for the rational planning of drugs. Thus, the objective of the study was to construct the three-dimensional model of the protein structure of the enzyme Pteridine reductase 1 from *Leishmania donovani* (LdPTR1). PTR1 is an enzyme used in the metabolism of pterin from GTP, being considered an excellent specific target of drugs of Leishmania. The target protein and template sequences were obtained from the National Center for Biotechnology Information database and the 3D template structures through the Protein Data Bank (PDB). Sequence alignment was performed on the FASTA, yielding 91.0% identity and 97.2% similarity to the *Leishmania major* template protein (LmPtr1). The LdPtr1 model was constructed using MODELLER software 9.18. The stereochemical quality was evaluated in PROCHECK and the structural quality in VERIFY 3D and WHAT IF software. The Discovery Studio Visualizer software was used for graphical visualization of the modeled protein. Due to the high level of identity and similarity of the target enzymes and template, the results revealed that a

	good model was constructed. The Ramachandran graph showed that the conformations of the main chain of amino acids are in allowed and favored regions. Besides that; 85.07% of the residues have the protein sequence compatible with their 3D structure and do not have unusual atomic contacts.

Introduction

The development of a three-dimensional structural model of protein homologues can be used to identify inhibitors for specific targets by modeling homology and molecular dynamics. Homology modeling is currently the most accurate method, able to predict the structure of a protein based on the general observation that proteins with similar sequences possess. The computational methods for protein structure modeling play an important role in homology modeling [1].

Homology modeling requires the structure of an established protein (template) generated by a homologue containing the target sequence, provided that it shares approximately 30% or more similarity in the sequence or structure of the template [2].

Pteridine Reductase 1 (PTR1) is an enzyme used in the metabolism of pterin and necessary for the survival of the parasite [3]. Previous studies have reported as the PTR1 as an important target chemotherapeutic [3,4]. Inhibitors of Pteridine Reductase 1 (PTR1) proved to be lethal to the parasites in Leishmania *spp*. and *Trypanosoma brucei*, but not in human cells [4].

Thus, the objective of the study was to construct the three-dimensional model of the structure of the enzyme Pteridine reductase 1 from *Leishmania donovani* (LdPTR1) to contribute to the study of anti-leishmania inhibitors.

Materials and Methods

Identification of target sequences and selection of protein template

The sequence of the target protein was obtained from the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/pubmed) and the identification of the resolved structures in 3D model candidates was done through a [5] and to obtain the structure, the RCSB Protein Data Bank (https : //www.rcsb.org/pdb/home/home.do) [6]. The template protein selected was LmPtr1 (PDB ID: 1E7W).

Alignment of template and target sequences

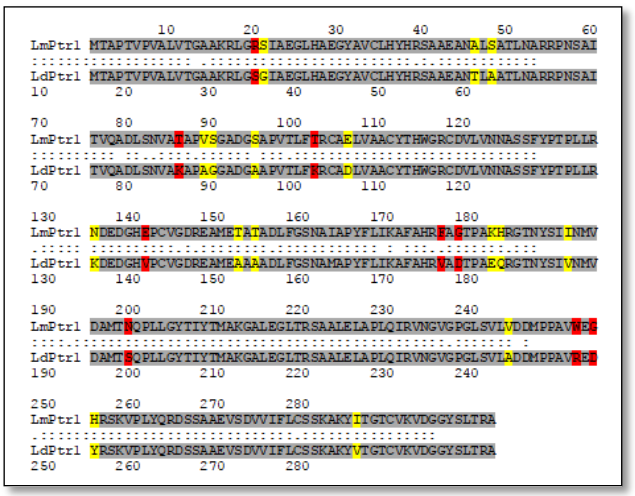
Alignment of multiple sequences was performed using FASTA (http://www.ebi.ac.uk/Tools/sss/fasta/) and obtained the following values for *Leishmania donovani* (LdPtr1): 91.0% identity and 97 , 2% similarity to *Leishmania major* Ptr1 (LmPtr1) (**Figure 1**). This was not the best score alignment in the FASTA, but it is the target organism of the study.

Construction and validation of the model

The LdPtr1 model was constructed using the homology molecular modeling method through MODELLER 9.18 software [7], which is based on the spatial constraints satisfaction modeling. Five models were generated and the lowest energy model was chosen. The stereochemical quality ofthe model was evaluated in the PROCHECK [8], which

evaluates several stereochemical parameters, such as torsional angles of the main chain, torsional angles of the side chain, bad contacts or steric impediments, flatness, among others. PROCHECK generates the Ramachandran graph [9] that verifies allowed and unallowed regions of the main amino acid chain. The structural quality was evaluated in VERIFY 3D software (http://services.mbi.ucla.edu/SAVES/) that analyzes the compatibility of the protein sequence with its 3D structure according to its chemical environments and WHAT IF (http: //swift.cmbi.ru.nl/servers/html/index.html) that analyzes various structural parameters, such as the atomic contacts between the residuals. The software Discovery Studio Visualizer [10] was used for graphic visualization of the modeled protein.

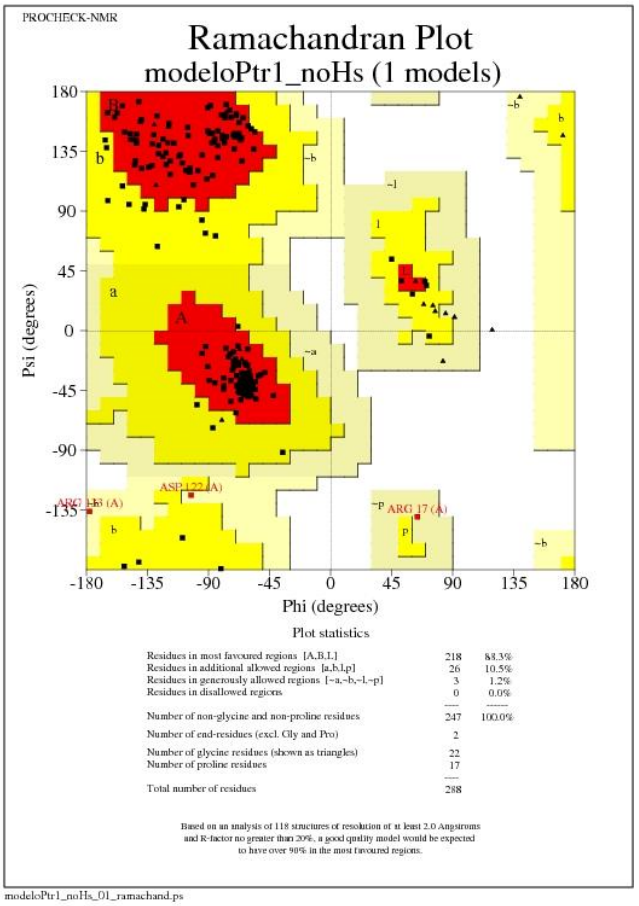
Figure 1 - Alignment between the template sequence (LmPtr1) and the target sequence (LdPtr1). The regions highlighted in gray correspond to the identical amino acids in the two sequences, in yellow are the similar residues and in red the non-identical and non-similar residues.



Results and Discussion

Molecular Modeling by Homology of the enzyme Ptr1 of *Leishmania donovani* was performed using the Ptr1 enzyme of *Leishmania major* as template. A good LdPtr1 template was obtained because of the high level of similarity between the target sequence (LdPtr1) and the template sequence (LmPtr1). To verify and validate the reliability and stereochemical quality of the modeled protein, data from the Ramachandran, VERIFY 3D and WHAT IF graph were considered. Analysis of the Ramachandran graph shows that the main chain conformations are 88.3% of the residues in the most favored regions, 11.7% allowed and 0% outlier (**Figure 2**). In this analysis, since there was no residue in the outlier region, the generated model was considered satisfactory. The G factors, which indicate the quality of covalent distance and bond angle, were 0.15 for dihedrons and 0.09 for phi / psi. Positive or slightly negative values indicate a model of good stereochemistry.

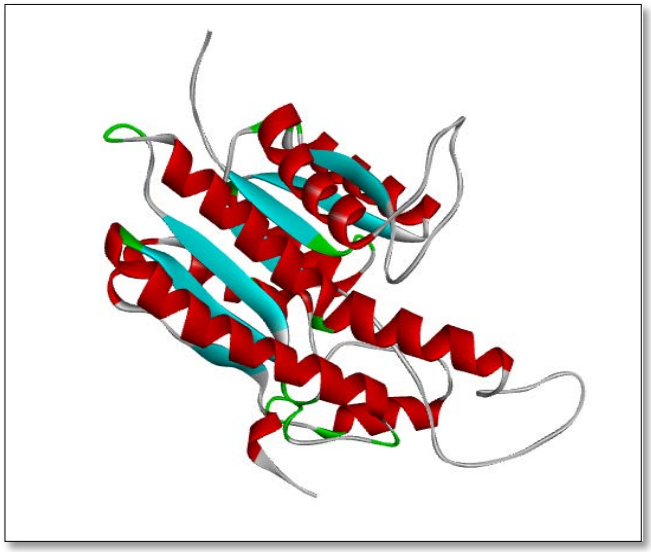
Figure 2 - Ramachandran plot of LdPtr1 using Procheck.



No VERIFY 3D; 85.07% of the residues had a mean 3D-1D score> = 0.2, which indicates

a reliable model, since it is superior to 80% of the amino acids that marked = 0.2 in the 3D / 1D profile. The quality of the atomic contacts involving the atoms of each residue was analyzed using the Fine Packing Quality Control module of the WHAT IF, which compares the distribution of atom positions around each residue. The mean score of all wastes is -0.687. A score of less than -5.0 for a residue means bad or unusual atomic contacts. The graphical visualization of the modeled protein is observed in figure 3.

Figure 3 - Graphical view of LdPtr1 in Discovery Studio Visualizer.



Conclusions

Molecular homology modeling is an excellent computational tool that performs the prediction of protein structures not yet crystallized, contributing to the rational planning of anti-leishmania inhibitors. The LdPTR1 model was considered satisfactory as observed in the validation results.

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Identification of triterpenic saponin by HPLC-DAD-MS/MS in *Zornia brasiliensis*

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Abstract: *Zornia brasiliensis* (Leguminosae) is a species popularly known as "urinária", "urinana" and "carrapicho" and its popular use is reported as a diuretic and for treatment of venereal diseases. It occurs in several states of Brazil, being abundant in the Northeast. Therefore, this study aims to contribute to the chemical knowledge of this species through an HPLC-DAD-MS/MS study for the rapid determination of saponins. Aerial parts of *Z. brasiliensis* were collected in Serra Branca, state of Paraiba. An exsicata was deposited in the Herbarium Arruda Câmara (ACAM) of Campus I of UEPB. After drying and ground, the plant material was subjected to a 95% ethanol maceration for 72 hours, and this process was repeated four times to obtain the crude ethanolic extract (CEE). An aliquot of the CEE (100.0g) was subjected to vacuum liquid chromatography (VLC) with silica deactivated with hexane, dichloromethane, ethyl acetate and methanol solvents. An aliquot of the 50% acetate-methanol fraction was subjected to a specific methodology for the concentration of saponins. This fraction was then analyzed by HPLC-DAD-MS^2, allowing the identification of a triterpenic saponin, suggesting to be the Soyasaponin IV, described for the first time for the genus *Zornia*. In this way, this work contributed to the chemistry *Z. brasiliensis* and corroborated with the chemitaxonomy of the family Leguminosae.

Keywords: Soyasaponin IV; *Zornia brasiliensis*; Saponin.

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1. Introduction

Zornia (Leguminosae) has about 80 species in the world, with 41 representatives in America, 16 in Africa, 13 in Oceania and 7 in Asia [1-2], is the second largest representative of the Adesmia clade.

Zornia brasiliensis is a species popularly known as "urinária", "urinana" and "carrapicho" and its popular use is reported as a diuretic and for treatment of venereal diseases [3]. It occurs in several states of Brazil, being abundant in the Northeast. In view of this, this study aims to contribute to the chemical knowledge of this species through a study by HPLC-DAD-MS/MS for the rapid determination of saponins.

2. Results and Discussion

The FrST fraction was analyzed by HPLC-DAD-MS / MS. First, the low resolution HPLC-DAD-MS analysis was performed in MS and MS^2 mode so that the retention times, the peaks of the molecular ions and their respective fragments can be obtained. Then, high resolution HPLC-DAD-MS analysis was performed in MS mode, similarly, retention times, molecular ion peaks, can be obtained. In addition to the molecular formulas.

Compound **1** showed a retention time of 32.5, *m/z* 765.4425 $[M-H]^-$ (*m/z* 765.4421, calcd) compatible with the molecular formula $C_{41}H_{65}O_{13}$. A sequenced loss of sugars with fragments in *m/z* 615.47 and 457.28, compatible with the loss of a pentose-like structure (150 Da) and a residue of glucuronic acid (158 Da) , respectively. The fragment with *m/z* = 457.28 was compatible with Soyasapogenol B, a triterpenic sapogenin, on the basis of these data it is suggestive that compound 1 is Soyasaponin IV [4].

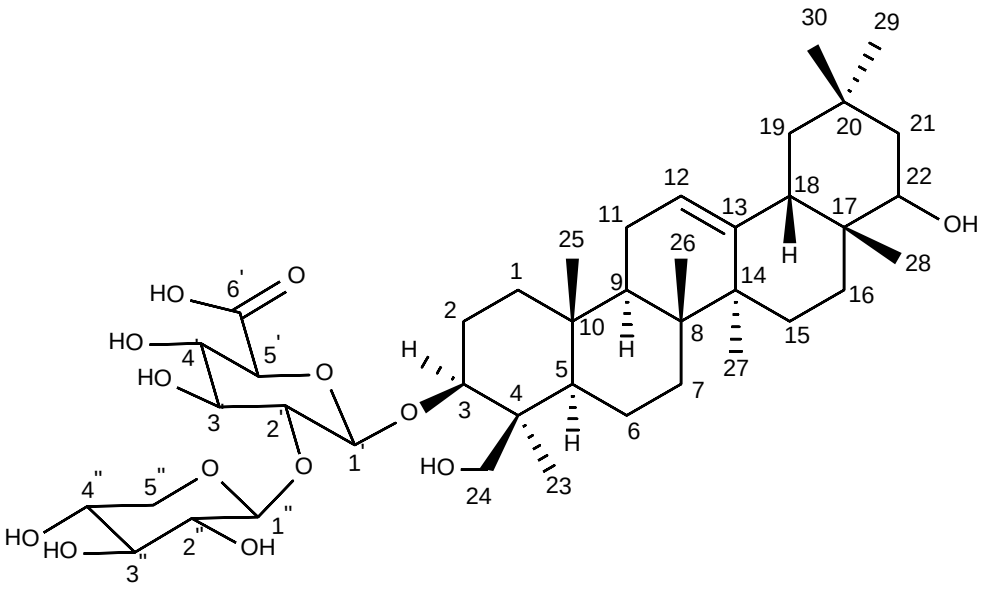

Figure 1. Chemical structure of Soysaponin IV.

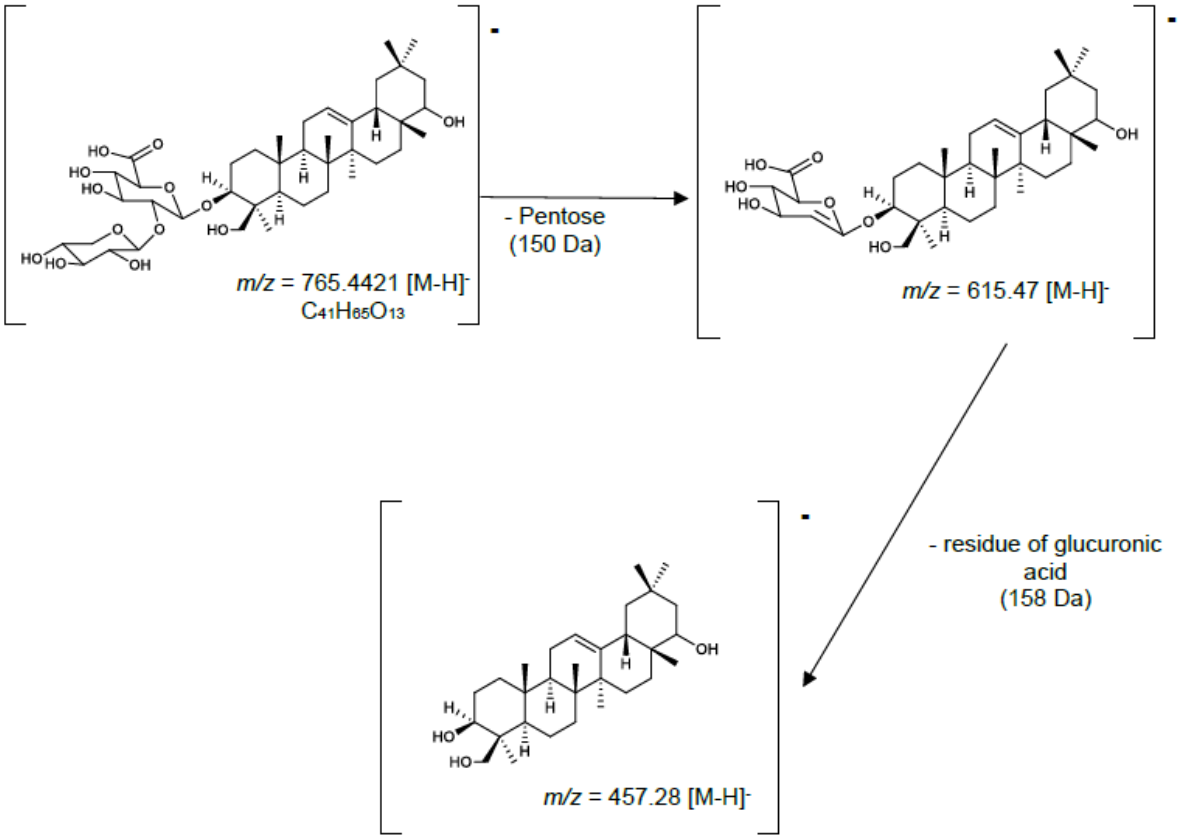

Figure 2. Proposed fragmentation of Soyasaponin IV.

3. Materials and Methods

Aerial parts of *Zornia brasiliensis* were collected in March 2016, the collection was carried out in Serra Branca, state of Paraíba. The botanical identification was carried out by Prof. Dr. José Iranildo Miranda de Melo from the State University of Paraíba (UEPB). An exsicata was deposited in the Herbarium Arruda Câmara (ACAM) of Campus I UEPB, under code 1862.

The botanical material (5 kg) was dehydrated using a circulating air oven at 45 ° C for 72 hours. After this, the material (3 kg) was shaken in a mechanical mill to obtain the powder. Then the powder was subjected to a thorough maceration with 95% ethanol (EtOH) for 72 hours in a stainless steel macerator, this process being repeated four times to obtain the extractive solution. The extractive solution was concentrated under reduced pressure on a rotary evaporator at a temperature of 40 ° C to remove the solvent to give the crude ethanolic extract (CEE).

An aliquot of CEE (100.0 g) was subjected to vacuum liquid chromatography (VLC) with 300 g of silica(ART 7734, MERCK, 0.060 – 0.200 mm e 70 - 230 mesh ASTM) deactivated with hexane, dichloromethane, ethyl acetate and methanol solvents. After fractionation the extractive solutions resulting from this process were concentrated in a rotary evaporator to give 0.73 g of the hexane fraction; 20.0 g of the dichloromethane fraction; 15.97g of the acetate fraction; 5.96 g of the 10% acetate-methanol fraction (AcOEt-MeOH 10%); and 59.81 g of the 50% acetate-methanol fraction (AcOEt-MeOH 50%).

An aliquot of 20 g of the 50% acetate-methanol fraction was dissolved in 300 ml of distilled water. It was then partitioned with n-butanol (300 mL) three times. The extractive solution of the n-butanolic phase was then concentrated on rotary evaporator under reduced pressure at a temperature of 50° C. After obtaining the dried material, it was dissolved in a solution with methanol-ethyl acetate (1:5, v/v) and then methanol was added until the precipitation of the saponins occurred. After precipitation the material

was allowed to decant for 72 hours at room temperature. The precipitate was then resuspended in methanol (200 mL) and concentrated on a rotary evaporator under reduced pressure at 40° C. Thus, obtaining a fraction rich in saponins and being encoded with FrST (1.1 g) [5].

A Shimadzu (Prominence) CLAE equipped with LC-20AT binary solvent pumping module, SIL-20A autoinjector, a degassing system DGU-20A, SPD-M20A diode array detector and CBM-20A were used. The column used was Phenomenex Gemini® C18 (250 mm x 4.6 mm i.d.,5 µm particles), with SecurityGuard Gemini® C18 pre-column (4 mm x 3.0 mm i.d., 5 µm particles). Method used for HPLC-DAD-MS / MS was water (0.1% formic acid) (A) and methanol (B). Gradient: 70% B (0.01 min) to 80% B (50.0 min), returning to 70% B (65.0 min) and remaining with 70% B to 80.0 min. Flow of the mobile phase: 0.6 mL / min. Injection volume: 5 µL. Detection: 205 nm.

The low resolution mass spectrometer of Bruker, model Ion Trap-amaZonX using the technique of ionization by Eletrospray. The parameters of the Ion-Trap analysis were: capillary 4.5 kV, ESI in negative mode, end plate offset 500 V, nebulizer 24.5 psi, dry gas (N_2) with flow of 4.5 L/h and temperature of 200° C. CID fragmentation was achieved in auto MS/MS mode using enhanced resolution mode for MS and MS/MS mode. The spectra (*m/z* 50-1000) were recorded every 2 sec. The high-resolution mass spectrometer model micrOTOF II (Bruker) using the Eletrospray Ionization technique. The micrOTOF II analysis parameters were: capillary 4.0 kV, ESI in the negative mode, end plate offset 500 V, 29.5 psi nebulizer, dry gas (N_2) with flow of 7.0 L/h and temperature of 200° C. The spectra (*m/z* 50-1000) were recorded every 2 sec.

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4. Conclusions

This work enabled the development of an analytical methodology by HPLC-DAD-MS/MS that enabled the suggestion of the presence of Soyasaponin IV in *Zornia brasiliensis*. This being the first report of saponins in this genus. In this way contributing with the chemistry *Z. brasiliensis* and corroborating with the quimitaxonomia of the family Leguminosae.

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Acknowledgments

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Author Contributions

YN, LA, RL, participated in phytochemical analysis. MS and JT participated in interpretation of the mass spectra. JIMM participated in botanical identification.

Conflicts of Interest

The authors declare no conflict of interest.

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Synthesis and Anti-mycobacterial evaluation of coumarin derivatives

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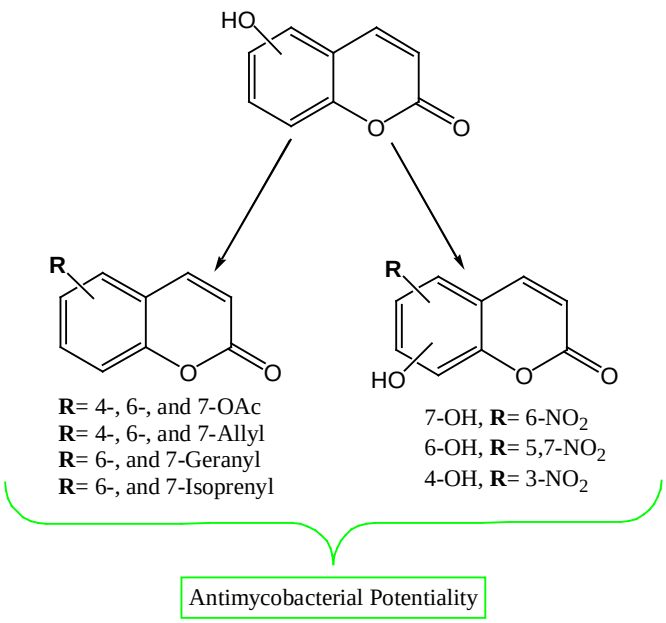
Graphical Abstract



Abstract.

Tuberculosis is one of the oldest known diseases of the world, remaining, within today, with the higher mortality rates caused by a single infectious agent, mainly represented by *Mycobacterium tuberculosis*. It's considered a greater public health problem, for reaching millions of people around the world, mainly in the developing countries, also highlighting their co-infection cases in immunocompromised patients, the example of HIV positive. Their high incidence rates are related to the non-adhesion to, and abandonment of, available treatment, due to its prolonged administration time, which allowed the appearance and increase of resistant strains causing tuberculosis. This evidences the need for the discovery and development of new drugs more efficient and powerful against pathogenic species of *Mycobacterium* genus. Classes of natural products, such as coumarins, have shown themselves to be powerful antimycobacteria agents in their evaluation against

Mycobacterium strains. Thus, this work shows the synthesis, from protocols of *O*-Alkylation, *O*-Acetylation and Nitration well described in the literature, of coumarin derivatives, in good to greater yields, in most cases. This semi-synthetic derivatives, together with some commercial coumarins were evaluated according their antimycobacterial activities against *M. tuberculosis* H37Rv strains, where all compounds demonstrated actives against the tested strains, inhibiting the growth of *M. tuberculosis* after eight weeks of observation (in in 100 μg/mL), being more active than standard-drug used, Isoniazid, which non-inhibiting the growth of pathogens after the fourth week. Showing themselves as therapeutic alternatives in the development of new antimycobacterial active compounds.

Introduction

Historically, tuberculosis is one of the oldest known diseases, remaining, within today, with the higher mortality rates caused by a single infectious agent[1], which is represented by species of the genus *Mycobacterium*, mainly by microorganisms of the *Mycobacterium tuberculosis* specie, and, secondarily, by *M. bovis* and *M. africanum* species[1-5]. This disease is characterized by affecting the airways of infected patients, being responsible for causing the death of about 2 million of people for year, all around the world[6,7], besides 8 million additional cases annually, especially in developing countries[8]. According to World Health Organization (WHO), these numbers should continue to grow in the coming years, if control of this is not strengthened[8].

This high incidence rate of tuberculosis is often related to co-infection in immunocompromised patients, such as individuals afflicted with HIV[9], to the point of being registered, by WHO, in the period from 2000 to 2006, about 700,000 cases of tuberculosis in Brazil, of which 60,000 infected they died, being 20% of these associated to patients co-infected with the HIV virus[2,8].

Their high incidence numbers made with that tuberculosis to be treated as a great public health problem, mainly by high rates of non-adherence to, and abandonment of, available treatments and/or by the appearance of multi-drug resistant strains of tuberculosis (MDR-TB)[6]. Failure to realization or complete treatment, considered as prolonged administration, appear as the main causes of the onset of mycobacterial resistance against the drugs used, as Isoniazid and Rifampicin, or combination of these with Ethambutol or Pyrazinamide, for example[10,11].

This facts reinforce the great need of development of potent new anti-tuberculosis agents more efficient and secure and with therapies of shorter duration when related at drugs currently utilized[7,12].

In this aspect, natural compounds and their derivatives has been shown as potent against *Mycobacterium sp.* Strains[7,12], such as coumarins, which are characterized for being formed by the fusion between benzene and α-pyrone rings[13], and are widely used according to their biological

potentialities, highlighting as antibacterial[14], antifungal[15], anti-inflammatory[16], antitumor[17], anticoagulant[18], antimycobacterial[19], among others.

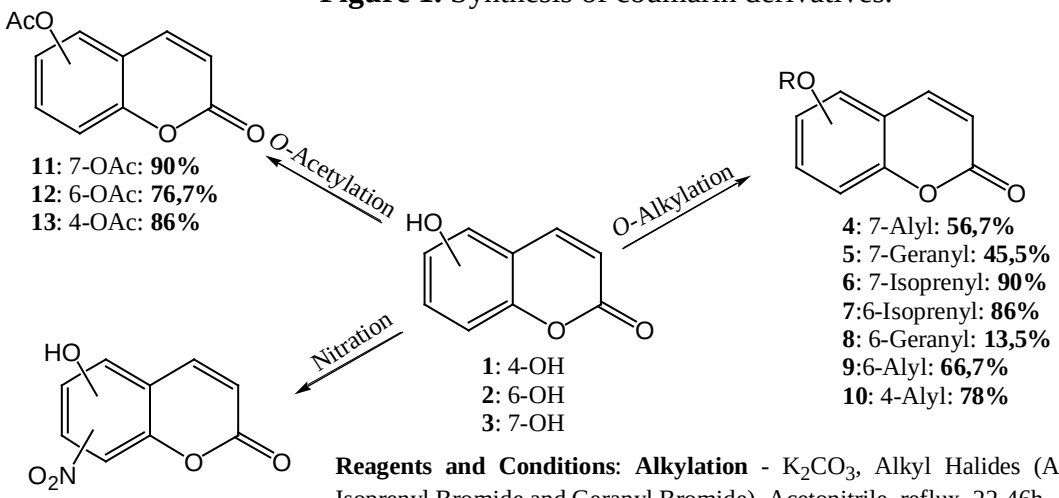
In view of the greater need in the discovery of new compounds with antimycobacterial potentiality, and the greater variety of biological activities of coumarin compounds, the aim of this work was the synthesis and evaluation of the antimycobacterial potential of coumarin derivatives against *M. tuberculosis* strains.

Materials and Methods

Synthesis of coumarin derivatives

The semi-synthetic coumarin derivatives was obtained through of standard procedures for *O*-alkylation, *O*-acetylation and Nitration of commercial coumarins (4-hydroxy-2*H*-1-benzopyran-2-one (**1**), 5-hydroxy-2*H*-1-benzopyran-2-one (**2**) and 7-hydroxy-2*H*-1-benzopyran-2-one (**3**)) (Figure 1), and previously described[15].

Figure 1. Synthesis of coumarin derivatives.

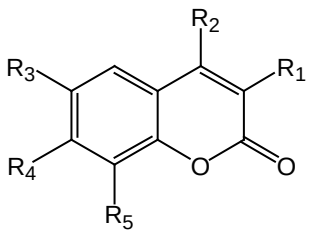


Reagents and Conditions: **Alkylation** - K_2CO_3, Alkyl Halides (Alyl Bromide, Isoprenyl Bromide and Geranyl Bromide), Acetonitrile, reflux, 22-46h. **Acetylation** - Acetic Anhydride, Pyridine, ultradound, room temperature, 15-30 min. **Nitration** - Nitric/Acetic Acids, Ice bath - room temperature, 120 min.

All final compounds had their structures proven by ^{1}H and ^{13}C NMR and physicochemical characteristics described[15].

Commercial coumarin derivatives also were analyzed according to their antimycobacterial activity (Figure 2).

Figure 2. Commercial coumarin derivatives analyzed.



17: $R_1=R_2=R_3=R_4=R_5=H$
18: $R_1=OH$, $R_2=R_3=R_4=R_5=H$
19: $R_1=R_2=R_5=H$, $R_3=R_4=OH$
20: $R_1=COOH$, $R_2=R_3=R_4=R_5=H$
21: R_1=Bis-4-OH-Coumarin, $R_2=OH$,$R_3=R_4=H$
22: $R_1=R_2=R_5=H$, $R_3=OMe$, $R_4=OH$
23: $R_1=R_2=H$, $R_3=OMe$, $R_4=R_5=OH$

Antimycobacterial Activity Assays

The antimycobacterial activity assays were performed using a concentration of 100 µg/mL of the test drugs in Löwenstein-Jensen medium. The H37Rv *Mycobacterium tuberculosis* strains were diluted in distilled water (Mc Farland scale – $3x10^8$ microrganisms/mL) and inoculated in the media containing the drugs to be tested. The controls were composed of simple and in the presence of

isoniazide in the concentration to 0.2 µg/mL Löwenstein-Jensen medium. The containers were placed in stove at 37°C, where were observed once a week until the 8th week for the analysis of bacterial growth or not. The reference drug used was isoniazid.

Results and Discussion

The semi-synthetic coumarin derivatives were obtained from *O*-alkylation, *O*-acetylation and Nitration procedures since 4-, 6- and 7-hydroxylated commercial coumarins, as previously described[15] in good to greater yields, in most cases, as demonstrated in Figure 1.

As demonstrated in the Table 1, all coumarin derivatives analyzed were able of inhibit the growth of pathogenic strains of *M. tuberculosis* after the observation period of eight weeks, demonstrating values more active than the standard-drug used, Isoniazid, which did not inhibit the *M. tuberculosis* growth after the 4th week.

Table 1. Inhibition of *M. tuberculosis* growth in the presence of the coumarin derivatives analyzed.

Drug/Week	1ª	2ª	3ª	4ª	5ª	6ª	7ª	8ª
1	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
2	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
3	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
4	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
5	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
6	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
7	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
8	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
9	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
10	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
11	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
12	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
13	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
14	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
15	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
16	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
17	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
18	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
19	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
20	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
21	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
22	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
23	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Isoniazid	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)

(-) - Inhibition of the pathogen growth; (+) - Non-inhibition of the pathogen growth

This results demonstrated the antimycobacterial potential of coumarin derivatives as growth inhibitors of *M. tuberculosis*, main etiological agent causing tuberculosis in humans, can present themselves as an alternative for the treatment of infections by this pathogen. Confirming the potentiality of coumarins against *Mycobacterium* pathogen strains results showed in the literature.

Conclusions

These results allowed the synthesis of semi-synthetic coumarin derivatives by *O*-Alkylation, *O*-Acetylation and Nitration in good to excellent yields, in most cases, which were evaluated according to their antimycobacterial activity against *Mycobacterium tuberculosis*, where all semi-synthetic and

commercial derivatives to be proved potential inhibitors of the *M. tuberculosis* growth. Can be an important alternative in the search of new antimycobacterial drugs.

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Analysis of the *ne bis in idem* principle in a court ruling.

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Abstract: The *ne bis in idem* principle establishes the impossibility of trying twice a person for the same criminal acts. In this short communication, we analyse the doctrine of the Spain Supreme Court, appreciating a conflict between the previous dualist system and the present vicarial system regarding this matter. The purpose of this study is to determine if the Spanish Supreme Court´s ruling nº 1332/2002 of the, 2nd courtroom (Criminal Chamber), 15th of July 2002, broke the *ne bis in idem* principle. Secondly, we define what this principle means by analysing the sentence. Finally, we made a critical commentary of the assessments of the Court.

Key words: *ne bis in idem* principle, vicarial system, dualist system, punishment, security measure.

Court ruling´s analysis:

The *ne bis in idem* principle, basically, consists on the fact that no one can be punished twice for the same incident, a breach that would mean arbitrariness and abuse of power. However, it is possible to punish someone for the commission of some criminal acts of the same nature many times.

Concerning the analysis of the Spanish Supreme Court´s ruling nº 1332/2002 of the, 2nd courtroom (Criminal Chamber), 15th of July 2002, it is necessary to clarify that it is an appeal for the convict, in which he argues three motives where the first and the last one are the same:

1. (and also 3.) contains the argument of violation of the article 25.2 from the Spanish Constitution because, the imposed criminal sanction does not respect the re-socializing principle contained in that article, and because the application of the security measure of the hospitalization in a detoxication centre in order to overcome the serious addiction is missing.
2. Contains infraction of the law´s report, specifically of the article 66.4 of the Spanish Criminal Code due to the imposition of a criminal sanction without enough motivation.

The Supreme Court states for the first and the third motives that, under the precedent dualist system, considering that punishments and security measures could consist in the same result, if finally there were a sentence of punishment, the *ne bis in idem* principle would be violated. But the new vicarial system overcomes this imperfection because the security measure can be an alternative to the punishment, even though this measure can consist in imprisonment. The the accused in the criminal case, asks for the implementation of the security measure of the hospitalization in a detoxification centre (it can be deduced from the Criminal Code that if the judge applies the extenuating circumstance or the exculpatory circumstance because of drug addiction, he should apply the security measure previously mentioned). However, the Supreme Court denies the request, based on the medical report that concluded that the criminal was in detoxification process.

For the second motive the Supreme Court argues that the decision of reducing the punishment in two degrees is optional, while reducing in one degree is mandatory, and that is why there is no reason to discuss about that.
To understand this, it is necessary to explain what these two systems consist of:

- Dualist system: the punishment responds to the culprit of the criminal, and the ration to its culpability. Punishment and measure respond to a different idea and purpose.
- Vicarial system: it is a variation of the previous one. During the execution of the punishment, the punishment can be replaced by a security measure, beginning with that measure, and the compliance would diminish the duration of the punishment. The remaining time of the punishment would be perform later or cancelled by the judge based on the social dangerousness of the convicted person. It has some objections: the excessive power conferred to the judge, the difficulty of the application, and the confusion between the punishment and the measure.

In this case, the convict was sentenced to prison, without any security measure. It is not an objective of this communication to discuss about will the possible arbitrariness of the judge in the moment to stablish the punishment. Nonetheless, I have to conclude that, as convict is a habitual drug consumer, the security measure should have been sentenced with no other option because the Spanish legal system has the aim to re-educate and reintegrate in society, two aspects that are not going to happen without the detoxification of the criminal.

Due to the dualist system by which offender was tried, the punishment and the security measure that were imposed to the criminal were both of them imprisonment. Consequently, the *ne bis in idem* principle is violated. It is not fair to sentence a criminal with two identical

consequences for the same act, which, as said in the content of the sentence, is something that happens in this type of system, and which should never do.

On the contrary, using the vicarial system the judge did not adjust it to the danger of the criminal, nor did the re-education and reintegration mentioned in the Spanish Constitution, as it did not impose any security measure that would have been validated with the punishment of imprisonment later. Thus, here is another conflict with the *ne bis in idem* principle considering that, as in the former case, the punishment is much bigger because it takes the part of the punishment and also the part of the security measure that is not applied. That's why it is possible to understand that the criminal has been sanctioned twice for the commission of the same crime and based on the same basis.

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Nanodesk project: development of a web platform for the selection of the nanoparticles of interest for the plastic industry

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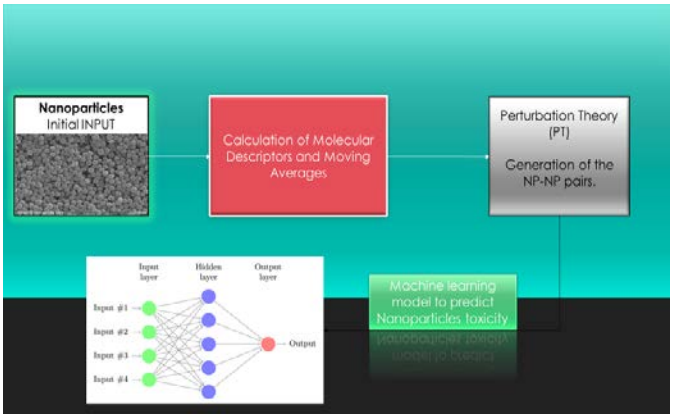
Graphical Abstract



Abstract.

Predict Nanoparticles (NP) toxicity is one of the biggest deal for both toxicologists and computational chemistries. The Nanodesk project is a SUDOE interreg project (Project Code: SOE1/P1/E0215) aimed at the development of an interactive and easy to use web-platform which will be help the plastic industry in the selection of the safer nanomaterials (NMs). The web-platform will implement a series of machine learning approaches which will be identify the safer NMs amongst a list selected by the industry and the scientific committee of the project. The project has started on July 2016 and will last for 36 month.

Introduction

The use of Nanoparticles (NP) in the plastic industry has increased significantly over recent years as demonstrated by the number of patents and products containing NP. On one hand the inclusion of these materials in classic plastic materials is leading to new product, with a high added value and incredible performance; on the other hand, there is also a growing concern regarding the hazard and the toxicity of these new materials. The Nanodesk project aims at creating a bridge to link the NP of interest in the plastic sector and its toxicity. In fact, one of the main aim of this project is to develop a set of QSAR materials in order to predict the toxicity of a set of selected NP. The QSAR models are implemented for the prediction of four properties of special relevance for the evaluation of the danger of the nanometer are implemented in the web platform NanoDesk, being one of its essential components.

The development of these models is essential in the context of this project, since its application will allow the determination of the hazard profile of the NMs, also making it an alternative to in vitro and in vivo tests, as recommended by international institutions such as the OECD and The ECHA.

Finally, these QSAR models will be implemented in an easy to use web-tool which will allow the plastic industry and other relevant stakeholders to select the safer NP.

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Nanodesk project: http://sudoenanodesk.europeanprojects.net/en/

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In silico evaluation of spiro-acridine derivatives as potential inhibitors of the Human Epidermal Growth Factor Receptor 2 (HER2)/ Epidermal Growth Factor Receptor (EGFR)

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Abstract: Breast cancer is one of the different types of cancer that most affect women around the world. Clinically breast cancer is characterized as the presence or absence of hormone receptors and as to the hyper-expression (or not) of the protein HER2 (Human Epidermal Growth Factor Receptor 2). Protein kinases play important roles in signal transduction pathways that regulate numerous cellular functions, including proliferation, differentiation, migration, apoptosis and angiogenesis. As signal transduction pathways are upregulated in many tumor cells, protein kinase inhibitors targeting these regulated pathways are attractive candidates for the search for new cancer therapies. An important class of compounds that has been outstanding for the planning and development of new drugs are the acridine derivatives, where more and more studies show that this class has promising activities for the therapeutic innovation of different diseases. In this study spiro-acridine derivatives (AMTAC-01 and AMTAC-02) that have potential activity antitumor on the MCF-7 (breast adenocarcinoma) line with a GI_{50} (concentration of the compound that inhibits 50% cell growth) of 2.09 and 0.69 μm respectively were subjected to energy minimization and Molecular Docking calculations in HER2 and EGFR with the software Molegro Virtual Docker. The results showed that spiro-acridine derivatives fit well into the active site of the EGFR and HER2 and also interact with the active site residues that appear to be important for their biological activity. Therefore, spiro-acridine derivatives may be a dual inhibitor of EGFR/HER2 and may be used as potential candidates for anticancer drugs, specifically as HER2-positive breast cancer agents.

Keywords: breast cancer; spiro-acridines; docking; dual inhibitor.

1. Introduction

Among the different types of cancers, breast cancer is the leading cause of cancer death in women worldwide, with an estimated 522.000 deaths estimated for 2012. This type of cancer is the second leading cause of cancer death in developed countries, and the leading cause of cancer death in developing countries [1].

Protein kinases play important roles in signal transduction pathways that regulate numerous cellular functions, including proliferation, differentiation, migration, apoptosis and angiogenesis. As signal transduction pathways are upregulated in many tumor cells, protein kinase inhibitors targeting these regulated pathways are attractive candidates for the search for new cancer therapies [2].

A group of receptors, such as the Epidermal Growth Factor Receptor (EGFR), has been the source of large studies because they are directly related to the development of some types of cancer. EGFR belongs to a superfamily of human proteins encompassing four members: EGFR (ErbB1/HER-1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). These receptors are glycoproteins contained in the membrane having the extracellular and intracellular sites, which possess the receptor tyrosine kinase (RTK). RTK activation is a common tumorigenic mechanism that has been associated with different types of cancer, such as breast, ovary and lung. EGFR and ErbB2 are the most important targets in HER2-positive metastatic breast cancer therapy [3].

An important class of compounds that have been outstanding for the planning and development of new drugs are the acridine derivatives, where more and more studies show that this class has promising activities for the therapeutic innovation of different diseases as well as potential antitumor agents [4].

Acridine derivatives are compounds characterized by a planar polycyclic system consisting of three or four rings and one or two flexible substituent groups [5].

Almeida *et al*., in a recent study found that the spiro-acridine derivatives AMTAC-01 and AMTAC-02 have promising anticancer activities, among them on the MCF-7 (breast adenocarcinoma) line with a GI_{50} (concentration of the compound that inhibits 50% of cell growth) of 2.09 and 0.69 μm respectively [6].

Thus, in this present work we use these two spiro-acridines derivatives due to its promising anticancer activities already identified and reported in the literature and was identified the potential of these derivatives to act as multitarget agents, being dual inhibitors of HER2 and EGFR.

2. Results and Discussion

When comparing the AMTAC-01 and AMTAC-02 compounds in the 3RCD and 3POZ enzymes (**table 1**), they showed a binding energy difference in both enzymes. AMTAC-02 shows lower binding energy (-114.482 and -125.79 respectively), being considered the best spiro-acridine compound, i.e., which has a better interaction with the two targets. AMTAC-02 differs from AMTAC-01 in that it has an OCH_3 (methoxyl) radical at the 4-position of the aromatic ring attached to the spiro-acridine ring. The substitution of an H by OCH_3, seems to have corroborated for a better stability of AMTAC-02 in both enzymes.

Lapatinib in both enzymes 3RCD and 3POZ (**table 1**) shows a better binding energy (-150.452 and -168.649) when compared to spiro-acridine derivatives, but when compared to TAK-285, it does not have better binding energy, since TAK-285 shows the best results in these enzymes (-168.353 and -202.237).

In **Figures 1** and **2**, we can observe the types of interactions between the compounds tested in the active site of the enzymes. Note the hydrogen bonds (dashed lines in blue) and steric bonds (dashed lines in red).

Figure 1 shows the interactions between the compounds and the enzyme 3POZ. Among the residues that are most important for the interaction with the active site of the enzyme, that is, the critical residue for biological activity are Met 793 and Thr 854, which is present in all the compounds tested, by steric interactions, except for TAK-285, which exhibits only a common interaction with Met 793, but with hydrogen bonding. Residues Val 726, Leu 844, Lys 745, are common to spiro-acridine derivatives. Only in AMTAC-01 was the

interaction of hydrogen with Asp 855 on the N atom of the acridine ring, as well as some residual interactions with Lapatinib, such as Leu 844, Lys 745, Ala 743, Thr 790, Cys 775, Gln 791. Only in AMTAC-02 was it possible to observe the interaction with Leu 788 with the carbonitrile N. In Lapatinib some residues are restricted to their interaction with the receptor as Met 766, Arg 776, Lys 852, Leu 858. In TAK-285, the restriction of sharing a residue with Lapatinib, Phe 856, which does not appear in AMTACs, but shares with them the residues Val 726, Asn 842, Arg 841.

Figure 2 shows the interactions between the compounds and the 3RCD enzyme. Among the residues that are most important for the interaction with the active site of the enzyme, that is, the critical residue for biological activity would be steric interaction of the Asp 863 residue, which occurs in all compounds tested, as well as Ser 783 (which does not appear only in Lapatinib). We can also observe that in figure 2 that the Leu 852 residue is common to spiro-acridine derivatives. Only in AMTAC-01 was the interaction of hydrogen with Asn 850 on carbonitrile N-atom as well as some residual interactions with Lapatinib, such as Thr 798, Val 734. Only in AMTAC-02 only new interactions with the residues Ileu 752, Thr 862, Leu 796, Val797, as well as shares some residual interactions with Lapatinib, as in residues Lys 753, Ala 751. In Lapatinib some residues are restricted to their interaction with the receptor as Gln 799, Gly 804, Gly 729.

Table 1. Energy Values of derivatives spiro-acridines and enzymes HER2 and EGFR interaction calculated from Molegro Virtual Docker program.

Enzymes / Compounds	MolDock Score (kcal mol^{-1})			
	AMTAC-01	**AMTAC-02**	**Lapatinib**	**TAK-285**
HER2	-98.2384	-114.482	-150.452	-168.353
EGFR	-104.101	-125.79	-168.649	-202.237

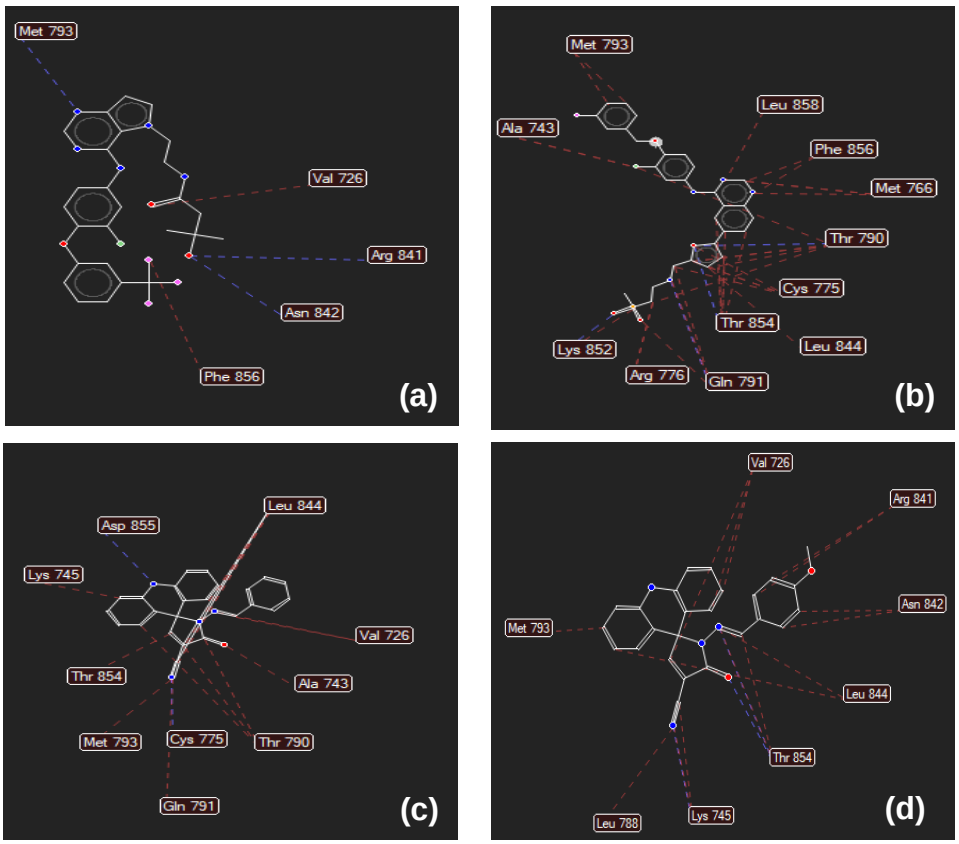

Figure 1. Interactions observed in the molecular docking of the EGFR enzyme (PDB ID: 3POZ) with its ligand TAK-285 (a), Lapatinib (b), AMTAC-01 (c) and AMTAC-02 (d).

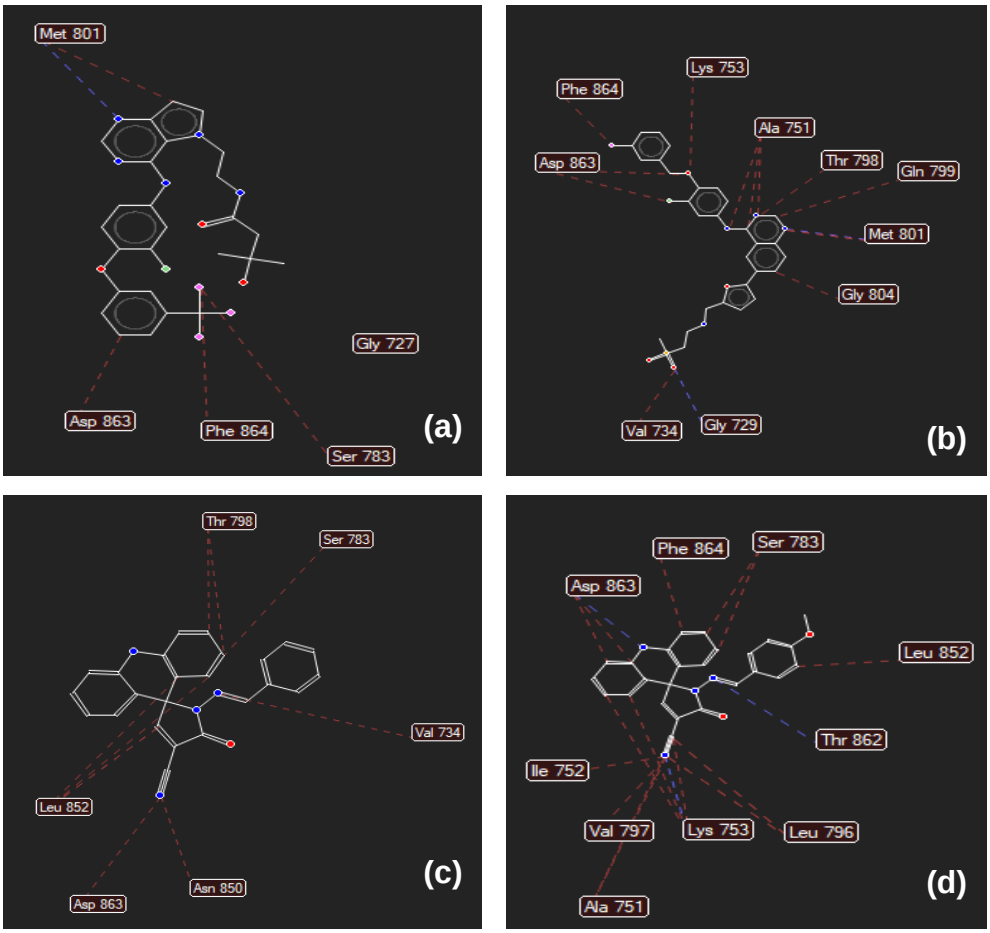

Figure 2. Interactions observed in HER2 (PDB ID: 3RCD) molecular docking with its ligand TAK-285 (a), Lapatinib (b), AMTAC-01 (c) and AMTAC-02 (d).

3. Materials and Methods

3.1 Molecular Model

The three-dimensional structures were designed using the HyperChem 8.0 program [7]; initially the structures had their geometry optimized by energy minimization, calculated using the method of molecular mechanics MM + (*Force Field Method*) without any restriction [8]. Subsequently, a new geometry optimization process, based on the semi-empirical method AM1 (*Austin Model 1*) was performed [9]. The optimized structures were subjected to conformation analysis using the random search method with 1000 interactions, 100 optimization cycles and 10 with lower reliability of minimum energy. [10,11]. The lower energy conforms were selected and imported into the Molegro program for further analysis.

3.2 Molecular Docking

Human epidermal growth factor receptor (HER2) and epidermal growth factor (EGFR) receptor complexes in complex with their respective linkers were downloaded from Protein Data Bank (http: //www.rcsb.org/pdb /home/home.do) and summarized in **table 2**.

Table 2. Data of the enzymes tested.

Enzyme	PDB ID	Resolution	Organism	Ligand	Ref.
HER2	3RCD	3.21 Å	*Homo sapiens*	TAK-285*	[12]
EGFR	3POZ	1.5 Å	*Homo sapiens*	TAK-285*	[13]

***TAK-285** (N-{2-[4-({3-chloro-4-[3-(trifluoromethyl)phenoxy]phenyl}amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl]ethyl}-3- hydroxy-3-methylbutanamide);

The spiro-acridine derivatives AMTAC-01 and AMTAC-02 were subjected to molecular coupling using the program Molegro Virtual Docker, v. 6.0.1 (MVD) [14]. All water molecules and cofactors were excluded from the enzyme structure, and the enzyme and compound structures were prepared using the same standard parameter settings in the same software package (scoring function: MolDock Score, Inner ES, Internal H-Bond and sp^2 -sp^2 Torsions, all

verified; number of runs: 10 Run; algorithm: MolDock SE; maximum interactions: 1500; maximum population size: 50; maximum steps: 300; neighbor distance factor: 1,00; maximum number of poses returned: 5). The docking procedure was performed using a GRID of 15Å in the radius and 0.30 in resolution to cover the binding site of the ligand of the structure of the proteins used. The coupling of models was used to focus the search for poses similar to the interactions and conformation of the ligand [14].

4. Conclusions

Molecular docking studies provided an estimate of inhibitory activities of the coupled ligands. The results showed that spiro-acridine derivatives fit well into the active site of the EGFR and HRE2 proteins and also interact with the active site residues that appear to be important for their biological activity. Therefore, spiro-acridine derivatives may be a dual inhibitor of EGFR/HER2 and may be used as potential candidates for anticancer drugs, specifically as HER2-positive breast cancer agents.

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CHEMICAL CHARACTERIZATION OF THE AGROINDUSTRIAL BY-PRODUCTS DESTINED FOR PIG FEEDING.

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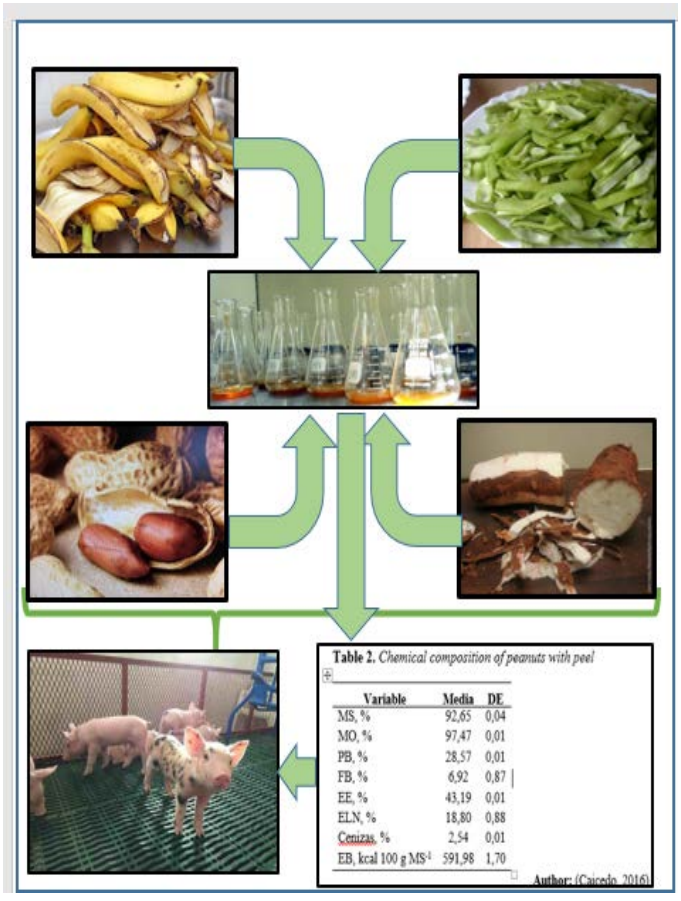
Graphical Abstract



Abstract.

The increase in the volume of agroindustrial, agricultural and domestic solid wastes entails a series of implications related to health and environmental aspects. The objective of this research was to determine the chemical composition of agroindustrial residues of shelled peanuts, peanuts in shell, banana peels, cassava peels, shelled beans and beans peas for use in pig feeding. The contents of matter (DM), organic matter (OM), crude protein (PB), crude fiber (FB), ashes, ethereal extract (EE), nitrogen free extracts, ELN and gross energy EB, were determined in agroindustrial residues by the use of descriptive statistics, and mean and standard deviation were determined. Peanut and shelled peanut residues showed the highest content of MS (91.65, 92.65%), PB (28.22, 28.27%), EE (40.49, 43.19%), and EB (577.68, 591.98 kcal kg MS^{-1}). Shelled beans had an acceptable ELN content (62.66%). In relation to the FB, the bean peel had the highest content (49.03 %). The banana peel and cassava presented the highest ash content (8.82 and 8.49 %) respectively. The agroindustrial by-products of shelled peanuts, peanuts, banana peels, cassava peels, peas and shelled beans presented a significant content of MS, MO, PB, ELN, EE, Ashes and EB, all suitable for use in pig feeding.

Key words: *pig feed, proximal analysis, agroindustrial residues.*

Introduction

According to GADPPz (2011), there are about 5.000 hectares of papa china in Pastaza province. It is considered as a traditional crop essential for small farmers survival. This fields reach a maximum yield of 38 tons per hectare. 60 % of this production is destined for export market and domestic consumption, while the other 40 % becomes by-products of tubers that are discarded in the countryside and turns into waste, consequently it becomes a problem for the environment and the crop.

The great growth of the population and the increase by the demand of food has caused a competition between humans and animals for the transcendental raw materials use for elaboration of feed, causing a rapid increase in its cost. This situation forces nutritionists to search for cheaper alternative foods for animal feed (Abdulrashid and Nnabuenyi, 2009).

The use of alternative raw materials in swine feeding, with the aim of replacing imports and reducing competition for human food to protect the environment, is a challenge for nutritionists, as well as for small and medium producers in the search for sustainable and efficient solutions in animal production systems (Agbédé *et al.*, 2002).

In Ecuador, there are viable agro-industrial byproducts for swine feeding that are not used due to lack of knowledge about their nutritional characteristics (Caicedo, 2013). Its nutrients can be raw material to generate products of interest, as food for pigs (Domínguez et al., 2012). In this regard, the use of rice husk (Martin, 2009), coffee pulp (Noriega *et al.*, 2008), apple waste (Díaz *et al.*, 2010), mangos (Rego *et al.*, 2010) taro tubers (Caicedo *et al.*, 2013) among others, has been reported with satisfactory results in the productive performance of the animals.

As reported by the GADPPz (2011), in the province of Pastaza there are approximately 5,000 hectares of Chinese potatoes, considered a traditional crop of importance for the survival of small farmers, this farm reaches yields of up to 38 tons per hectare, of which 60% is used for the export market and domestic consumption, while the remaining 40% are by-products of tubers that are discarded in the field and do not receive any use, so it becomes more of a problem for the environment and the cultivation

The objective of this research was to determine the chemical composition of the agro-industrial residues of peanuts without peel, peanuts with peel, banana peel, cassava peel, beans without peel and husk of beans for use in swine feeding.

Materials and Methods

Origin of agroindustrials waste. - The research was conducted in the city of Puyo, province of Pastaza, Ecuador. This area has a semi-warm or humid subtropical climate, with rainfall ranging between 4000 and 4500 mm per year. It is located at an altitude of 900 meters above sea level, with an average relative humidity of 87% and minimum and maximum average temperature of 20 to 28 ºC (IGM, 2016).
The by-products were obtained from the Artisanal Association (CONFERIB). Randomly, 2 kg of sample of each type of agro-industrial by-products were collected, these residues were treated and conditioned separately in the bromatology laboratory of the Amazon State University, in the later samples were dehydrated at a temperature of 60 ° C for a 5 hours to perform the grinding and subsequent analysis.

Chemical characterization of agroindustrial waste. - The content of dry matter (MS), ash, crude protein (PB), ether extract (EE), nitrogen-free extracts (ELN) and crude fiber (FB) was determined according to the procedures of the AOAC (2005). Organic matter (OM) was obtained by subtracting (100 -% ash). The gross energy (EB) was evaluated using a Parr brand adiabatic calorimetric pump, model 1241.

The data on the chemical composition of the byproducts were analyzed using the descriptive statistical module and the mean and standard deviation (SD) were determined, using the statistical program Infostat Version 1.0 for Windows (Di Rienzo et al., 2012).

Results and Discussion

Table 1. *Chemical composition of peanuts without shell.*

Variable	Media	DE
MS, %	91,65	0,16
MO, %	96,74	0,21
PB, %	28,22	0,12
FB, %	5,85	0,02
EE, %	40,49	0,76
ELN, %	22,19	0,65
Cenizas, %	3,27	0,21
EB, kcal 100 g MS^{-1}	577,68	4,65

Author: (Caicedo, 2016)

The peanut without peel, **Table 1,** presented high contents of MS (91.65%), MO (96.74%), PB (28.22%), EE (40.49%), EB (577.68 kcal 100 g MS-1) and low levels of FB (5.85%), ELN (22.19%) and ashes (3.27%).

Table 2. *Chemical composition of peanuts with peel*

Variable	Media	DE
MS, %	92,65	0,04
MO, %	97,47	0,01
PB, %	28,57	0,01
FB, %	6,92	0,87
EE, %	43,19	0,01
ELN, %	18,80	0,88
Cenizas, %	2,54	0,01
EB, kcal 100 g MS^{-1}	591,98	1,70

Author: (Caicedo, 2016)

As well as shelled peanuts, peanuts in shell have excellent nutritional qualities for its use in pig feeding as a protein supplement (Pozza *et al.*, 2005). However, we must take into consideration the high level of fat in this food and there is no way to prevent or eliminate the presence of mycotoxins and aflatoxins in the process of manufacturing balanced meals, even if a "sequestrant" of mycotoxins and aflatoxins, which may have adverse effects on the performance of animals (Etienne and Dourma, 1994; Schwarzer, 2002), the product should be stored in a clean and dry place to minimize the entry of these pathogens into the food (Jouany, 2007).

Table 3. *Chemical composition of the banana peel*

Variable	Media	DE
MS, %	14,06	0,11
MO, %	91,19	0,01
PB, %	7,93	0,70
FB, %	5,85	0,02
EE, %	5,02	0,07
ELN, %	72,40	0,76
Cenizas, %	8,82	0,01
EB, kcal 100 g MS^{-1}	378,15	0,37

Author: (Caicedo, 2016)

In **Table 3**, the chemical composition of the banana peel is observed, this by-product presented a good content of PB (7.93%), MO (91.19%), EE (5%), ELN (72.40) %), ash (8.82%), EB (378.15 kcal 100 g MS-1) and low levels of FB (5.85%) and MS (14.06%).

The banana peel presented high content of OM, ELN, ash, EB and low levels of MS and FB. In this regard, Campabadal *et al.*, (1988) and Valdivié (2008) showed that it is feasible to use banana peel flour in a 10% inclusion limit in the diet of pigs from 10 to 20 kg without affecting the productive behavior of the animals.

Table 4. *Chemical composition of cassava peel*

Variable	Media	DE
MS, %	23,77	0,59
MO, %	91,52	0,26
PB, %	5,92	0,12
FB, %	8,23	0,08
EE, %	1,21	0,08
ELN, %	76,16	0,31
Cenizas, %	8,49	0,26
EB, kcal 100 g MS^{-1}	355,65	0,79

Author: (Aguiar, 2016)

In relation to cassava peel, Table 4, it presented a high content of MO (91.52%), ELN (76.16%), ash (8.49%), EB (355.65 kcal 100 g MS- 1) and low levels of MS (23.77%), FB (8.23%), PB (5.92%) and EE (1.21%).

Cassava peel is a good source of MO, ELN and EB. To use these residues in the diet of monogastric animals, it is necessary to apply physical, chemical and biological methods to improve nutritional conditions such as increased protein and digestibility (Caicedo *et al.*, 2015), agroindustrial waste is an important source of sugars, starch and structural carbohydrates (Gómez *et al.*, 2013). In research on pigs, Buitrago (1990) established that the flour of these byproducts can be used with an inclusion of up to 30% in the diet without affecting the weight gain of the animals.

Table 5. *Chemical composition of the bean peel*

Variable	Media	DE
MS, %	32,74	0,01
MO, %	97,64	0,04
PB, %	5,03	0,11
FB, %	49,03	0,42
EE, %	0,16	0,01
ELN, %	43,43	0,26
Cenizas, %	2,36	0,04
EB, kcal 100 g MS^{-1}	293,28	0,71

Author: (Caicedo, 2016)

In **Table 5**, the chemical composition of the bean rind is observed, this by-product showed high MO content (97.64%), appreciable MS content (32.74%), FB (49.03%), ELN (43.43%), EB (293.28 kcal 100 g MS-1) and low PB (5.03%), EE (0.16%) and ashes (2.36%).

In correspondence to the tenor of MS, MO, PB, EE, ELN and EB from the nutritional point of view it is probable that there is no detrimental effect on the performance of pigs in the fattening stage (Almaguel

et al., 2008; Almaguel *et. al.,* 2013). However, a high content of FB was found in the swine species. The higher its concentration is, the lower its utilization becomes (Ly *et al.*, 1998).

Table 6. *Chemical composition of the shellless bean*

Variable	Media	DE
MS, %	32,74	0,13
MO, %	96,85	0,24
PB, %	31,48	0,13
FB, %	0,93	0,23
EE, %	1,79	0,23
ELN, %	62,66	0,83
Cenizas, %	3,15	0,24
EB, kcal 100 g MS^{-1}	394,47	0,25

Author: (Aguiar, 2016)

The peeled bean is a good source of MO, PB and ELN. Among the factors that must be taken into consideration for the use of this by-product is the content of secondary metabolites that these foods possess (Buntha *et al.,* 2008). In monogastric animals, the inclusion limit of these raw materials must be taken into account so as not to affect consumption and thus the normal performance of the animals (Lezcano *et al.,* 2014).

Conclusions

Peanut byproducts without shell showed a high level of PB, EE and EB. In pig feeding, it is recommended not to include more than 25% of peanuts in the diet because of the high EE content of this resource, it can have a laxative effect in pigs (Rostagno *et al.,* 2011). Among protein supplements, it is perhaps the most palatable.

Agroindustrial byproducts of peanuts without peel, peanuts with peel, banana peel, cassava peel, rind of beans and shelled beans presented good content of DM, MO, PB, ELN, EE, Ash and EB, all suitable for use in the swine feeding.

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Author Contributions

All authors have the same contribution**.**

Conflicts of Interest

There is no conflict of interest of the authors.

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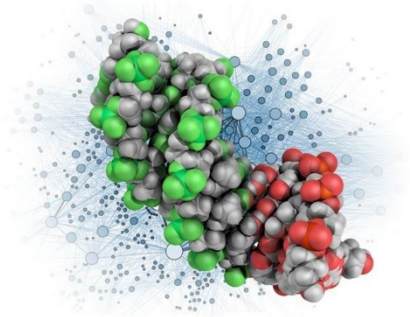

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Development of computational tools to enhance the study of catalytic mechanisms

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Graphical Abstract

Abstract

Computational methods have been widely used to characterize the catalytic mechanisms of several chemical systems namely enzymes. However, enzymes are studied using big chemical systems containing several thousands of atoms generating huge amounts of data that are hard to manipulate and analyze efficiently. Therefore, we developed molUP that is a user-friendly plugin for VMD to handle QM and ONIOM calculations performed using Gaussian software.

MolUP allows loading output files from Gaussian calculations and performs analysis concerning the structure of the chemical system as well as their energies and vibrational frequencies. Furthermore, molUP provides a graphical interface to manipulate the length of atomic bonds and the amplitude of angles and dihedral angles. Users can also easily choose which atoms belong to each ONIOM layer and the atoms that are free to move during a geometry optimization. At the end, molUP is capable of saving the new structure as a new Gaussian input file, ready to run a new calculation. Since molUP is a VMD extension, users can also benefit from the many features and resources available on VMD.

In order to demonstrate the potential of MolUP, we will also present the results that have been carried out in our research group regarding

	the catalytic mechanism of Serine HydroxyMethylTransferase (SHMT), using a QM/MM approach. SHMT is a pyridoxal-5'-phosphate (PLP)-dependent enzyme [1-3] that catalyzes the α-elimination of *L*-serine, where a methyl group is transferred from the substrate to a second cofactor, tetrahydrofolate (THF). The reaction occurs in six sequential steps from which the first one is the rate-limiting step with an activation barrier of 18.3 kcal/mol that closely fits the experimental k_{cat} of 0.98±0.06 s^{-1} [4] ($\Delta G^{\ddagger} \approx 18.2$ kcal/mol). This first step involves the nucleophilic attack of nitrogen from THF to the β-carbon of the substrate, promoting the α-elimination of the CH_2OH group of the substrate. The subsequent steps involve an intramolecular cyclization within the THF cofactor where the elimination product of the first step is incorporated, generating the 5,10-methyl-THF. At the end, the quinonoid intermediate (substrate + PLP) is protonated, producing glycine.

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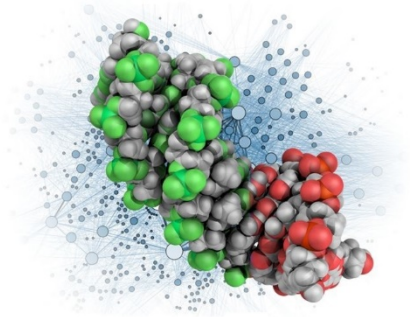

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Computational studies addressed to the catalytic mechanism of the alpha sub-unit of Tryptophan Synthase

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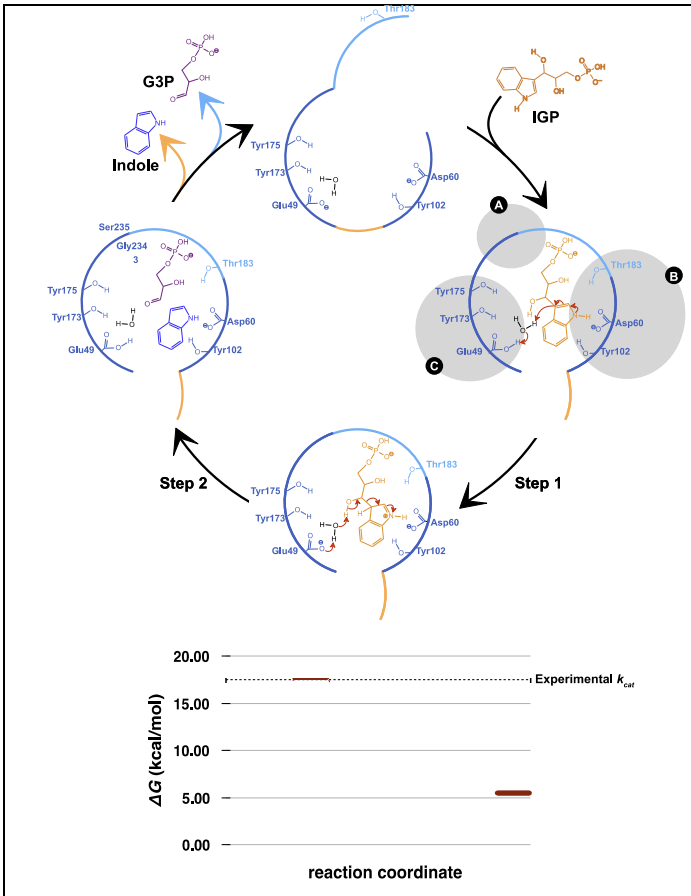

Abstract.

Tryptophan Synthase (TSase) is a bi-functional enzyme that catalyzes the last two steps in the synthesis of tryptophan (trp), in different actives site. The active site of the α-subunit catalyzes the formation of indole and gliceraldeyde-3-phosphate (G3P) from indole 3- glycerolphosphate (IGP). Indole is then transported through a 25Å physical tunnel to the active site of the β-subunit where it is added to a molecule of acrylate, derived from serine, to produce trp, in a PLP dependent reaction [1].

In this work, we studied the reaction that takes place in the α-active site of TSase using computational means and QM/MM hybrid methodologies [2]. The results show that the reaction occurs in a stepwise general acid-base mechanism. The first step requires the participation of a water molecule that protonates C3 of the indole ring and receives a proton from αGlu49. In the second step, αGlu49 abstracts a proton from the glycerolyl hydroxyl of IGP through a water molecule, triggering the C–C

	bond cleavage to give indole and G3P. The rate-limiting step of this reaction is the first one that requires an activation free energy of 17.74 kcal/mol. This result agrees extremely well with the available experimental data that predicts reaction rate of 3.0-3.7 s-1, which corresponds to a free energy barrier of 17.37-17.50 kcal/mol. The results obtained in this work provide important details about TSase that can now be used for the development of new transition state analogues inhibitors targeting TSase – an important drug target used in the treatment and prophylaxis of tuberculosis that is caused by the *Mycobacterium tuberculosis* pathogen.

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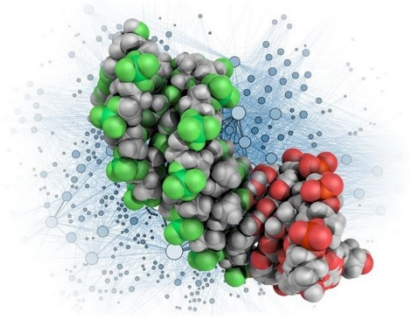

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Predicting HIV-1 resistance to protease inhibitors: A new structure-based algorithm exploring binding-site Molecular Interactions Field dissimilarities

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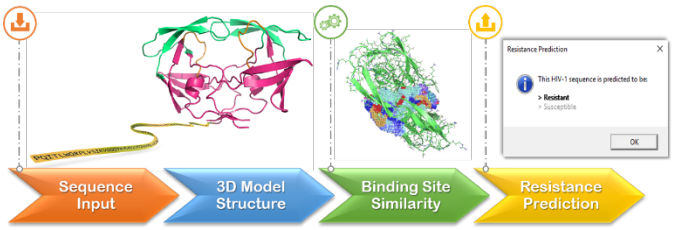
Graphical Abstract



Abstract.

Over the last 30 years, HIV has grown to a pandemic status with more than 36 million people infected worldwide. Current therapies provide a significant improvement in the quality of patients' lives, specifically the Highly Active Anti-Retroviral Therapy (HAART). Yet, viral resistance development towards anti-HIV medication stands as the main obstacle to an effective therapy, having also a substantial economic impact on healthcare systems worldwide. Such viral resistance is primarily related to mutations occurring mainly on the active site of viral key enzymes, capable of decreasing the pocket's capability to establish the necessary non-covalent interactions with the drugs. Even so, mutations outside the enzyme's active site can also lead to resistances, by causing changes on its structure and/or chemical environment. Among the two HIV virus types, HIV-1 stands as the most studied and prominent, with HIV-1 protease being one of the main viral targets for therapy.[1]

Given the ease of quickly and affordably sequence HIV from infected individuals, considerable progress

	– in the sense of predicting resistance towards drugs – could be made by developing tools to link specific genetic mutations with the resulting structural and chemical alterations in the active site of the target enzymes.[2] In recognition of a serious medical need identified by a team of virologists working at the University of Coimbra teaching Hospital and with the intent of helping rationalize and personalize the choice of anti-HIV therapies, we set out to develop a new computational algorithm to predict resistance to protease inhibitors in HIV-1 via detection of binding-site Molecular Interactions Field (MIF) dissimilarities. Briefly, the algorithm works by 1) automatically generating high-quality 3D protein model structures from HIV-1 protease sequences; 2) capturing subtle, mutation-induced, chemical perturbations within the binding sites of resistant and non-resistant HIV-1 protease structures using a MIF-based approach; and 3) quantifying binding site dissimilarities based on MIF analysis, and translating these into a *resistance score*. In terms of its predictive power, preliminary testing of the algorithm using several different HIV protease sequences showed promising levels of sensitivity and specificity. Despite both sequence- and structure-based computational approaches to the prediction of HIV drug resistance have been proposed in the past, our present work stands out from other known algorithms as a first implementation of a fast structure-based algorithm capable of discriminating between HIV sequences that may be susceptible or resistant to commercially available protease inhibitors. Since the problem of mutation-induced resistance cuts across virtually all pathogenic virus, we believe that our approach may be extended to a wide range of viral targets besides HIV-1.

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Analysis of Microbial Communities Reflect Diel Vertical Migration in the Gulf of Mexico

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Graphical Abstract

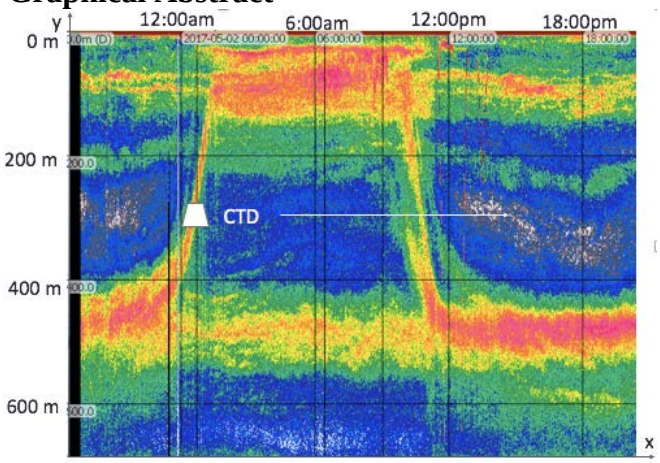

Figure 1. The figure shows the Vertical Migration occurring in real-time with the use of a multifrequency echosounder system that measures acoustic backscatter**.** Warmer colors represent higher densities of microzooplankton. However, we don't exactly know what all of these organisms are. The **Y-axis** is the depth, and the **X-axis** is time. (Image courtesy of Kevin Boswell, FIU)

Abstract: Marine Diel Vertical Migration (DVM), perhaps the largest movement of animals on Earth, is composed of mesopelagic species migrating vertically every night to feed in epipelagic depths and return to deeper water during the day. The objective of this study infers potential taxonomic identity of organisms in the DVM via their association with bacterioplankton signatures. High throughput sequencing of the 16S rRNA V4 region and the use of bioinformatics and statistics provided evidence to which bacteria appeared associated with either upward or downward vertical migration during two cycles. DNA was extracted from water samples and sequenced. Data analysis was performed using R Studio software. Our results confirm the vertical movement of bacterial taxa throughout the pelagic depths. The most abundant bacteria present during the Vertical Migration were of Genera Marinobacter, Alteromonas, Prochlorococcus, and class Gammaproteobacteria. These taxa occurred at depth of 320 meters which is the mesopelagic zone. Proteobacteria was only found during the vertical migration at mesopelagic depths, whereas Cyanobacteria was only found during the vertical migration at epipelagic depths. This indicates that these two phyla of bacteria are distinct to their respective zones.

Introduction

The earth's oceans hold vast amounts of water, with great depths and complex dynamics making them difficult to study. To better characterize the Gulf of Mexico, a relatively deep ocean basin, the DEEPEND consortium (www.deependconsortium.org) was formed. DEEPEND stands for Deep-Pelagic Nekton Dynamics. This consortium began after the Deepwater Horizon Oil Spill (DWHOS) in 2010. The BP/Deepwater Horizon (DWH) discharge in 2010 was the largest marine open water hydrocarbon discharge to date. The DWH well blowout at the seafloor discharged approximately 5 million barrels of oil and at least 250,000 metric tons of natural gas to the deep water (about 1,500m) of the Gulf of Mexico (Jove et al,).

Teams of DEEPEND scientists go on cruises to collect water and organismal samples and bring them back to the lab for molecular studies. The last cruise the team went on was in May, and they

collected water samples for DNA extraction. However, this was not to check for oil contamination. This was to check another phenomenon of nature. This phenomenon is marine *Diel Vertical Migration* (DVM), perhaps the largest movement of animals on Earth. The Vertical Migration is composed of mesopelagic species migrating vertically every night to feed in epipelagic depths and return to deeper water during the day. The objective of this study infers potential taxonomic identity of animals in the DVM via their association with bacterioplankton signatures. High throughput sequencing of the 16S rRNA V4 region and the use of bioinformatics and statistics provided evidence to which bacteria appeared associated with either upward or downward vertical migration during two cycles.

Materials and Methods

Seawater samples were collected during a DEEPEND consortium research cruise in May 2017. Water samples were collected using a niskin bottle array from 0-326 meters depth. Realtime acoustic echosounder data was used to direct sample collection in order to capture seawater samples above, during, and below the vertically migrating organisms (Figure 1). Collected seawater was filtered through a 0.45um membrane on the ship, and preserved.

DNA sequencing and clustering

DNA was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (QIAGEN) PCR was used for amplification of the DNA targeting the V4 region of the 16S rRNA gene. An Illumina MiSeq sequencing platform using a V2 chemistry 500 cycle cartridge was used for sequencing. Initial processing of sequence data was performed in MacQIIME version 1.9.1("MacQIIME - Werner Lab," 2016). Raw sequences were quality filtered to remove all chimeric and low quality (quality score < 30) sequences. These sequences were then clustered into 97% similar Operational taxonomic units (OTUs) using a combination of open and closed reference OTU clustering strategies.

Data analysis

Data analysis was performed using the R Studio software (versions 3.3.2 and 3.4.0) with the use of the library "vegan".

Results and Discussion

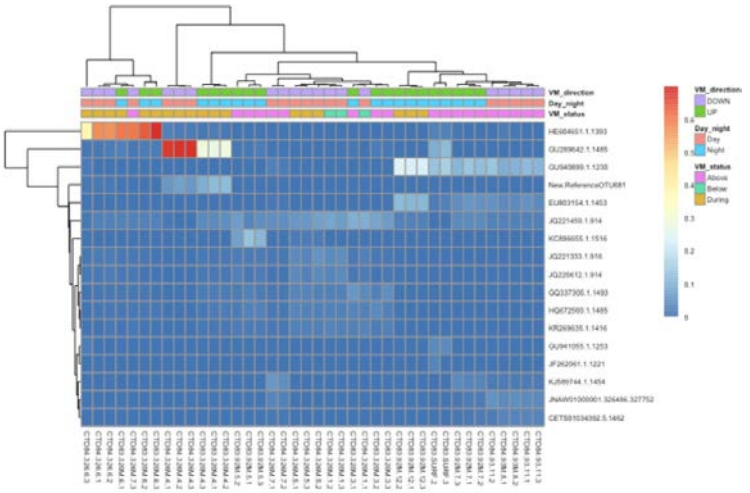

Figure 2. Heat map of abundant OTU's. Generated in R Studio version 3.4.0

The heat map above (Figure 2) shows the relative abundance of taxa with red indicating high and blue low relative abundance. The top three rows indicated vertical migration direction, time, and status, respectively. Two taxa at each depth (epipelagic and mesopelagic) were significantly more abundant in samples taken from within the vertically migrating layer. From the 40,872 different OTUs that were collected, we decided to observe in greater detail some of those OTUs that drove the change in our samples. The selected OTUs were those that were during the Vertical Migration and had greater abundance than the rest.

Table 1. Abundance chart and Taxonomy of OTU's. Generated in R Studio version 3.3.2.

Vertical Migration Status	Pelagic Zone	OTU ID	Taxonomy	Abundance	Depth
During	Epipelagic	GU940899.1.1230	Phylum - Cyanobacteria; Genus - Prochlorococcus	13.40%	92m
During	Epipelagic	EU803154.1.1453	Phylum - Cyanobacteria; Genus - Prochlorococcus	6.30%	92m
During	Mesopelagic	HE604651.1.1393	Phylum - Proteobacteria; Genus - Marinobacter	19.80%	320m
During	Mesopelagic	New.ReferenceOTU681	Phylum - Proteobacteria; Class - Gammaproteobacteria	2.90%	320m
During	Mesopelagic	GU289642.1.1485	Phylum - Proteobacteria; Genus - Alteromonas	14.80%	326m
Above	Mesopelagic	HE604651.1.1393	Phylum - Proteobacteria; Genus - Marinobacter	6.10%	326m

The abundance chart (Table 1) shows an abundance of Proteobacteria in the Mesopelagic zone, and an abundance of Cyanobacteria in the Epipelagic zone.

Conclusions

Diel vertical migration, perhaps the largest movement of animals on Earth is remarkable and well-organized. Every night both prokaryotes and eukaryotes come to mesopelagic and epipelagic depths to feed, and every morning they go back down to deeper depths. “The distinct diel vertical migration of scattering layers is believed to be due to changing ambient light conditions” (D’elia et al, 2016) as well as feeding and predation factors. The approach of extracting DNA from the water samples collected and analyzing the data into taxonomic categories of bacteria highlighted the vertical movement of these organisms throughout the pelagic depths.

We found that for prokaryotes there were a few distinct and abundant bacteria present during this phenomenon. The most abundant bacteria present during the Vertical Migration were OTU ID’s HE604651.1.1393, GU289642.1.1485, GU940899.1.1230, and New.ReferenceOTU681. The depth they were around was 320 to 326 meters, which is the mesopelagic zone. All of the bacteria that were distinct during the vertical migration and in the mesopelagic zone were proteobacteria. These were the most abundant bacteria in this pelagic zone. OTU HE604651.1.1393 was the most abundant bacterium that was found in the mesopelagic zone at 320 meters. Furthermore, the bacteria that were distinct during the vertical migration and in the epipelagic zone were cyanobacteria. These were the most abundant bacteria in this pelagic zone. OTU GU940899.1.1230 was the most abundant bacterium that was found in the epipelagic zone at 92 meters. With this information we can distinguish that these two phyla of bacteria are distinct to their pelagic zones. Proteobacteria is only found during the vertical migration at mesopelagic depths, whereas Cyanobacteria is only found during the vertical migration at epipelagic depths.

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- R package “picante”

Acknowledgements

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Preliminary Antioxidant Activity Analysis of Brazilian Pepper Tree (Schinus terebinthifolius) Extracts via TLC, FRAP, and DPPH

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Graphical Abstract

Fig. 1. Braxilian Pepper Tree: leaves, bark and berries from the Organic Garden at St. Thomas University.

Abstract. In the present study, Schinus Terebinthifolius (Brazilian Pepper Tree) extracts were evaluated for antioxidant activity using free radical scavenging activity, and ferric reducing power. The plant was collected in the organic garden at St. Thomas University and the extracts were prepared by maceration of three parts of the plants, the leaves, the berries, and the bark. The extracts were made using varying proportions of ethanol and hexane solvents. All the samples were analyzed using thin layer chromatography (TLC). Multiple extract samples were submitted to DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to determine the free radical scavenging (FRS) capacity, and absorbance was read at 517 and 520 nm in a plate reader. A control sample was prepared containing the same volume of solvent and DPPH without any extract and reference ascorbic acid. Percent scavenging activity of the DPPH free radical is expressed as an ascorbic acid (AA) equivalent antioxidant capacity (mg AA/100g). A Ferric reducing anti-oxidant power assay (FRAP) was performed and absorbance was measured at 700 nm to quantify the total antioxidant activity. FRAP and DPPH assays indicated that the bark has significantly higher free radical scavenging ability than any other part of the plant.

Introduction

Schinus Terebinthifolius has been widely used in South America in herbal remedies and was reported to have anti-bacterial and antioxidant properties. High levels of antioxidant capacity in plants are believed to decrease oxidative stress and free radicals in the body..

Background research of the Brazilian Pepper Tree indicated that certain parts of the plant exhibited higher antioxidant and anti-bacterial levels than others. In order to compare the antioxidant activity levels, the plant was categorized into leaves, bark, and berries for testing. Also be used to evaluate total phenolic content and concentrations of antioxidants in different parts of the vine.

Materials and Methods

Extractions of Samples

Fresh leaf samples were dried before placing in an oven for 48 hours. Then, were crushed into fine powder using a mortar and pestle. Different sample extracts were prepared, each with 5 grams of the powder in different percent mixtures of 50/50 ethanol to hexane, 75/25 ethanol/hexane and ethanol alone. Each solution was subjected to maceration at room temperature for 24 hours.

Thin Layer Chromatography

Plant extracts were analyzed by TLC (Thin Layer Chromatography) using polar and non-polar solvents, and the spots were developed and visualized with Iodine and UV light. Multiple TLC plates like the one shown in Figure 2 demonstrated a variety of nonpolar as well as slightly polar compounds within the plant extracts. The various colors displayed indicate presence of chlorophyll A B, carotene, and other pigments.

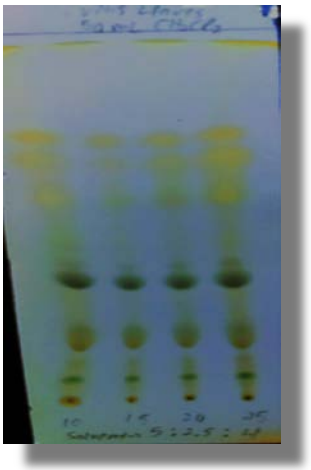


Fig.2. TLC of Muscadine grapes.

Ferric Reducing Power Assay (FRAP)

2.5 mL 0.2 M phosphate buffer, 2.5 mL potassium ferricyanide, 2.5 mL trichloroacetic acid, was added to 2.5 mL of extracts at different concentrations. The mixtures were incubated at 50°C for 20 min, and centrifuged at 3000rpm for 10 min. 2.5 mL of methanol and 0.5 mL of ferric chloride were added before absorbance values were taken at 700 nm.

Total Phenolic Content Assay (TPC)

2 mL of ethanolic extract solutions were mixed with 2.5 mL of 7.5% sodium bicarbonate ($NaHCO_3$), and 2.5 mL of 10% Folin-Ciocalteu reagent. The test tubes were placed in an incubator shaker for 45 min at 45°C before the absorbances were taken at 765 nm in the spectrophotometer.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging (FRS) Assay

This assay was used to determine radical scavenging activity of the extracts. 1mL of different extract concentrations were mixed with 1mL of DPPH. The test tubes were placed in a dark chamber for 30 min at room temperature. 0.2 mL of each solution were added into a well plate and read in a computer programming (Gen-5).

Results and Discussion

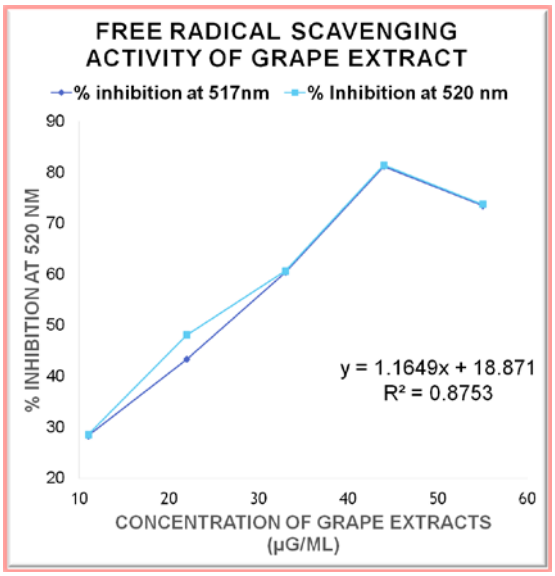
75 EtOH/ 25 Hex leaves Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	3507 ± 1348	0.474 ± 0.177
sample 2	8699 ± 3283	1.566 ± 0.156
sample 3	21355 ± 3804	3.419 ± 0.292
sample 4	33881 ± 3628	5.649 ± 0.401
sample 5	55036 ±4490	10.07 ± 0.911

75 EtOH/25 Hex Roots Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	1747.9± 812.8	0.295 ± 0.0319
sample 2	11366 ± 2203	1.455 ± 0.0337
sample 3	21272 ± 2045	3.524 ± 0.321
sample 4	34992 ± 4200	5.349 ± 0.174
sample 5	51703 ± 5292	9.353 ± 0.641

75 EtOH/25 Hex Grapes Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	252.2 ± 45.86	0.0549 ± 0.017
sample 2	1160 ± 39.29	0.264 ± 0.0533
sample 3	2589 ± 226.3	0.598 ± 0.0402
sample 4	4610 ± 272.2	1.007 ± 0.1044
sample 5	7219 ± 508.8	1.657 ± 0.1475

Table 1-3. Total Phenolic Content (mgGAE/g) and Ferric reducing Power (mgAAE/g)..Mean ± Standard Deviation

Conclusions



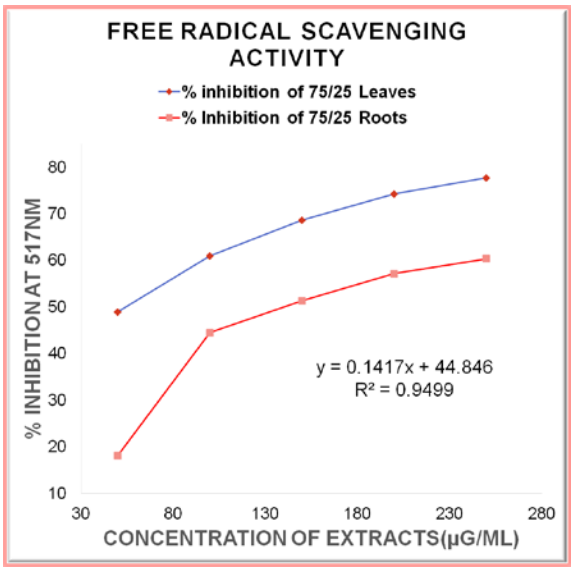


Fig 3. and **Fig 4**. DPPH free radical scavenging Activity (%) Inhibition of grapes, leaves, and roots extracts in 75/25 EtOH/hexane

FRP

According to **Table 1**, Vitis leaves exhibited the highest TPC and FRP of all the parts of the plant tested in this study (TPC= 55036 ±4490 mgGAE/g; FRP= 10.07 ± 0.911 mgAAE/g) . These values correspond to higher concentration of the leaf extracts, varying from 50 to 250µg/mL compare to the grapes with lower concentrations ranging from 10 to 54 µg/mL. Concentrations were very similar among the roots and leaves extracts. Both FRP and TPC measurements were very close in value. Grapes showed the highest value in the TPC with 7219 ± 508.8 mgGAE/g.

DPPH

The antioxidant activities of the extracts in terms of free scavenging activity were expressed as % inhibition ranging from 18 to 81. The graphs show a direct correlation between the concentration of the samples and their scavenging activity. As the concentration increased, the percent inhibition also increased. Results showed up that the 75/25 EtOH/hexane grape extract exhibited the greatest antioxidant activity with a value of 81.27 ± 0.180% compared to the leaves which were 77.80 ± 0.111% and the roots with 60.49 ± 0.204% scavenging activity. This findings indicates that both the Vitis leaves and grapes extracts have high antioxidant activities and they are the most potent sources of antioxidants

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Evaluating medicinal plants for anticancer properties: testing plant extracts for cytotoxicity.

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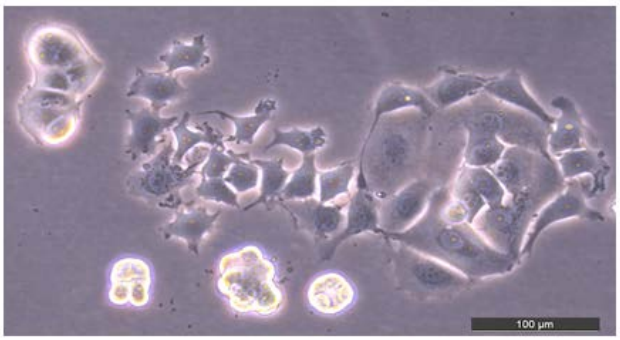
Graphical Abstract



MCF7 breast cancer cells 7 *days in vitro*.

scarlet bush
Hamelia patens

moringa
Moringa oleifera

Abstract

Cancer describes a class of diseases which involve the uncontrolled growth of abnormal cells and the spread or metastasis of those cells to other sites in the body. Natural products derived from plants are valuable sources for anticancer drug discovery. The long term goal of this project is to isolate potential anticancer compounds from medicinal plants using bioassay guided fractionation, a process through which components are purified from an extract by multiple rounds of chemical separation and biological activity tests. Towards this purpose, we commenced our research by performing cytotoxicity assays on chemical extracts obtained from plants with medicinal properties or health benefits. The plants included in this study are commonly known as muscadine, scarlet bush, Brazilian pepper tree, anamú, moringa, guanábana, oyster plant, and Okinawa spinach. Plant extracts, prepared with aqueous and/or organic solvents (including dimethyl sulfoxide, ethanol and hexane), were tested on MCF7 breast cancer cells cultured *in vitro*. Methylthiazol tetrazolium (MTT) assays were used to quantify cytotoxicity. Preliminary data indicated the extracts were not cytotoxic at the concentrations tested. On the contrary, extracts from each type of plant improved cell viability. These data provide valuable dosing information regarding extract concentrations for upcoming experiments, including cell invasion assays, which model metastatic processes, and studies on other human cancer cell lines.

Introduction

Treating cancers requires an integrative approach, utilizing multiple therapies that complement one another. Cancer development and progression has been described as exhibiting the following characteristics: (1) maintained proliferative signaling, (2) evasion of growth suppressors, (3) genomic instability and mutation, (4) replicative immortality, (5) avoidance of cell death, (6) tumor-promoting inflammation, (7) activation of invasion and metastatic pathways, (8) induction of angiogenesis, (9) reprogramming of cellular metabolism, and (10) escape from immune destruction [1]. Plants traditionally used for medicinal purposes contain a rich repository of bioactive compounds, which have the potential to therapeutically target these features of cancer biology [2].

Materials and Methods

MCF7 breast cancer cells (American Type Culture Collection) were plated on 96-well plates at a density of 15,000-20,000 cells per well. Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12, 10% fetal bovine serum, and 1X penicillin/streptomycin.

Plant extracts were prepared from muscadine (*Vitis rotundifolia*), scarlet bush (*Hamelia patens*), Brazilian pepper tree (*Schinus terebinthifolius*), anamú (*Petiveria alliacea*), moringa (*Moringa oleifera*), guanábana (*Annona muricata*), oyster plant (*Tradescantia spathacea*), and Okinawa spinach (*Gynura bicolor*) utilizing aqueous and/or organic solvents [3-10]. Dimethyl sulfoxide (DMSO) was added to extracts at the concentrations listed for the control (Table 1) to improve solubility and cellular internalization. Extracts were then filter-sterilized and diluted in media as described in Table 1. Concentrations varied between extracts because the aim was to maximize extract concentration, not to test uniform extract concentrations.

Cells were first cultured for 48 hours and then treated with plant extracts for 72 hours. Methylthiazol tetrazolium (MTT) assays were conducted to evaluate cytotoxicity. Culture media was replaced with RPMI1640 (without phenol red), 10% fetal bovine serum, and 0.5 mg/mL MTT. Formazan crystals were solubilized in 0.1 N HCl (diluted in isopropanol). Samples were read on a Synergy H1 (BioTek) plate reader set to 570 nm with a 630 nm reference background subtraction.

Table 1: Plant extract concentrations tested

Extract	mg/mL
Control (DMSO)	110 or 154
Anamú Leaves	7.1
Anamú Roots	9.4
Braz. Pepper Bark 75 EtOH/25 Hex	0.1
Braz. Pepper Bark (dried)	1.9
Braz. Pepper Berry (dried)	9.4
Braz. Pepper Berry 100 EtOH	8.8
Braz. Pepper Berry 75 EtOH/25 Hex	2.4
Braz. Pepper Leaves (dried)	9.4
Braz. Pepper Leaves 75 EtOH/25 Hex	3.3
Braz. Pepper Leaves 50 EtOH/50 Hex	20.0
Braz. Pepper Leaves CH2Cl2	0.9
Guanábana Leaves	1.0
Moringa Bark	1.5
Moringa Leaves	1.5
Moringa Seeds	9.3
Muscadine Fruit (dried)	1.4
Muscadine Fruit (whole)	1.4
Muscadine Fruit Outer Shell	39.5
Muscadine Fruit Pulp	28.3
Muscadine Leaves 100 EtOH	13.3
Muscadine Leaves 75 EtOH/25 Hex	10.0
Muscadine Leaves 50 EtOH/50 Hex	7.8
Muscadine Leaves CH2Cl2	3.6
Muscadine Roots 75 EtOH/25 Hex	11.6
Oki. Spinach Leaves Aqueous	9.3
Oyster Plant Leaves 50 EtOH/50 Hex	3.6
Scarlet Bush Leaves	61.7

Each extract was tested using 5-16 replicates in one or two independent experiments. Data from extract-treated cells were compared to that obtained from untreated controls with the same DMSO concentration. Absorbance values were averaged and normalized to controls. Standard deviations of normalized values were calculated. Two-sample, two-tail *t*-tests assuming unequal variances were utilized to calculate *p*-values. In the case of multiple experiments, the largest *p*-value was reported.

Results and Discussion

Figure 1 summarizes preliminary data regarding the effect of plant extracts on MCF7 breast cancer cells. Extracts were not cytotoxic at the concentrations tested. The greater the absorbance, the higher the concentration of formazan, a purple product generated in live cells by mitochondrial succinate dehydrogenase (SDH) enzyme reduction of MTT. Variability in the data, especially evident in the larger error bars observed at higher values, are primarily attributed to the challenge of consistently solubilizing higher formazan concentrations with manual trituration.

Wells treated with extracts from each type of plant had significantly higher SDH activity during the MTT assay than untreated wells (Fig. 1). This activity is proportional to the number of live cells in a well, which is related to cell survival and cellular proliferation. Hence, overall the extracts reduced cell death. These results are not surprising given that extracts from these plants have been reported to have medicinal effects [reviewed in 3-8]. Moreover, recent studies suggest several of the extracts demonstrate antioxidant activity in chemical reactions [3-8]. In general, antioxidants improve cell viability, including that of cancer cells, which tend to exhibit elevated levels of reactive oxygen species due to metabolic and signal transduction aberrations related to tumorigenesis [11]. Antioxidants provided by the plant extracts could reduce electron leakage during mitochondrial respiration and superoxide formation, increasing the number of live cells in treated samples.

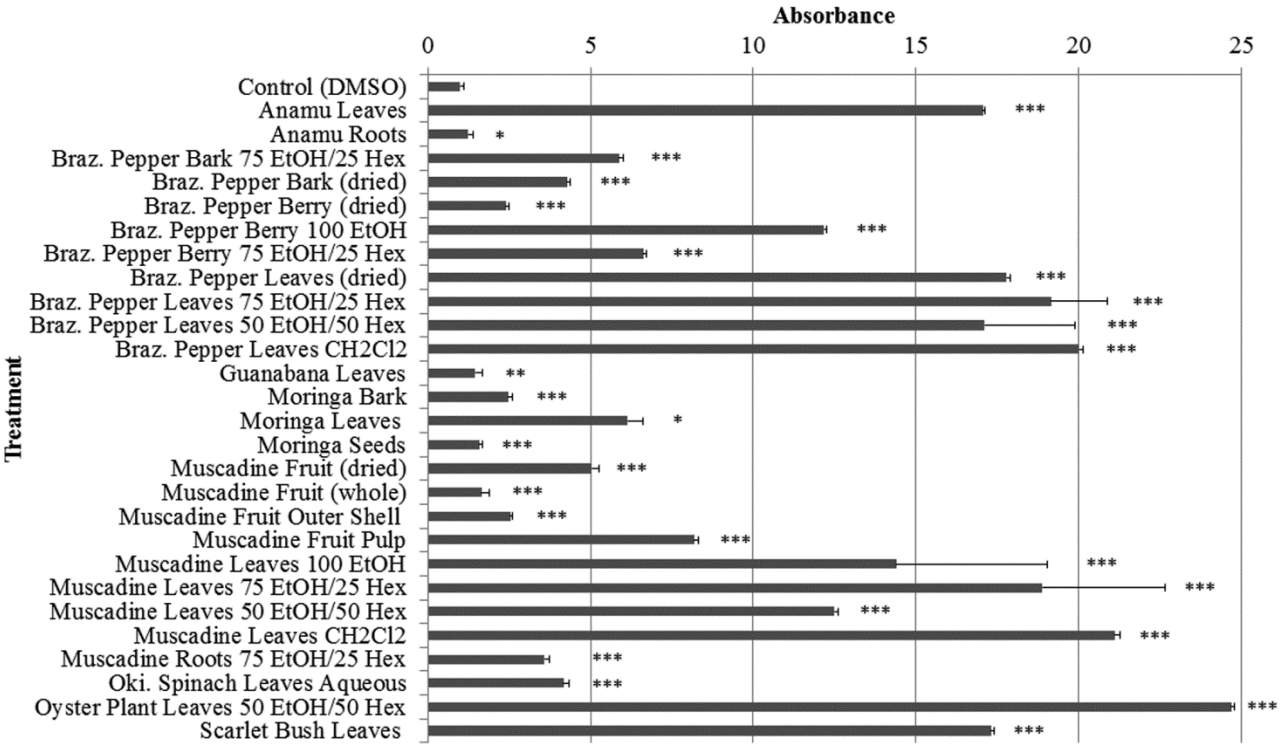


Figure 1: MTT Cytotoxicity Assay. The number of live cells is proportional to absorbance values. Bars indicate the mean absorbance of each treatment normalized to the control. Error bars represent standard deviations. Asterisks correspond to *p*-values: * $p<0.01$, ** $p<0.001$, *** $p<0.0001$.

Conclusions

In summary, plant extracts derived from muscadine, scarlet bush, Brazilian pepper tree, anamú, moringa, guanábana, oyster plant, and Okinawa spinach were not cytotoxic to MCF7 breast cancer cells. These findings are relevant, as they indicate the listed concentrations can be used in other assays to study potential anticancer properties present in the plant extracts. Future experiments will test how the plant extracts affect invasion-associated processes, such as cell migration, cell adhesion, and cell aggregation in breast cancer cells, as well as other cancer cell types.

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Antioxidant Properties of Oyster Plant (Tradescantia Spathacea) Extracts using Different Methods.

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Graphical Abstract

Figure 1. Oyster Plant (*Tradescantia spathacea*) from the Organic Garden at St. Thomas University.

Abstract

Plants are a large source of antioxidant compounds. This project presents the determination of the antioxidant capacity of the Oyster Plant (Tradescantia Spathacea). The samples consisted of ethanol/hexane extracts of the stems, roots, and leaves. The antioxidant activity was measured by three different assays: ferric reducing anti-oxidant power assay (FRAP), DPPH free radical scavenging (FRS) method *in vitro* antioxidant activity and is expressed as ascorbic acid (AA) equivalent antioxidant capacity (mg AA/100g). The total phenolic content (TPC) was determined with the Folin-Ciocalteau reagent and expressed as mg/g gallic acid equivalents (GAE). This study showed that Tradescantia Spathacea extracts contain a number of health promoting bioactive compounds, such as phenolic compounds, and are potential sources of natural antioxidants.

Introduction

Medicinal plants and plant derived products have been part of the health-care system since ancient human civilization. Traditional medicine is widely used, and plants are a large source of antioxidant compounds such as phenols, carotenoids, and flavonoids with potent antioxidant properties that have received much attention recently. The Oyster Plant (Tradescantia Spathacea) is a fleshy or succulent perennial garden herb ornamental plant and is found in many tropical countries. Medicinally, the plant is used for colds, sore throat, whooping cough, nasal bleeding, and is also used as an anti-inflammatory. The plant was grown in the organic garden at St. Thomas University and the ethanol/hexane extracts via maceration of the roots and leaves were analyzed to measure the antioxidant activity by three different assays: Ferric reducing power (FRAP), DPPH free radical scavenging (FRS) and total phenolic content (TPC).

Materials and Methods

Plant components were separated into three parts: leaves, roots, and stems. Extracts were prepared in different solvents: ethanol/hexane 3:1; ethanol/hexane 1:1; and ethanol alone for 24 hours. The wet portion was placed in refrigeration to prevent it from evaporating and receiving any sunlight. Ferric Reducing Antioxidant Power **(FRAP)** Assay**.** 2.5mL 0.2M phosphate buffer, 2.5mL potassium

ferricyanide, 2.5mL trichloroacetic acid, was added to 2.5mL of extracts at different concentrations. The mixtures were incubated at 50°C, centrifuged and added 2.5mL of methanol and 0.5mL of ferric acid. Absorbance values were taken at 700 nm.

2,2-diphenyl-1-picrylhydrazyl (**DPPH**) Free Radical Scavenging **(FRS)** Assay. 1 mL of different concentrated extracts were mixed with 1mL of DPPH. The test tubes were placed in a dark chamber for 30 min and 0.2 mL of each solution were placed into a well plate and read in a computer program (Gen-5).

Total Phenolic Content Assay **(TPC).** 2mL of methanolic extract solutions were mixed with 2.5mL of 7.5% sodium bicarbonate ($NaHCO_3$), and 2.5 mL of 10% Folin-Ciocalteu reagent. The test tubes were placed in an incubator shaker for 45 min at 45°C before the absorbance quantities were taken at 765 nm with a spectrophotometer.

Results and Discussion

The leaves and roots of the Oyster Plant (*Tradescantia spathacea*) were washed with tap water, dried for 2-3 days and made into a fine powder using the laboratory blender. Following that, the powder was extracted with different organic solvents by maceration to prepare the extracts. They were filtered to remove plant insoluble residue, protected from the light, and use immediately in the antioxidant assays procedures.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay:

% Inhibition vs Concentration µg/mL
Expressed as ascorbic acid equivalent antioxidant capacity (mgAAmg/100 mg)

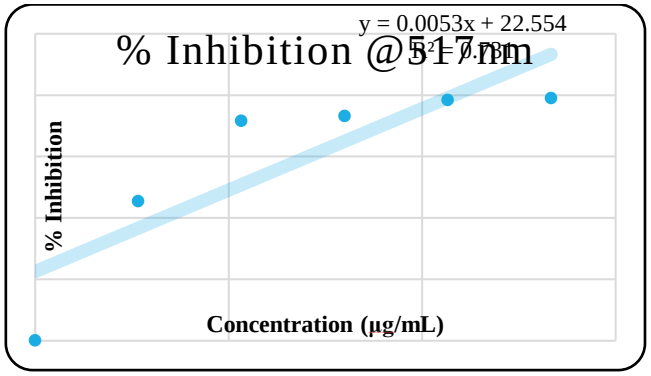

Figure 2. % Inhibition of Roots in 75/25 EtOH/hexane measured at 517 nm

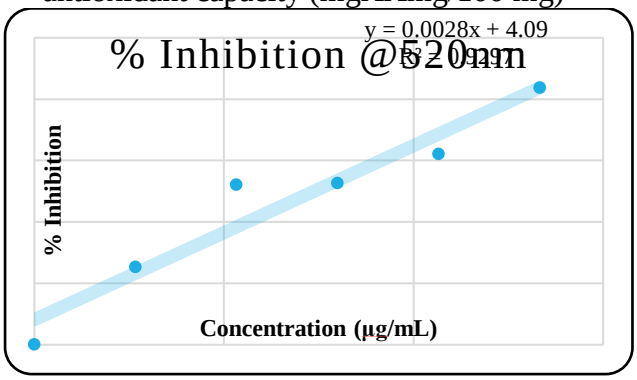

Figure 3. % Inhibiton of Roots in 50/50 EtOH/hexane measured at 517 nm

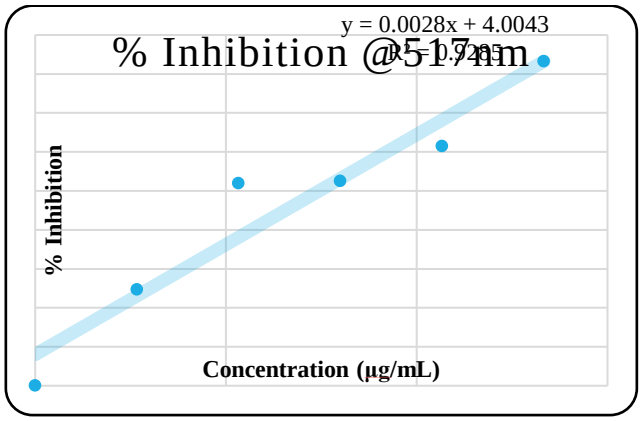

Figure 4. % Inhibition of Leaves in 50/50 Eth/Hex

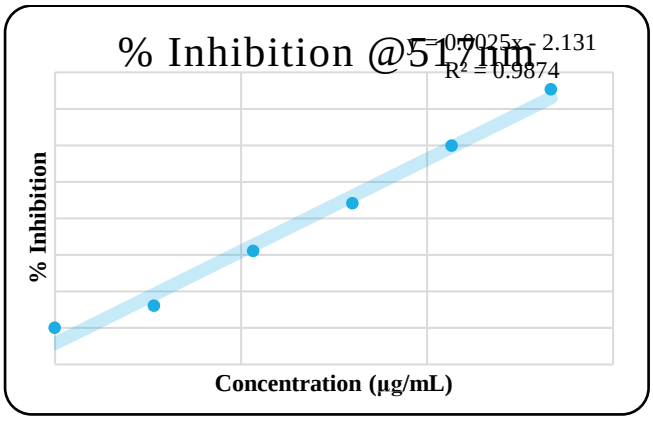

Figure 5. % Inhibition of Leaves in Ethanol

The spectrophotometric method with DPPH free readical scavenging assay applied to antioxidant capacity determination of the root extracts in 75/25 ethanol:hexane mixture generated the highest percent inhibition when we compare with other parts of the plant.

The ferric reducing power assay showed that the oyster plant extract of leaves which was placed in 100% ethanol had the highest antioxidant power. The oyster plant extract of roots which was placed in the mixture of 50:50 ethanol/hexane resulted with the highest antioxidant power.

The total phenolic content assay (**TPC**) exhibited that the roots and leaves of ethananolic/hexane solvent mixtures indicated very high values of antioxidant activity expressed in mg of gallic acid equivalent per gram of vegetal material.

Ferric Reducing Antioxidant Power (FRAP) Assay	
Plant Extract	mgAAE/g
75/25 EtOH/ Hex Leaves	3.37056 ± 0.914208266
50/50 EtOH/ Hex Leaves	2.46198 ± 0.333236353
100 EtOH Leaves	3.47914 ± 0.157170108
75/25 EtOH/ Hex Roots	3.82032 ± 0.752064523
50/50 EtOH/ Hex Roots	3.82252 ± 0.802147245
100 EtOH/ Hex Roots	2.83234 ± 0.701867310

Table 1 . Ferric Reducing Antioxidant Power (FRAP) Assay. Results shown in Mean ± SD

Total Phenolic Content Assay (TPC) Assay	
Plant Extract	mgGAE/g
75/25 EtOH/ Hex Leaves	770.32 ± 91.96666244
50/50 EtOH/ Hex Leaves	555.64 ± 33.02526306
100 EtOH Leaves	707.78 ± 44.09900226
75/25 EtOH/ Hex Roots	879.84 ± 311.7297916
50/50 EtOH/ Hex Roots	163.29 ± 120.6394879
100 EtOH/ Hex Roots	983.80 ± 103.4242960

Table 2. Total Phenolic Content (TPC) Assay. Results shown in Mean ± SD

Conclusions

The increasing interest gained by antioxidants is due to the health benefits provided mainly by natural sources. This consists in preventing the occurrence of oxidative-stress related diseases, as a consequence of the attack of free radicals in different biocomponents in the human body.

Extraction, thin layer chromatography, and various analytical methods using spectrophotometric measurements were employed for determination the antioxidant content and total antioxidant capacity. We report the preliminary study and further analysis will be made taking into account the present results.

The roots contained the highest antioxidant activity of the plant components tested. This could explain the ethno pharmacological applications of the plant. It is possible that most of the medicinal properties that have been observed could come mostly from the root components of the plant.

Further analysis of cytotoxicity and anticancer property evaluation has been started with different concentrations of plant extracts.

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Preliminary Study of the Antioxidant Activity of Vitis rotundifolia (Muscadine Grape) Extracts Using Different Methods.

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Graphical Abstract

Fig. 1. Fresh Vitis leaves, roots and grapes from the Organic Garden at St. Thomas University.

Abstract. Vitis rotundifolia (muscadine grape) is a grapevine species found in humid subtropical climates. Previous researches have reported the bioactivity of the muscadine grapes and our purpose is to look for valuable compounds from plants with medicinal properties or health benefits. The plant used in this research was collected in the organic garden at St. Thomas University. Muscadine plant extracts were analyzed by TLC (Thin Layer Chromatography) using polar and non-polar solvents, and the spots were visualized with Iodine and UV light. The antioxidant activity assays were performed with the extracts of oven and freeze-dried leaves, grapes, and roots. The free radical scavenging activity (FRS) was evaluated with the DPPH reagent, and is reported as ascorbic acid equivalent antioxidant capacity, measured spectrophotometrically at 517 and 520 nm. The estimation of the ferric reducing power (FRP) with potassium ferricyanide was obtained, and the absorbances of the colored complex in the extracts were taken at 700 nm. Total Phenolic Content (TPC) assay of the samples using Folin-Ciocalteu reagent for all parts of the plant showed up the grapes exhibiting the highest antioxidant content compare with the leaves and roots.

Introduction

Some species of Vitis rotundifolia, especially the grapes, are widely used in ancient and modern medicine because of their notable pharmacological effects. Vitis vines are rich of polyphenols, large chemical compounds consisting of hydrocarbon rings bonded to a hydroxyl group. (**Fig. 1**). Polyphenols are the most abundant antioxidants, very essential to the human body. The potential of muscadine grape as a strong natural antioxidant can contribute to the development of treatments for certain diseases. Antioxidants are compounds capable of hindering the process of oxidation caused by reactive oxygen species and raiding free radicals. Thus, they prevent deterioration of diseases cause by oxidative stress in an organism, cancer being an example. This study will primarily aim at quantifying antioxidant activity of the leaves, grapes, and roots of the Muscadine grapes using the 1,1-diphenyl—1-picrylhydrazyl (DPPH) radical scavenging method. In addition, spectrophotometric methods will also be used to evaluate total phenolic content and concentrations of antioxidants in different parts of the vine.

Materials and Methods

Extractions of Samples

Fresh leaf samples were dried before placing in an oven for 48 hours. Then, were crushed into fine powder using a mortar and pestle. Different sample extracts were prepared, each with 5 grams of the powder in different percent mixtures of 50/50 ethanol to hexane, 75/25 ethanol/hexane and ethanol alone. Each solution was subjected to maceration at room temperature for 24 hours.

Thin Layer Chromatography

Plant extracts were analyzed by TLC (Thin Layer Chromatography) using polar and non-polar solvents, and the spots were developed and visualized with Iodine and UV light. Multiple TLC plates like the one shown in Figure 2 demonstrated a variety of nonpolar as well as slightly polar compounds within the plant extracts. The various colors displayed indicate presence of chlorophyll A B, carotene, and other pigments.

Fig. 2: TLC of Muscadine grapes.

Ferric Reducing Power Assay (FRAP)

2.5 mL 0.2 M phosphate buffer, 2.5 mL potassium ferricyanide, 2.5 mL trichloroacetic acid, was added to 2.5 mL of extracts at different concentrations. The mixtures were incubated at 50°C for 20 min, and centrifuged at 3000rpm for 10 min. 2.5 mL of methanol and 0.5 mL of ferric chloride were added before absorbance values were taken at 700 nm.

Total Phenolic Content Assay (TPC)

2 mL of ethanolic extract solutions were mixed with 2.5 mL of 7.5% sodium bicarbonate ($NaHCO_3$), and 2.5 mL of 10% Folin-Ciocalteu reagent. The test tubes were placed in an incubator shaker for 45 min at 45°C before the absorbances were taken at 765 nm in the spectrophotometer.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging (FRS) Assay

This assay was used to determine radical scavenging activity of the extracts. 1mL of different extract concentrations were mixed with 1mL of DPPH. The test tubes were placed in a dark chamber for 30 min at room temperature. 0.2 mL of each solution were added into a well plate and read in a computer programming (Gen-5).

Results and Discussion

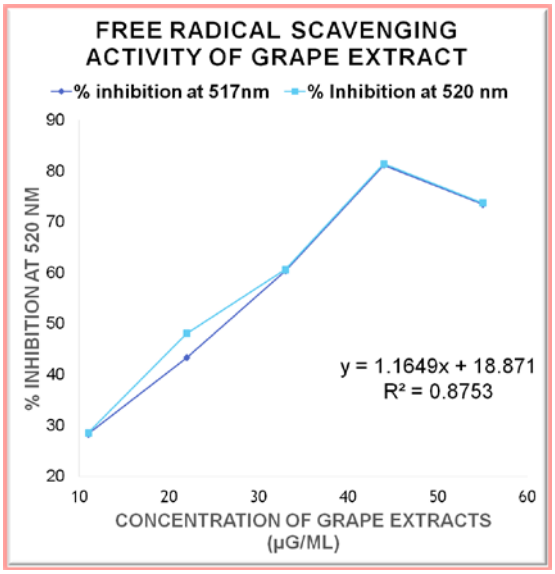
75 EtOH/ 25 Hex leaves Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	3507 ± 1348	0.474 ± 0.177
sample 2	8699 ± 3283	1.566 ± 0.156
sample 3	21355 ± 3804	3.419 ± 0.292
sample 4	33881 ± 3628	5.649 ± 0.401
sample 5	55036 ±4490	10.07 ± 0.911

75 EtOH/25 Hex Roots Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	1747.9± 812.8	0.295 ± 0.0319
sample 2	11366 ± 2203	1.455 ± 0.0337
sample 3	21272 ± 2045	3.524 ± 0.321
sample 4	34992 ± 4200	5.349 ± 0.174
sample 5	51703 ± 5292	9.353 ± 0.641

75 EtOH/25 Hex Grapes Extract	Total Phenolic Content	FRP Total Antioxidant
sample 1	252.2 ± 45.86	0.0549 ± 0.017
sample 2	1160 ± 39.29	0.264 ± 0.0533
sample 3	2589 ± 226.3	0.598 ± 0.0402
sample 4	4610 ± 272.2	1.007 ± 0.1044
sample 5	7219 ± 508.8	1.657 ± 0.1475

Table 1-3. Total Phenolic Content (mgGAE/g) and Ferric reducing Power (mgAAE/g)..Mean ± Standard Deviation

Conclusions



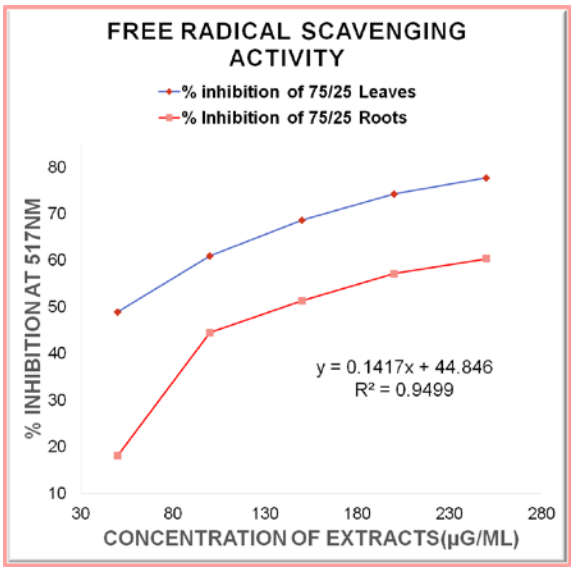


Fig 3. and **Fig 4**. DPPH free radical scavenging Activity (%) Inhibition of grapes, leaves, and roots extracts in 75/25 EtOH/hexane

FRP

According to **Table 1**, Vitis leaves exhibited the highest TPC and FRP of all the parts of the plant tested in this study (TPC= 55036 ±4490 mgGAE/g; FRP= 10.07 ± 0.911 mgAAE/g) . These values correspond to higher concentration of the leaf extracts, varying from 50 to 250µg/mL compare to the grapes with lower concentrations ranging from 10 to 54 µg/mL. Concentrations were very similar among the roots and leaves extracts. Both FRP and TPC measurements were very close in value. Grapes showed the highest value in the TPC with 7219 ± 508.8 mgGAE/g.

DPPH

The antioxidant activities of the extracts in terms of free scavenging activity were expressed as % inhibition ranging from 18 to 81. The graphs show a direct correlation between the concentration of the samples and their scavenging activity. As the concentration increased, the percent inhibition also increased. Results showed up that the 75/25 EtOH/hexane grape extract exhibited the greatest antioxidant activity with a value of 81.27 ± 0.180% compared to the leaves which were 77.80 ± 0.111% and the roots with 60.49 ± 0.204% scavenging activity. This findings indicates that both the Vitis leaves and grapes extracts have high antioxidant activities and they are the most potent sources of antioxidants

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Differential Expression of Native Potatoes Genes in Response to Drought Conditions

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Graphical Abstract

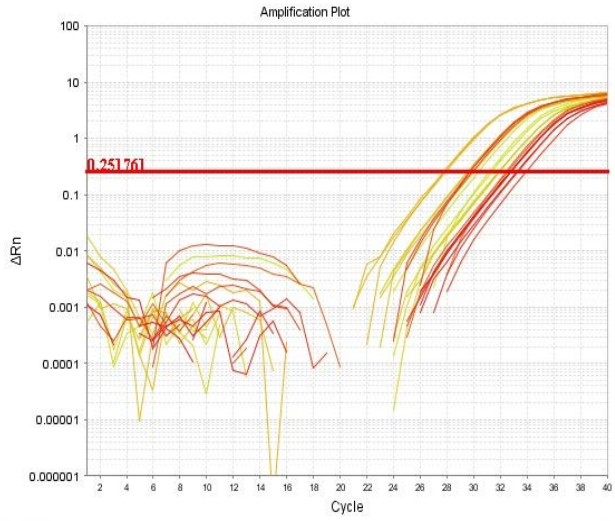

Figure 3. RT-PCR amplification plots representing gene expression for a fatty acid desaturase after drought treatments in native potato plants (drought-tolerant represented in red and drought-susceptible plants represented in yellow.

Abstract.

The exposure of plants to drought conditions increases production of toxic reactive oxygen species (ROS), and negatively affects photosynthesis and carbohydrate metabolism. Few studies of drought tolerance in potatoes have been based on native potato species. Because of their high genetic diversity, native potatoes from the Andean regions of Peru, Ecuador and Bolivia, are well adapted to the harsh environmental conditions that prevail in the Andes, including drought. This makes them ideal candidates for gene expression studies associated with drought tolerance. The identification of drought tolerance traits and genes for potato will facilitate breeding for high yield stability under drought conditions. A drought experiment with both tolerant and susceptible native potato species, using an aeroponics growth system was conducted. Selected drought-associated candidate genes from RNA sequence analysis were used in primer design and quantitative RT-PCR analysis. Differential gene expression in tolerant vs. susceptible cultivars was confirmed for chloroplastic heat shock protein 1, for triacyl glycerol lipase, and for a fatty acid desaturase.

Introduction

Potato, the fourth most important crop in the world, is generally considered to be drought-sensitive. Drought susceptibility in potato impacts all stages of the crop, from emergence to tuber initiation (Evers *et al.*2010). Although there have been various studies of the global changes in gene expression profiles during drought conditions in potatoes, few studies have been based on native potato species. Because of their high genetic diversity, native potatoes from the Andean regions of Peru, Ecuador and Bolivia, cultivated at altitudes as high as 11,480 ft. (3500m) above sea level, are well adapted to the harsh environmental conditions that prevail in the Andes, including drought. This makes them ideal candidates for gene expression studies associated with drought tolerance. In a previous

phase of this study conducted at the Universidad Peruana Cayetano Heredia (UPCH), RNAseq of both a drought-tolerant- and a drought- resistant native potato species was used to identify a large set of drought-tolerant genes. The purpose of this study was to confirm differential gene expression in drought-associated candidate genes obtained from RNAseq data during drought response and recovery.

Materials and Methods

Plant Material.- *Solanum tuberosum* ssp.*andigena,* Negrita 703 671 (drought-tolerant) and Wila - HuakaLajra 703 248 (susceptible to drought) plants were used in the aeroponics drought experiment. Previously propagated in vitro, rooted and transferred to an aeroponics growing system at the INIA Experimental Station in Huancayo, Peru, they received misted water on the roots every 15 minutes, for 10 continuous minutes. After both varieties were adapted to the aeroponics system, a drought experiment was conducted. The four treatments consisted on, control, early, late response and recovery (Table 1). Following the treatments, three plants were randomly selected for each of the four groups in the two varieties. Three leaves were collected from each of the selected plants and immediately frozen in liquid nitrogen inside 50 mL tubes.

Table 1.Drought treatments on native potato plants. Plants were grown on an aeroponics system in which they were normally watered by misting the roots with water every 15 min for 10 continuous minutes.

Drought Treatment:	Abbrev.	Water Deprivation:
Control	T0	None
Early Response	T1	40 min
Late Response	T2	120 min
Recovery	T3	120 min followed by 20 min of mist in the roots

RNA Extraction.- After RNA was extracted from native potato varieties (tolerant and susceptible) using the TRIzol modified protocol, they were DNased using the DNA free kit (Ambion).

Primers for Drought-Associated Candidate Genes.- Genes were selected from the RNA sequencing data showing differential expression in drought-tolerant vs. susceptible varieties. Primer Express 3.0 software (Applied Biosystems) was used to design the primers. Glyceraldehyde 3-phosphate dehydrogenase (GADPH) gene were included as the internal control for gene expression experiments.

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR).- For gene expression analysis, two-step RT-PCR was used. RNA was first reverse transcribed using the ThermoScript RT-PCR System (Invitrogen). The Fast 2X SYBR Green Master Mix (Applied Biosystems) together with the Step-One Plus Fast Real-Time PCR System (Applied Biosystems) was used for qPCR analysis. Primer efficiency was determined with the software included with the instrument. The gene expression analysis was done using the Pfaffl method.

Results and Discussion

Chloroplastic small heat shock protein was affected by the drought treatments in both susceptible and resistant varieties, with a drop in transcript abundance during late drought as well as after recovery (Table 2). This drop was more pronounced in the susceptible variety, perhaps indicating that the resistant variety was more capable of expressing this gene at normal levels even under the stressful drought conditions. Heat shock proteins are chaperone molecules that stabilize structures of proteins by preventing denaturation during stress (Evers *et al.*, 2010). Triacylglycerol lipase is almost unchanged in transcript level in the susceptible variety but shows a decrease during the drought recovery period in the resistant variety. Exposure to environmental stress leads to the generation or

reactive oxygen species (ROS). ROS cause lipid peroxidation with the subsequent destruction of membrane lipids affecting cell viability. Triacylglycerol lipase has a role in mobilization of triacylglycerol, lipid signaling and membrane degradation (Eastmond, 2006). While the susceptible variety showed a drop in transcript levels during late drought for the fatty acid desaturase gene, the resistant variety showed up to a two-fold increase during drought late response. This generated a 3.2 fold difference between both varieties pointing to a potential association of this gene to drought tolerance. Fatty acid desaturases regulate the overall level of fatty acids in plants and have been found associated with defense signaling pathways (Kachroo *et al*. 2001).

Table 2. Gene Expression Analysis of selected candidate genes. The Pflaff method was used to estimate fold difference between samples following qRT-PCR analysis. Data was normalized using GAPDH as housekeeping gene. Left: Chloroplastic heat shock protein 1; Center: Triacylglycerol lipase; Right: Fatty acid desaturase.

Treatment	Susceptible	Resistant	Resistant vs. Susceptible
Time 0	1.0	1.0	-
Time 1	1.1	1.0	-
Time 2	0.4	0.7	1.8
Time 3	0.2	0.5	2.5

Treatment	Susceptible	Resistant	Resistant vs. Susceptible
Time 0	1.0	1.0	-
Time 1	1.3	1.1	0.8
Time 2	1.0	0.9	0.9
Time 3	1.0	0.6	0.6

Treatment	Susceptible	Resistant	Resistant vs. Susceptible
Time 0	1.0	1.0	-
Time 1	1.1	1.4	1.3
Time 2	0.6	2.0	3.2
Time 3	1.0	1.7	1.7

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Acknowledgements

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Twitter Data Mining and Predictive Modeling in R

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Graphical Abstract

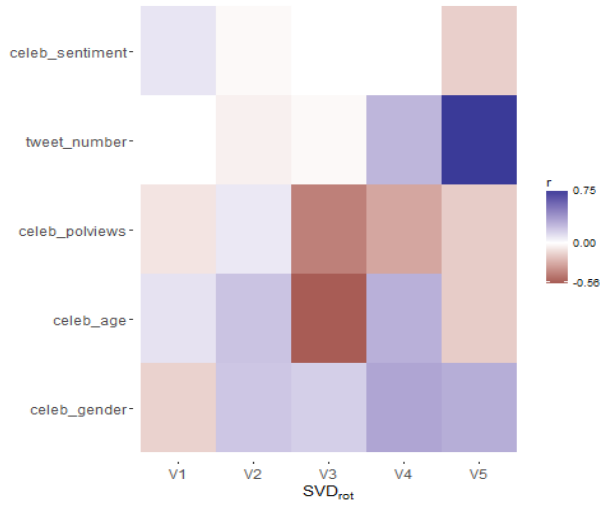

Fig. 2: Correlation of Singular Value Decomposition (SVD) dimensions and psych demographic traits

Keywords: *social media, sentiment analysis, Twitter data mining.*

Abstract.

The open source statistical programming language R can be used to gather information from the social media platform Twitter, to collect tweets from various news sources, celebrities, political figures, official colleges accounts, among others. Information such as screen names, number of tweets, number of followers, list of friends, and locations can be collected using the twitteR package in combination with the Twitter application programming interface (Twitter API). With such data, one can perform text mining by counting the word frequency in news sources' tweets, creating data visualizations to represent frequency of words, and conduct a sentiment analysis to understand and measure the impact of certain topics and opinions expressed in this social media venue. This project explores the various ways that Twitter can be used to gather information on certain topics and how this data could be used to help predict some of the behaviors and characteristics on how people communicate through this social media outlet.

Introduction

We used similar methods presented by M. Kosinski et. al [1] in their work on mining big data (from Facebook users) to predict real-life outcomes, performing a data analysis of Twitter data using the open source statistical programming language R [2]. Some of the mathematical techniques used in this work include Singular Value Decomposition (SVD), and Logistic Regression models. A training matrix was generated gathering different data from celebrities including the number of people they follow, their age, gender, political party, and number of tweets. The data was retrieved using the Twitter API and the twitteR package [3] during July 2017.

Results and Discussion

We created a heatmap showing the correlation between the SVD dimensions and the psychological demographic traits of each celebrity in the dataset we created. We focused on $k = 5$ SVD dimensions. In Fig. 2 we can notice that the SVD dimension V5 correlates positively with the number of tweets and gender while it has a negative correlation with the sentiment, political views, and age.

	Name	Gender	Age	Pol. View	Tweet Num.
[1,]	"Adam Sandler"	"M"	"50"	"R"	"180"
[2,]	"Ben Affleck"	"M"	"44"	"D"	"395"
[3,]	"Beyonce"	"F"	"35"	"D"	"10"
[4,]	"Blake Lively"	"F"	"29"	"D"	"25"
[5,]	"Chris Hemsworth"	"M"	"33"	"R"	"170"
[6,]	"Christina Aguilera"	"F"	"36"	"D"	"979"
[7,]	"Cristiano Ronaldo"	"M"	"32"	"D"	"2916"
[8,]	"Hugh Jackman"	"M"	"48"	"D"	"2942"
[9,]	"Jason Mraz"	"M"	"40"	"D"	"3460"
[10,]	"Karim Benzema"	"M"	"29"	"D"	"1156"
[11,]	"Kourtney Kardashian"	"F"	"38"	"D"	"12200"
[12,]	"Liam Hemsworth"	"M"	"27"	"D"	"149"
[13,]	"Luis Suarez"	"M"	"30"	"R"	"838"
[14,]	"Madonna"	"F"	"58"	"D"	"2452"
[15,]	"Mariah Carey"	"F"	"47"	"D"	"7365"
[16,]	"Mark Wahlberg"	"M"	"46"	"D"	"1030"
[17,]	"Michelle Obama"	"F"	"53"	"D"	"798"
[18,]	"Sandra Oh"	"F"	"45"	"D"	"535"
[19,]	"Sofia Vergara"	"F"	"44"	"D"	"6819"
[20,]	"Sylvester Stallone"	"M"	"70"	"R"	"1251"
[21,]	"Tom Hanks"	"M"	"60"	"D"	"799"
[22,]	"Vicoria Beckham"	"F"	"43"	"R"	"3156"
[23,]	"Zac Efron"	"M"	"29"	"D"	"1585"

Fig. 1: Sample of celebrities' data matrix and their Twitter accounts (July 2017)

Programming was essential in the development of this project. R is an open source language widely used in the data science community, with focus on statistical data analysis, data visualization and machine learning methods. During this project, tools from the tidyverse package [4] were used for data wrangling and data visualization with the help of RStudio, an open source integrated development environment (IDE) for R. Sentiment analysis can be thought of as the exercise of taking a sentence, paragraph, document, or any piece of natural language, and determining whether that text's emotional tone is *positive*, *negative* or *neutral*. Using the Twitter API, we collected information on the Twitter accounts of celebrities following less than 100 accounts (as of July 2017), and performed sentiment analysis on the messages they posted on this social media outlet. Fig. 3 shows the distribution of sentiment scores from different accounts.

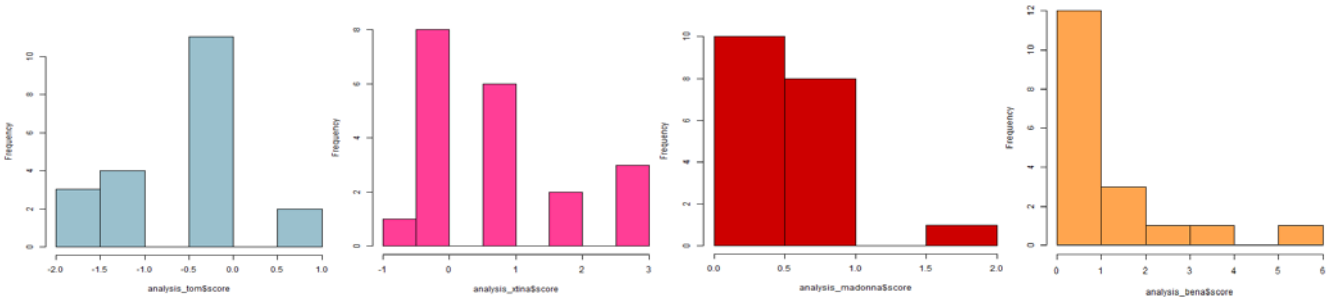

Fig. 3: Sentiment score for tweets by Tom Hanks, Christina Aguilera, Madonna, and Ben Affleck (as of July 2017)

Using the twitteR package in R, we gathered information of users who follow the official Twitter account of St Thomas University (https://twitter.com/StThomasUniv). Using the geolocation information associated to each user, longitude and latitude coordinates can be easily mapped, to have a measurement of the diversity of followers of any account. In Fig. 4 we show the locations of the @StThomasUniv followers as an example, both in North America and Worldwide.

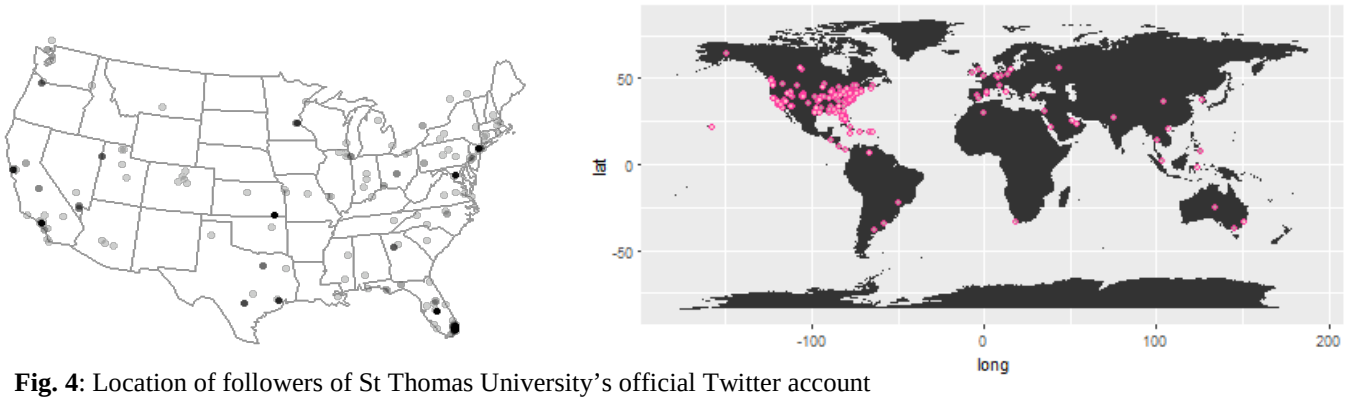

Fig. 4: Location of followers of St Thomas University's official Twitter account

Conclusions

The conjunction of R for data analysis and different APIs makes for a powerful tool for data mining and visualization of social media outlets data like Twitter.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

Authors want to thank St. Thomas University facilities for completing this work during the SRI 2017. This project was supported, in part, by U.S. Department of Education grant award P03C1160161 (STEM SPACE), P031c160143 (STEM EngInE), P120A160036 (STEM ISLE), 1161177 (STEP Up), P120A140012 (SPARC).

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Creating a Model to Predict Student Success Using WeBWorK Data

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Graphical Abstract

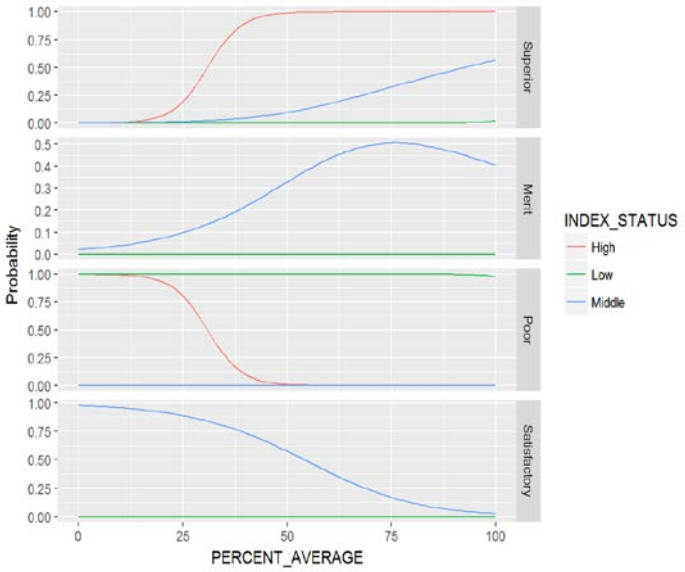

Fig. 3: Multinomial Logistic Regression model for a Calculus I course.

Abstract.

Student success is a major focus in the educational system, where a variety of predictors are used to estimate and measure how well students do in their different classes at the end of the academic year. Our research project aims towards proposing a model capable of demonstrating how student success can be predicted based on a series of indicators gathered from work submitted by the student throughout the semester. We studied the student's performance in an online homework assignment system for a mathematics course, taking into account the final score in a given assignment, but also the number of times every problem was tried by the student before obtaining a correct answer.

Keywords: *WeBWorK, data mining, logistic regression, predictive analytics.*

Introduction

For some of the mathematics courses at St. Thomas University, instructors use the open-source online homework system WeBWorK [1]. WeBWorK is supported by the Mathematical Association of America (MAA) and the National Science Foundation (NSF), and it provides students with a system that lets them work at their own pace and helps reinforce the different topics covered in a given course.

Results and Discussion

WeBWorK stores information of the scores for each assignment, as well as a variable called "*success index*", an indicator of the number of attempts made by the student to complete the assignment. This information was used to create our data sets, run a clustering analysis and differentiate between students that are very efficient in the assignments from those who need more trials before completing a given homework set. Data from one Pre-Calculus and two Calculus I courses at St Thomas University was anonymized and the open source statistical programming language R [2] was used for the data analysis. The main indicators used were labeled the "Homework Percent", which represents the score obtained by a student in a given assignment, and the "Homework Index", recorded by WeBWorK to measure the success indicator for the corresponding problem set. We used all the records from the Calculus course

and created a graph to help us visualize the different groups in the students. As observed in Fig. 2 we identified four groups, using a *K*-means algorithm in R: Students with high index and percent, students with average scores, students with low index but a high percent and students with low index and low percent.

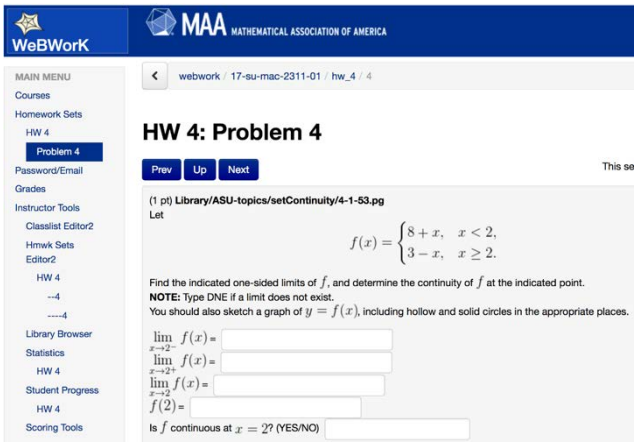

Fig. 1: Sample calculus assignment using WeBWorK

After all the data was successfully processed and having identified patterns, we created a mathematical model to interpret this analysis, grouping the grades into 4 categories: *Superior*, *Merit*, *Satisfactory*, and *Poor*. We divided the average of the index for every student into the categories of *High*, *Middle*, and *Low*. Using this information, we created a multinomial logistic regression model, capable of calculating the probability of a student to succeed on the course based on the average homework percent and the average homework index. The four different categories created can be then translated into an estimated final grade in the course. The method studied in this project showed high accuracy in the predictions and allowed for an easy interpretation of the model outputs as shown in Fig. 3.

Conclusions

In this research project we designed a model capable of predicting a student's probability of success in a given course, based on student's work submitted throughout the semester in the form of online homework assignments. The model takes into account the student's scores in all assignments during a semester, as well as the student's "success index" per assignment, a fairly good indicator of how well the student is grasping the concepts evaluated in every assignment.

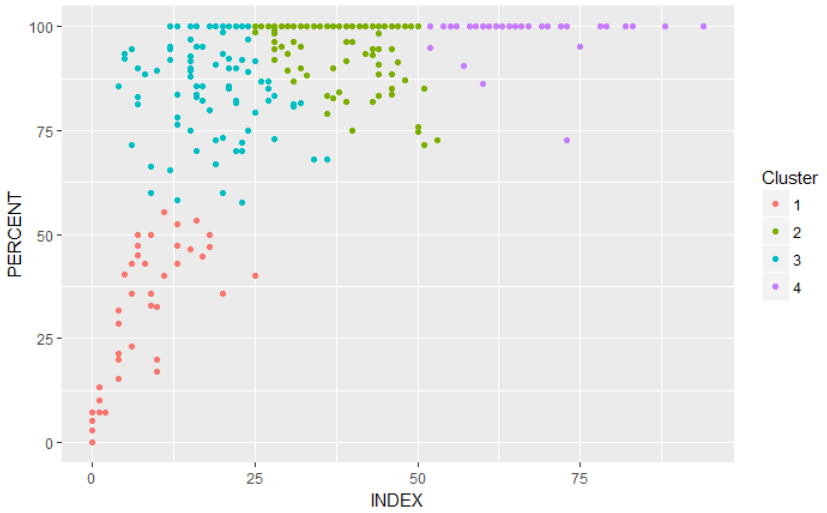

Fig. 2: K-means clustering of the different work submitted by students in a Calculus I course.

The model can be extended to include other predictors, and additional observations could be easily added to our framework for an even more robust model and prediction accuracy.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

Authors want to thank St. Thomas University facilities for completing this work during the SRI 2017. This project was supported, in part, by U.S. Department of Education grant award P03C1160161 (STEM SPACE), P031c160143 (STEM EngInE), P120A160036 (STEM ISLE), 1161177 (STEP Up), P120A140012 (SPARC).

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Analysis of the Gentrification Process in the City of Miami using Publicly Available Data

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Graphical Abstract

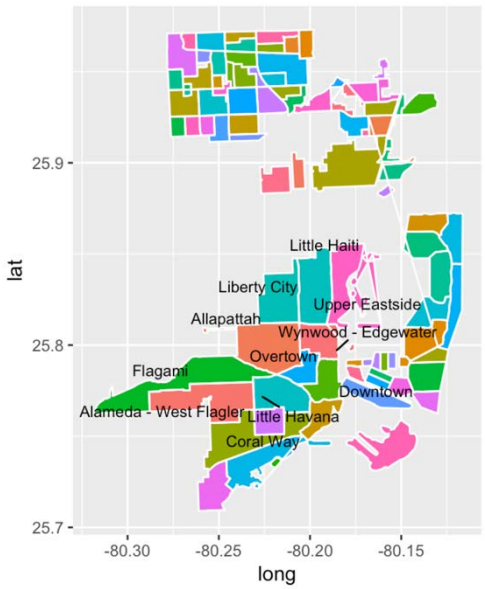

Fig. 1: Neighborhoods in the City of Miami that were considered in this study (labeled).

Keywords: *gentrification, data mining, sentiment analysis.*

Abstract.
Gentrification is a process involving the movement of a high-income group to working-class neighborhoods nearby amenities, and that usually results in the displacement of the original inhabitants. Changes like alteration of local services, increasing prices, and unaffordable housing are derived from the process, and the negative repercussions it might have in our community, grant great significance to its forecasting. Our goal for this project was to offer a comprehensive analysis of the main indicators of the gentrification process in Miami. The data that was collected and analyzed, along with the different patterns found for the indicators considered in this study, will help in the development of a forecasting model for gentrification of the neighborhoods in the City of Miami.

Introduction

Using data publicly available for the City of Miami, an analysis of the gentrification process was performed in a subset of neighborhoods in the city, taking into account several indicators that could be used as predictors in a gentrification forecasting model. By finding significant patterns of the main indicators of gentrification in the different communities, local governments could intervene in an effort to balance development and tradition. Movement of a high-income group to a working-class neighborhood might also be encouraged by government policies in the form of tax incentives, local economic developments (public transportation, malls, stadiums, amenities), programs to rehabilitate public housing, among others.

Results and Discussion

In order to analyze the impact of gentrification in the City of Miami, three of the main indicators were chosen: local tax rates [1], records of building permits [1], and housing prices [2]. The datasets conveying such information were filtered and analyzed using R [3], an open source statistical

programming language widely used in the data science community. Furthermore, the public opinion of each of the neighborhoods was obtained from social media, specifically, from Twitter. For this endeavor, sentiment analysis was implemented. Data analysis was performed using R, an open source language widely used in the data science community, with focus on statistical data analysis, data visualization and machine learning methods. Tools from the "tidyverse" package [4] were used for data wrangling and data visualization with the help of RStudio, an open source integrated development environment (IDE) for R. For example, using data published by Zillow [2], an online real estate database company that has data on 110 million home across the United States, we compared the median home value in Fig. 2, that shows the different trends from 2011 to 2016.

Sentiment analysis can be thought of as the exercise of taking a sentence, paragraph, document, or any piece of natural language, and determining whether that text's emotional tone is *positive*, *negative* or *neutral*. Using the Twitter API, we collected a set of tweets during the month of July, in which any mention to the neighborhoods being analyzed in this study was made. The data was transformed, arranged, and analyzed in R, and a sentiment analysis was performed to compare the different public opinions via social media. In Fig. 3 we show a snippet of the code used for performing sentiment analysis, and Fig. 4 shows the sentiment score for the different neighborhoods. Similar approaches could be used to extend a gentrification model and social media will continue to be used as a strong indicator of the public opinion in future studies.

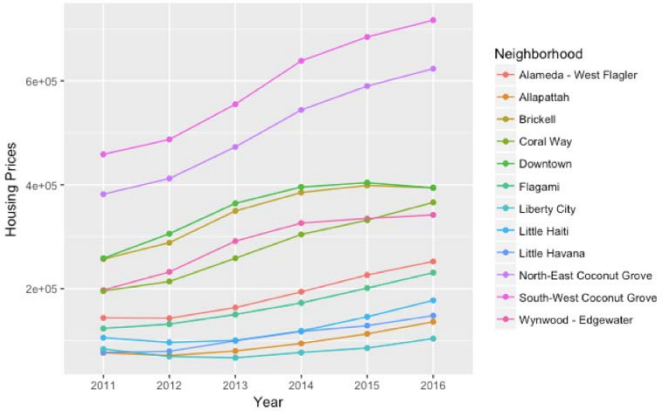

Fig. 2: Median Housing Prices in Miami's neighborhoods

```r
score.sentiment = function(tweets, pos.words, neg.words){
require(plyr)
require(stringr)
scores = laply(tweets, function(tweet, pos.words, neg.words) {
tweet = gsub('https://','',tweet)
tweet = gsub('http://','',tweet)
tweet=gsub('[^[:graph:]]', ' ',tweet)
tweet = gsub('[[:punct:]]', '', tweet)
tweet = gsub('[[:cntrl:]]', '', tweet)
tweet = gsub('\\d+', '', tweet) # removes numbers
tweet=str_replace_all(tweet,"[^[:graph:]]", " ")
tweet = tolower(tweet)
word.list = str_split(tweet, '\\s+')
words = unlist(word.list)
pos.matches = match(words, pos.words)
neg.matches = match(words, neg.words)
pos.matches = !is.na(pos.matches)
neg.matches = !is.na(neg.matches)
score = sum(pos.matches) - sum(neg.matches)
return(score)
}, pos.words, neg.words )
scores.df = data.frame(score=scores, text=tweets)
return(scores.df)
}
```

Fig. 3: Sample R script to compute the "sentiment" of each tweet mentioning the neighborhoods that were part of this study.

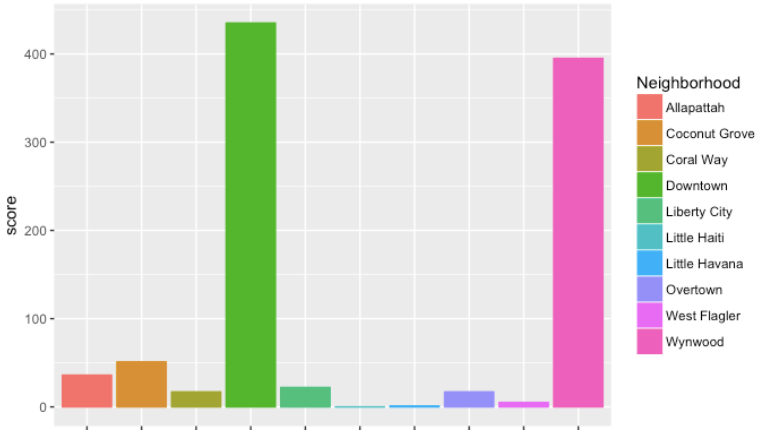

Fig. 4: Sentiment Analysis. Public opinion of each neighborhood from Twitter during July 2017.

Conclusions

Using open access datasets and data mining techniques, results from our analysis confirm the high development standards of the Downtown, Wynwood, Coconut Grove, and Brickell neighborhoods. Further research including data on crime rates, demographics, tax incentives tools, public transportation and other indicators, will help to build a forecasting model of gentrification.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

Authors want to thank St. Thomas University facilities for completing this work during the SRI 2017. This project was supported, in part, by U.S. Department of Education grant award P03C1160161 (STEM

SPACE), P031c160143 (STEM EngInE), P120A160036 (STEM ISLE), 1161177 (STEP Up), P120A140012 (SPARC).

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Biomedical modeling of Magnetic Nanoparticles Fluid Hyperthermia for Cancer treatment

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Graphical Abstract

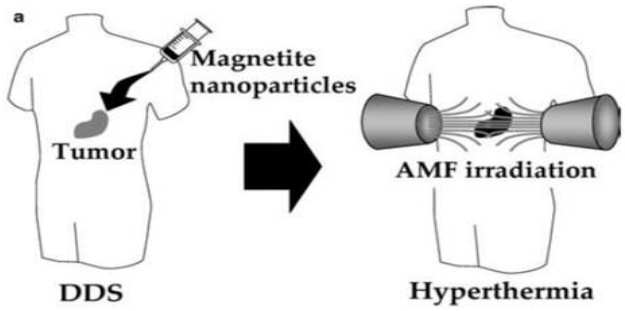

Fig 2: MNP based on polymer-coated magnetite have been used in magnetic fluid hyperthermia (MFH): A nanofluid containing the MNP is injected directly into the tumor or is injected to the tumor vasculature.

Abstract.

Magnetic Fluid Hyperthermia is called to be a promising method for cancer lesions, constituting an alternative pathway to other medical approaches. Despite of these promising possibilities, a critical problem of hyperthermia is the direct control of the heat source and the distribution of MNP in order to induce necrosis within cancerous cells with the minimum negative impact to the surrounding healthy cells. In the current project, the biomedical modeling of the process of hyperthermia is carried on for cancer cells of different geometries appealing to the modified Penne's bioheat equation and the Finite Element Method (FEM). Special attention was paid to the size and spatial distribution of nanoparticles. The results from numerical solutions have permitted to establish guidance towards optimal conditions for its use.

1. Introduction

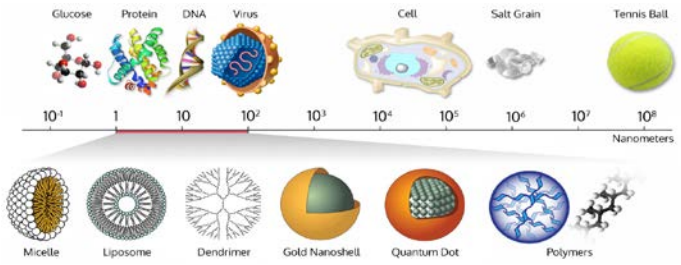

Fig 1: Nanoparticles are particles with sizes from fractions to hundreds of nanometers. They have high reactivity and a large surface to volume ratio.

Nanoparticles are particles with dimensions of the order of one nanometer (10^{-9} m) to few hundreds of nanometers. These dimensions are comparable to those observed among entities studied by molecular biology (see Fig. 1).

Among nanoparticles, Magnetic Nanoparticles (MNP) have received a considerable attention because of their potential applications in medicine, as bactericides, drug carriers, and agents for non-invasive localized therapies [1]. Cancer treatment using MNP is considered a promising therapy based on the thermal ablation of cancerous cells using the heat generated by these particles when they are placed in Alternating Magnetic Fields (AMF) (see Fig. 2) [2]. Despite of these promising possibilities, a critical problem of hyperthermia is the direct control of the heat source and the distribution of MNP in order to induce necrosis within cancerous cells with the minimum negative impact to the surrounding healthy cells. In the current project, the biomedical modeling of the process of hyperthermia is carried on for cancer cells of different geometries appealing to the modified Penne's bioheat equation and the Finite Element Method (FEM). Special

attention was paid to the size and spatial distribution of nanoparticles. The results from numerical solutions have permitted to establish guidance towards optimal conditions for its use.

2. Mathematical model and Numerical methods

In order to simulate the heating of cancer cells the modified Penne's bioheat equation [3] is used. It is a heat-type equation complemented with terms responsible for the heat exchange between the cell and blood, and also the metabolic heat and the one produced by the embedded MNP. Equations can be cast into:

$$\rho_t c_t \frac{\partial T_t}{\partial t} = k_t \nabla^2 T_t + \rho_b c_b \omega_t (T_b - T_t) + Q_t + P$$

$$\rho_h c_h \frac{\partial T_h}{\partial t} = k_h \nabla^2 T_h + \rho_b c_b \omega_h (T_b - T_h) + Q_h$$

where the sub indices t and h refer to tumor and healthy cells respectively, ρ is the tissue density, c is the specific heat for the tissue, k is the thermal conductivity, ρ_b is the blood density, c_b is the specific heat of the blood, ω_t (ω_h) is the blood perfusion rate, and T_b is blood temperature. The terms Q_t and Q_h are metabolic sources of heat for tumor and healthy cells respectively. The function P(T,H) is the rate of heat production by the MNP and includes the information about the static and dynamic components of the power dissipated by MNP. It is given by the following equation:

$$P(T,H) = \frac{1}{2}\mu_0 \chi_0 H_0^2 \omega \frac{\omega \tau_{eff}}{1 + (\omega \tau_{eff})^2}$$

where H_0 is the intensity of the applied magnetic field, ω is the frequency of the AC applied magnetic field, μ_0 is the magnetic permeability of the vacuum, χ_0 is the static component of the magnetic susceptibility, τ_{eff} is the effective relaxation time of MNP due to Brown (rotation of MNP in the viscous medium) and Neel (rotation of magnetic moments) mechanisms. The explicit mathematical expressions are:

$$\tau_{eff} = \tau_N^{-1} + \tau_B^{-1}; \quad \tau_N = \tau_0 \exp\left(\frac{KV}{k_B T}\right); \quad \tau_B = \frac{3\eta V_H}{k_B T}$$

$$\chi_0(T,H) = \chi_i \frac{3}{\xi}\left[\coth(\xi) - \frac{1}{\xi}\right]; \quad \chi_i = \frac{\mu_0 \phi M_d^2 V}{3 k_B T}; \quad \xi = \frac{\mu_0 M_d H V}{k_B T}$$

where η is the dynamic viscosity of a medium where particles are suspended, K is the effective anisotropy constant, V_H is the particle hydrodynamic volume, V is the volume of the magnetic core, M_d is the domain magnetization of the MNP, and ϕ is the volume fraction solid.

3. Results and Discussion

The parameters used for simulations are summarized in tables 1 and 2.

	Tumor	Healthy Tissue	Blood
Density – ρ (kg/m^3)	1045	1045	1060
Heat capacity – c (J/kg K)	3760	3760	3770
Thermal conductivity – k (W/m K)	0.51	0.51	----
Blood perfusion rate – ω (1/s)	0.0095	0.003	----
Metabolic heat – Q (W/m^3)	31872.5	6374.5	----

Table 1: Physical parameters characterizing each medium that were used in simulations.

Magnetic NP radius R_p= 9.5 x 10^{-9} m	Volume fraction solid ϕ = 0.071	k_B = 1.38 x 10^{23} J/K
Effective anisotropy constant K = 1.0 x 10^4 J/m^3	Dynamic viscosity η = 1.0 x 10^{-3} kg/m s	f = 300 kHz
Hydrodynamic volume V_H = 5.08 x 10^{-22} m^3	Attempt time τ_0 = 10^{-9} s	μ_0= 4π x 10^{-7} T m/A
Domain magnetization M_d = 446 kA/m	Field strength H_0 = 5518 A/m	

Table 2: Physical parameters and constants used in simulations.

The above system of partial differential equations (PDE) was discretized according to the Finite Differences and solved numerically following the Crank-Nicholson method. The inclusion of the function P(T,H) transforms the system from linear to non-linear, which demands a more careful attention. Solutions were found and plotted

with Wolfram Mathematica (Figs 3 and 4). In Fig. 3 the static magnetic susceptibility has been computed including a convolution by the distribution of particle's radii assumed to be Log-normal. Curves in red and blue correspond to particle's radii of 9.46 and 9.11 nm respectively, while the green one corresponds to 10.30 but different standard deviation, making it to appear narrower than the other two. The choice for a Log-normal distribution is based on results from [2].

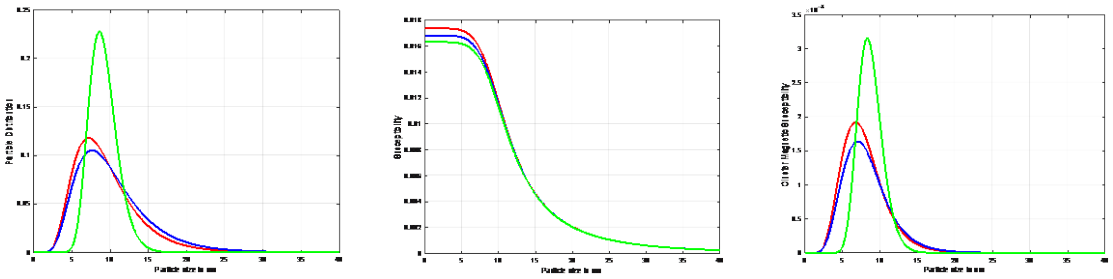

Fig 3: Computation of the Probability Distribution Function (PDF) (left) for nanoparticles, the magnetics susceptibility (center), and the cluster susceptibility (right).

The computation of the heat distribution was done for tumor cells of circular shape embeeded into a ractangular frame, as can be seen from Fig. 4. The plots show the ratio of the actual temperature to a characteristic temperature, in order the make the system of equations dimensionless. As the time goes the tumor cell is heating from the center, where the nanoparticle cluster was located. More irregular geometries are in progress and are more suitable for invasive tumor cells of rapid growth. Likewise, different nanoparticle cluster's geometries are impacting the effectivenes of using MFH.

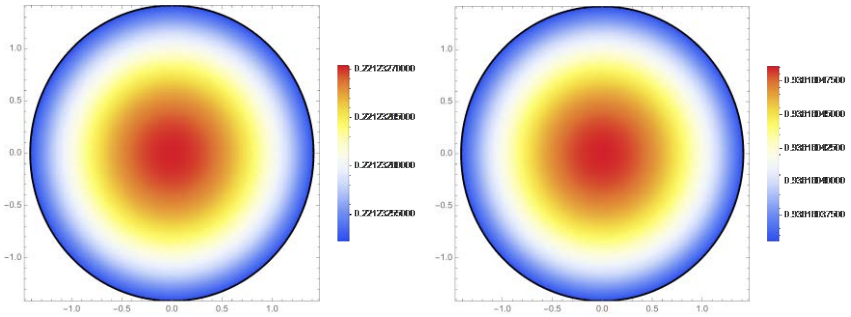

Fig 4: Numerical solution of the Modified Pennes Bio-heat equation for spherical cell model. The temperature profiles at two different moments are shown, noticing the gradual increase in temperature.

4. Conclusions

The distribution of heat inside a tumor tissue has been computed and the effect of the distribution of MNP on the intensity of the heat was determined. The heat increases initially and then drops towards the boundary of the tumor tissue preventing neighbor healthy tissues of being affected.

Acknowledgments

Authors appreciate the support received from both, St. Thomas University and Miami Dade College, as wells as from the Department of Education grant P03C1160161 (STEM-SPACE).

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Assessment of Microclimatic conditions of St. Thomas University forest

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Graphical Abstract

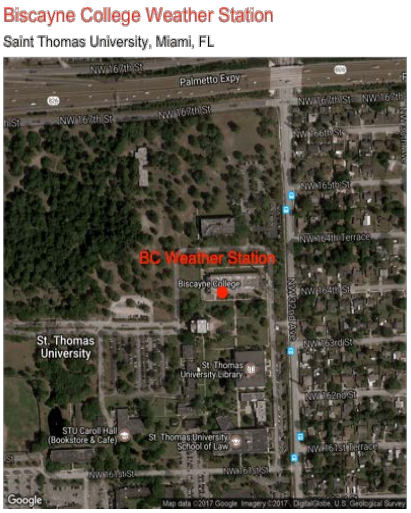

Fig. 1: Aerial view (from Google Earth) of St. Thomas University campus, the canopy layer, and nearby community and highway.

Abstract.

Urban meteorology and biometeorology have become very important fields nowadays due to the high rate of urbanization worldwide. Motivated by this situation this project is aimed at assessing the microclimatic conditions of St. Thomas University forest and evaluates the impact of canopy on the distribution on weather parameters within and around green areas as well as the extent of dispersal of pollutants from the Palmetto Expressway as a result of automobile exhausts. Observations from both, in campus Automated Weather Station (AWS) and mobile sensors (Xplorer from PASCO) are put together and mapped based on GIS information for points of measurements. The statistical analysis was done through the software R-Studio and packages for data visualization. As a result, a full characterization of soil and atmospheric conditions within the forest was done.

1. Introduction

Starting from the second half of the 20th Century an increase in urbanization rates worldwide have been witnessed such that, around half of the world population lives now in urban areas [1] rather than in rural communities. Therefore, the study of urban weather and climate is very important for urban health management. Health forecasting might provide a reliable warning health care system that would facilitate the coverage and the quality of delivered services, as well as to address the problem of the increasing cost of medical care. The ability to anticipate episodes of medical outbreaks of any nature, as well as to properly manage the peak will facilitate taking the necessary steps during extreme events.

In this context, the high load in services and resources in large urban areas impacts the quality of the environment through modification of the physical and chemical properties of the atmosphere and the covering of the soil surface [2]. The high burning of fossil fuels, the secondary pollution derived from automobile exhausts, industries and further chemical transformations have released into the atmosphere a considerable amount of Sulfur Dioxide (SO_2), Carbon Monoxide (CO), Nitrous Oxides (NO_x), Ozone (O_3), Polycyclic Aromatic Hydrocarbons (PAH), secondary organic aerosols (SOA), and Particulate Matter of different sizes ($PM_{2.5}$ and PM_{10}), which taken combined pose a serious problem for human health. A further complication with all these chemicals and aerosols is coming from the variation of mixing ratios over time, a fact expressed quantitatively through the resident time. Resident times range from few minutes (NO_3) to days (SO_2, O_3, aerosols) and even

years (N_2O) [3]. The extent of the influence also varies spatially; being the micro-scales and meso-scales the most relevant to issues related with urban health effects.

St. Thomas forest offers a unique opportunity as a test bed for the above-mentioned effects. It covers a land area where both micro-meteorological and air quality effects might be observed. Its proximity to a highway (Palmetto –SR 826) in Metropolitan Miami allows observing the effect of diesel exhausts spread over nearby communities and the importance of a canopy layer on such dynamics (see Fig. 1).

2. Data and Methods

Fig. 2: St. Thomas forest observational grids location.

Observations from both, in campus Automated Weather Station (AWS) running in partnership with Earth-Networks (Weatherbug) and mobile sensors (Xplorer from PASCO) are put together and mapped based on GIS information for points of measurements. The statistical analysis was done through the software RStudio and packages for data visualization. The forest was divided into sections, such that data might be gridded and comparison between different grids was performed (see Fig. 2).

In a data set of nearly 28000 observations done from March 1 to June 4, 2017 field campaign, cleaning data properly and efficiently requires computational functions. To clean the weather station data, a combination of Boolean functions and graphical tests were used. The models were created with the help of R packages ggplot2, EBImage, and ggmap.

3. Results and Discussion

The variation of temperature inside the forest is shown in Fig. 3 (a). As can be seen from Fig. 3 (a), temperatures are lower in the densest part of the forest, since the canopy layer blocks sunlight from reaching the ground. Ground level temperature measurements were performed every two hours from 10:00 am to 4:00 pm every day.

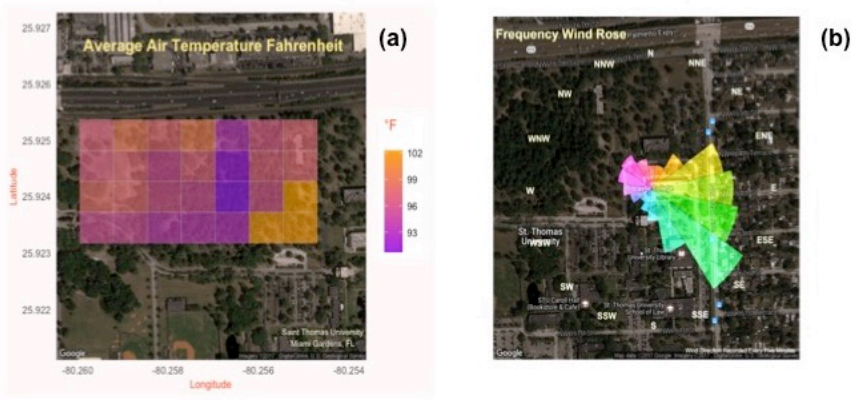

Fig. 3: (a) Distribution of ground temperatures within the forest, (b) wind rose, showing the prevalent winds and their intensity.

Additionally, AWS recorded variations of air temperature every hour daily and year round. A comparison of both types of measurement was performed and rapid variations are observed within forest, which are averaged when larger scales are considered. Wind direction and intensity reading from the AWS were mapped into a wind rose (Fig. 3 (b) – polar plot showing the directions from where most likely the wind is blowing from). During the period of analysis the wind pattern is most South-South-East (SSE). Therefore, most of the automobile exhausts from Palmetto expressway tend to spread towards the North-North-West (NNW).

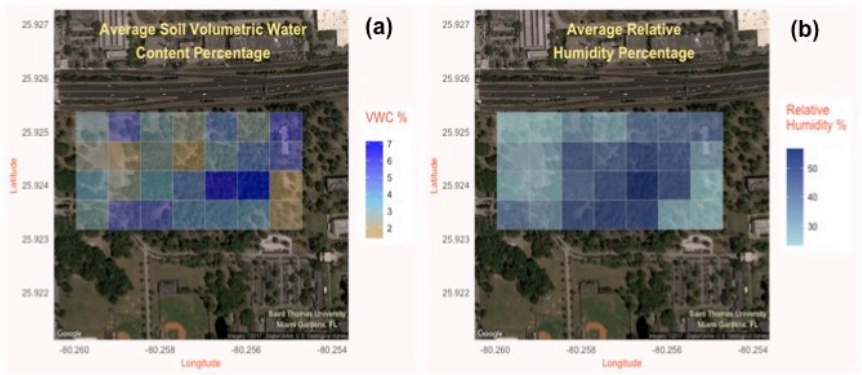

Fig. 4: (a) Average soil water content, (b) Average relative humidity within the forest.

Another important component to look for is the volumetric water content which is a measure of soil moisture. The GLX device uses capacitance between two metal probes to measure the dielectric permittivity of sampled soil. Due to

water's high dielectric compared to other materials within soil, the GLX will measure a significant change of capacitance in the presence of water. The percentage represents the amount of water compared to other soil components. The choropleth (see Fig. 4 (a)) shows greater soil moisture in the densest forest areas. Urban forests appear to reduce the risk of soil aridity by allowing greater runoff infiltration and reducing evaporation of soil moisture due to canopy cover and organic floor cover. The effect of trees on increased humidity is shown in Fig. 4 (b). Evapotranspiration from the trees releases water vapor into the surrounding atmosphere, which can have cooling effects. The regions with greatest humidity are also the coolest, which is related with the fact that low temperature allows more room for water molecules within the air. This phenomenon happens because a change in air temperature alters the air's saturation vapor pressure. If the air temperature decreases, so does the air's saturation vapor pressure. As the saturation vapor pressure approaches the actual vapor pressure, the relative humidity increases as the air approaches saturation [4].

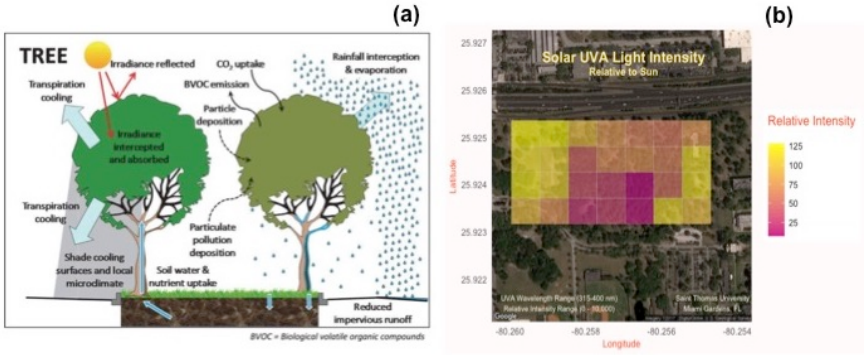

Fig. 5: (a) Schematic diagram showing physical processes within the canopy layer, (b) UVA intensity within the forest.

It is noteworthy that the above picture is consistent with results from Fig. 3(a) and also the measurements of the UVA-light included in Fig. 5(b). All physical processes occurring within the canopy layer are schematically represented in Fig. 5 (a).

The above discussed results have the potential to be guidance for the Urban Tree Project, aimed at developing sustainable smart urban environments, where canopies might help the cleaning of the air while support the cooling of the many urban areas preventing the excess of Urban Islands [5,6,7].

4. Conclusions

Microclimate modeling can provide helpful insights across many disciplines. Understanding both natural and anthropogenic factors influencing local climate conditions through statistical modeling can aid in making decisions which might reduce climate change, reduce costly storm damage, and create healthier citizens. This research could be furthered developed with longer observation periods of current measurements and the additional measurements of carbon dioxide levels and transpiration levels by tree species.

Acknowledgments

Authors appreciate the support received from both, St. Thomas University and Miami Dade College, as wells as from the Department of Education grant P03C1160161 (STEM-SPACE).

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Time series analysis of the EEG signals for Epilepsy seizure forecast

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Graphical Abstract

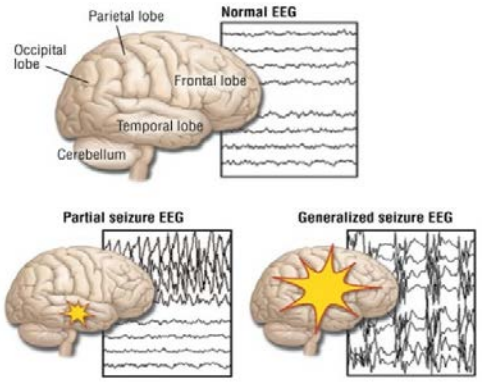

Fig. 1: The intensity and brain area affected subdivides Epileptic seizures. EEG is able to record the evolution of it.

Abstract.

Epilepsy is a Central Nervous Disorder that is affecting millions of people with a different degree of severity. This project is aimed at studying the feasibility to use the software R for statistical analysis of EEG signals in order to perform statistical forecast of epileptic seizures by constructing functional networks based on the cross-correlation of time series from different electrodes. Such functional associations are a result of an emergent neuronal activity of a large amount of neurons, thus, they will be guidance to physicians. A further understanding of the causes will require a combination of biomedical modeling and sensing with fMRI and EEG combined.

1. Introduction

Epilepsy is a Central Nervous Disorder that is affecting millions of people with a different degree of severity. Epilepsy is the 4th most common neurological problem in the USA, followed by migraines, strokes and Alzheimer disease. The average incidence of this condition each year in the USA is estimated at 48 incidents for every 100,000 people [1]. Young children and older adults are the groups with the highest rates. In addition, the prevalence of this condition is estimated at 2.2 million people or 7.1 for every 1000 people in the USA. Depending on the level the patient is at the moment, seizures can be controlled either with medications or with surgical procedures. However, during surgical procedures many complications might show up, mainly due to the lack of knowledge of functional neuronal networks operating behind epileptic seizures [2]. Therefore, a reliable methodology capable to predict in advance the beginning of seizures would have a tremendous impact on the quality of life of these patients and might prevent further complications with the management of this condition. In this end, EEG provides a reliable method to detect the seizures with very good temporal resolution [3,4] (see Fig. 1).

This project is aimed at learning about available epileptic databases, the format of the data, the RStudio package eegkit to do EEG signal processing, and principles of network science. It is rather a review project rather than a computational one and constitutes an entry-level mathematics-oriented project for Biology majors.

2. Methodology

One of the challenges new applied biomedical-oriented star-ups face is the cost of technology oriented software. In the Biomedical fields, MATLAB has a long tradition of being use. Despite of that “gained seniority”, there are new possibilities that are based on community open source projects. R software and its Integrated Development Environment (IDE) RStudio are potential alternative to MATLAB. Within R, the package **eegkit**

[5] allows the processing of EEG signals. Thus the first objective of this project was to get introduced and familiar with such package.

The second objective was to access available epileptic EEG databases in order to apply eegkit to the existing datasets. In that regards, the following sources were used: European Database on Epilepsy – Epilepsiae [6], Free accessible database of 23 patients with MRI and EEG information for both phases, pre and post-operatory from Warsaw Memorial Child Hospital [7], and EEG and ERP data available for public download [8]. One common trouble encountered with accessing to data was the standardization of the output format for EEG and its further translation into a readable by R binary. At this point, it is worth to mention that MATLAB toolbox eeglab [9] is more versatile and easy to use, thus the further use of R will be determined by the ability to create a more easy-to-use interface competing with eeglab.

Third objective was to create within R the cross-correlation matrix between different time series obtained from different electrodes. Once the matrix is created, a criterion for a strong connectivity is set. For instance, Pearson correlation above 0.6 thresholds is considered a strong correlation between different electrodes. Connectivity above 0.6 thresholds is also segmented in order to see the strongest links and how far electrodes and consequently patches of neurons are connected.

3. Results

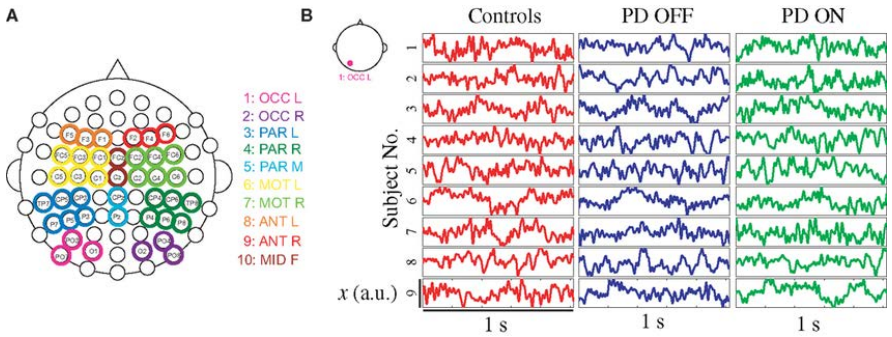

Fig. 2: (A) Positions of the electrodes and its planar representation within eegkit, (B) time series from specific patient before and after were stimulated.

Working with the scheme of 64 electrodes, they can be represented and selected at out convenience within eegkit (see Fig. 2 A). Likewise, time series from specific patient and from a specific electrode can be selected and plotted (see Fig. 2 B). The mining of the data from within eegkit still need to be worked out better. It was one of the most difficult moments with the package. Another important trouble encountered with eegkit was the exporting of graphs. In many cases screenshots were needed because a regular export procedure did not work. The cross-correlation analysis was not performed because of the above-mentioned troubles with eegkit and lack of standardization of the datasets. Nevertheless, the familiarity with the desired goal is exemplified in Fig. 3.

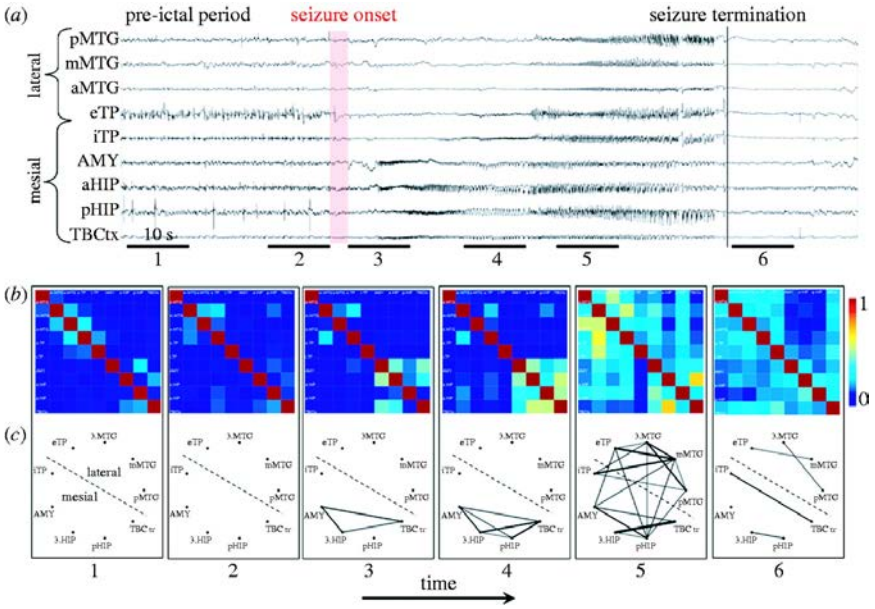

Fig. 3: Schematic representation of the obtention of electrode-correlated network from EEG time [1]

4. Conclusions

Access to medical records it is a difficult task for several reasons: availability, reliability, and the standardization of the output formats.

Despite of the implementation of the application “eegkit” is not at the stage that datasets might be uploaded easily and the obtained graphs exported to other applications. Therefore, the full analysis expected to be done from the very beginning it could not be accomplished in most. Even though, it might be seen initially as a negative moment, it opens new opportunities for further developments and collaborations.

Acknowledgments

Authors appreciate the support received from both, St. Thomas University and Miami Dade College, as wells as from the Department of Education grant P03C1160161 (STEM-SPACE).

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Assessment of the temperature comfort of a model house

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Graphical Abstract

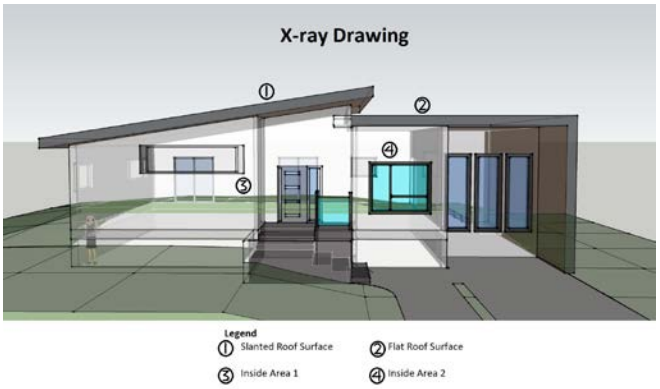

Fig. 1: Model house designed with AutoCAD. The geometry facilitate circulation and cooling

Abstract.

The growing concerns about global climate changes and mitigation strategies constitute a technical challenge for civil engineers and architects altogether. In this project, a model house was designed appealing to AutoCAD, and a scaled version was assembled using low cost materials. The model design pretends to enhance cooling within the house taking advantage of the geometry of it and the circulation pattern around it. Measurements of the thermal load were done with a Thermal Infrared Camera and a set of sensors located within the model house. The temperature distribution was compared with the one obtained from solving the heat equation with Wolfram Mathematica. Recommendations for homebuilders are presented.

1. Introduction

Urban developments nowadays demand from civil engineers and architects creativity. Megacities are suffering from large urban heat island effect (UHI). It is defined as the rise of temperatures in any man-made area, resulting in a change of urban temperature profiles, wind and rain patterns when these parameters are contrasted with those from rural areas [1]. Changes in albedo that are associated with UHI yield to changes in transport of energy and mass within urban areas which combined with particular geometry of urban canyons will produce specific spatio-temporal patterns of pollution dispersal too [2]. A further exacerbation of UHI effect is coming from global climate changes. Variations in weather conditions around urban areas are sensible to further amplification effects when they are contrasted to similar variations in rural environments. These changes might impact the ecology of cities and stimulate the proliferation of vector-borne diseases in addition to the thermal loads already associated with climate changes [3]. Altogether, they tend to increase the demand on energy in order to cool houses and other facilities and maintain a level of acceptable comfort. In these circumstances, the consideration of geometries that facilitate the air circulation might lead to an effective cooling and therefore to energy saving while maintaining the comfort standards.

This project is aimed at designing and testing a model house for insulation. It is combining design with educated choice of materials and geometries, as well as the internal layout of the house.

2. Materials and Methods

Fig. 2: Model house and the collection of spotlights simulating the sunlight. The emitted power from the lamps was about

A model is created using AutoCAD (see Fig. 1) and then created to scale using thermo-insulating materials such as: insulating polystyrene foam, acrylic, silicon sealer, and Masonite hardboard (see Fig. 2). This model will be tested by exposing it to a radiating source of heat, which follows a temporal pattern similar to that exhibited by the sun in that it raises from the east and sets on the west. We will measure the temperature at different regions of the house using a laser sensor as stated in the X-ray Drawings (see Fig. 1). By comparing the outside and inside temperatures after the model has been exposed to the heat source for 1 hour, the efficiency of the insulation can be assessed. In order to estimate how much heat is capable to enter the house for a given radiating source output, the Fourier law of thermal conduction [4] will be used and it can be cast into:

$$\frac{dQ}{dt} = \frac{kA}{L}(T_H - T_E)$$

where L is the width of the wall, A is the surface area of the wall, k is the coefficient of thermal conductivity, T_H is the temperature at the interior of the house, while T_E is the value outside of it. In Table 1, the values for the physical parameters are summarized.

Description	Values	Unit Conversion	Conversion Factor	Values in SI Units
Themal conductivity of polystyrene	0.027 W/(m*K)	-	-	0.027 W/(m*K)
Cross-Sectional Area	372 in^2	sq inches to sqmeter	1 in^2 = 0.000645 m^2	0.24 m^2
Thickness of Wall	1.25 in	inches to meter	1 inch = 0.0254 m	0.03 m
Temperature of Interior	20°C	Celsius to Kelvin	T(°C)+273.15	293.15 K
Temperature of Exterior	23°C	Celsius to Kelvin	T(°C)+273.15	296.15 K
Time elapsed	1h	Hours to seconds	1 h = 3600 s	3600 s

Table 1: Values of the physical parameters used to estimate the heat transfer across the model house and following the Fourier law of thermal conduction.

3. Results and Discussion

The first round of measurements focused on the outside distribution of temperatures while moving the radiating source following a sun-like pattern. It is noteworthy that environmental (background) temperature at the Physics Laboratory was around 69 F (20.56 C or 293.71 K) for most part of the day. It conditioned the time interval required to observe significant changes while heating the model house (see Fig. 3).

Fig. 3: Thermal distribution obtained from the Thermal Infrared Camera for different positions of the source of heat (spotlight emitting about 500W of power). Left-most panel corresponds to the source located to the eats, the central panel corresponds the source located at a center, while the right-most pane corresponds to the source located westward.

Once the outside part was characterized, measurements at the inside locations were performed with a infrared diode laser thermometer. The obtained results were included in Table 2.

Temperature at Different Regions (East)			
		Temperature (°C)	
		Min	Max
Exterior	Slanted Roof	20	22
	Flat Roof	21	24
Interior	Inside Area 1	20	21
	Inside Area 2	20	21

Temperature at Different Regions (Center)			
		Temperature (°C)	
		Min	Max
Exterior	Slanted Roof	20	23
	Flat Roof	21	23
Interior	Inside Area 1	19	20
	Inside Area 2	20	21

Temperature at Different Regions (Center)			
		Temperature (°C)	
		Min	Max
Exterior	Slanted Roof	20	23
	Flat Roof	21	23
Interior	Inside Area 1	19	20
	Inside Area 2	20	21

Table 2: Temperature measurements for different geometries and comparison of in and out values.

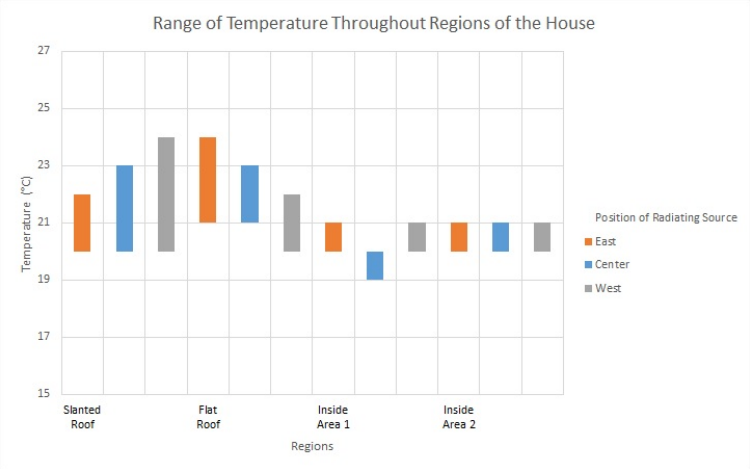

Fig. 4: Summary of the range of temperature variations in different parts of the model house.

Taking into account all measurements together, a summary of the temperature ranges in different parts of the model house can be seen in Fig. 4. It is clear from this picture that the largest range is observed in the junction of the flat roof and slanted roof (see Fig. 1).

According to the results, we can say that our insulation system is very reliable for small projects like this. These materials may not be optimal for real-life construction, yet the polystyrene insulation works very well to insulate thermal transmission through the conduction method, which makes it optimal to place along masonry units. Other small size applications of this experiment include cool (cold) boxes, packaging delicate electronics, and flooring. From our experiment we can see that not even a thermostat will be needed to prevent the interior of the house from heating up. However, real life always has more variables that implicate the results of experiments. A legitimate, not model, house under changing environment conditions will release or absorb heat; in which case, there are many other methods that reduce this leak or absorbance of heat like manipulating air flow through vents or using specialized panels that have high thermal resistance R = L/k ratings.

4. Conclusions

The joint effort of civil engineers, architects and applied physicists might help to find optimal solutions in the construction industry. The thermal and fluid environment may be characterized nowadays much better than it happened years before, thus, physics-comfort-oriented designs might become a reality, saving energy and resources, and transforming construction industry into a smart and sustainable one.

Acknowledgments

Authors appreciate the support received from both, St. Thomas University and Miami Dade College, as wells as from the Department of Education grant P03C1160161 (STEM-SPACE).

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Time series analysis and forecast of respiratory conditions in Florida

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Graphical Abstract

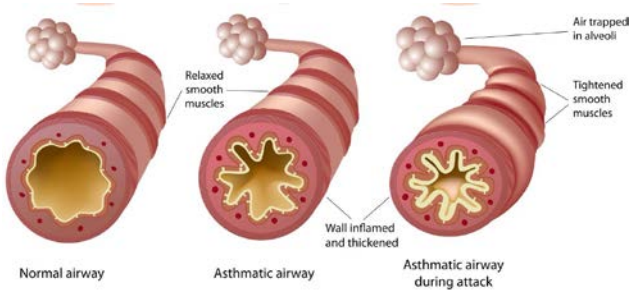

Fig. 1: Schematic representation of the airways' blockage due to an asthma episode.

Abstract.

In an effort to understand conditions triggering asthma episodes and therefore create a asthma risk index that might be valuable to both patients and medical practitioners, 6 different counties in Florida were chosen, 3 of them in the southeast region and 3 located in the central region. The number of cases at emergency rooms due to asthma and other respiratory conditions were provided by the Department of Health BRACE project and analyzed statistically looking for potential associations with weather and environmental conditions.

1. Introduction

Asthma is a chronic inflammatory lung disease that narrows the airways producing large amounts of mucus, making it difficult to breathe [1] (see Fig 1). Asthma may be mild or may interfere with daily activities. In some cases, it can lead to deadly attacks. The exact cause of this disease has not yet been found, however, it affects people of all ages, races and gender [2]. Researchers think that genetics and environmental factors have a critical role in the origin of asthma cases, especially during early childhood [3]. This project can help researchers with the identification of factors triggering asthma attacks and therefore, the creation of new applications to prevent and manage them.

2. Health data and Methods

Health data were obtained from the Department of Health of Florida through the Florida Asthma Coalition and the BRACE project (Building Resilience Against Climate Effects). The data set consists of eight years of daily cases of asthma and other respiratory conditions reported at Emergency Departments from six Counties in Florida: Miami Dade, Broward, Palm Beach, Hillsborough, Osceola, and Polk. The first three located in the southeast portion of the State, while the last three are in the center of the State. The data set contains information from January 1, 2005 to December 31, 2012.

The incidence of asthma was normalized to 10,000 people. The results are presented in Fig. 3. Despite of the large number of cases reported in the southeast region the largest prevalence is occurring in the central part of Florida, in particular in Polk County.

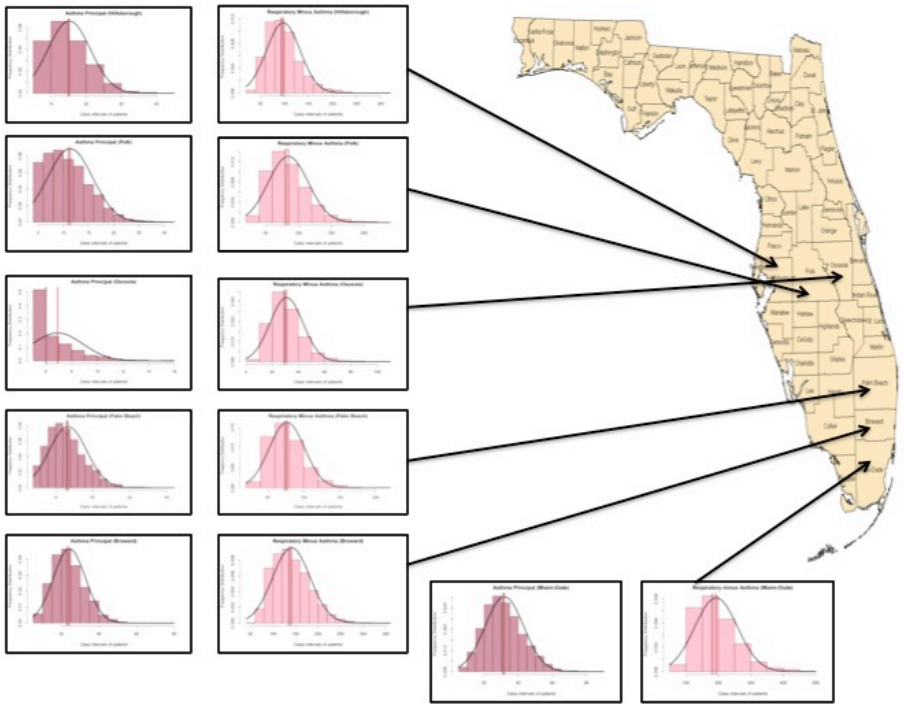

Fig. 2: Frequency distribution of the number of asthma (left panel) and other respiratory conditions (right panel) from six Counties in the State of Florida from January 1, 2005 to December 31, 2012.

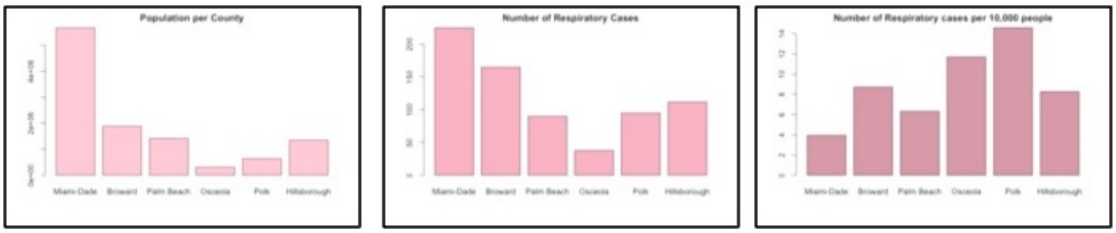

Fig. 3: Prevalence of asthma through the State of Florida.

3. Results and Discussion

The time series of asthma cases was analyzed with software RStudio for statistical computing. The results are shown in Fig. 4, and it is clear there is a seasonal component that peaks between December and February every year and drops between April and June. It seems to be associated with the thermal stress upper respiratory track undergoes during the "Florida winter" [4], causing a larger likelihood of being infected by upper respiratory track infections, which induce inflammatory processes in many people [5,6]. The overall trend of the series shows a gradual and steadily increases in the number of asthma cases since 2005. Such behavior might be associated with the gradual shift of temperatures and relative humidity towards higher values causing an increase of natural allergens. Health data were contrasted with meteorological and environmental data. The correlation between the number of asthma cases and the minimum temperature for the day is shown in Fig. 5.

4. Conclusions

Asthma in Florida follows a seasonal pattern with peaks in late fall and winter that coincide also with the peaks of upper respiratory tract infections at Emergency departments. The week association with minimum temperature is an indication of nonlinear threshold response, due in much to the cold stress load facilitating the entrance of infection and the further triggering of inflammatory episodes.

Acknowledgments

Authors appreciate the support received from both, St. Thomas University and Miami Dade College, as wells as from the Department of Education grant P03C1160161 (STEM-SPACE).

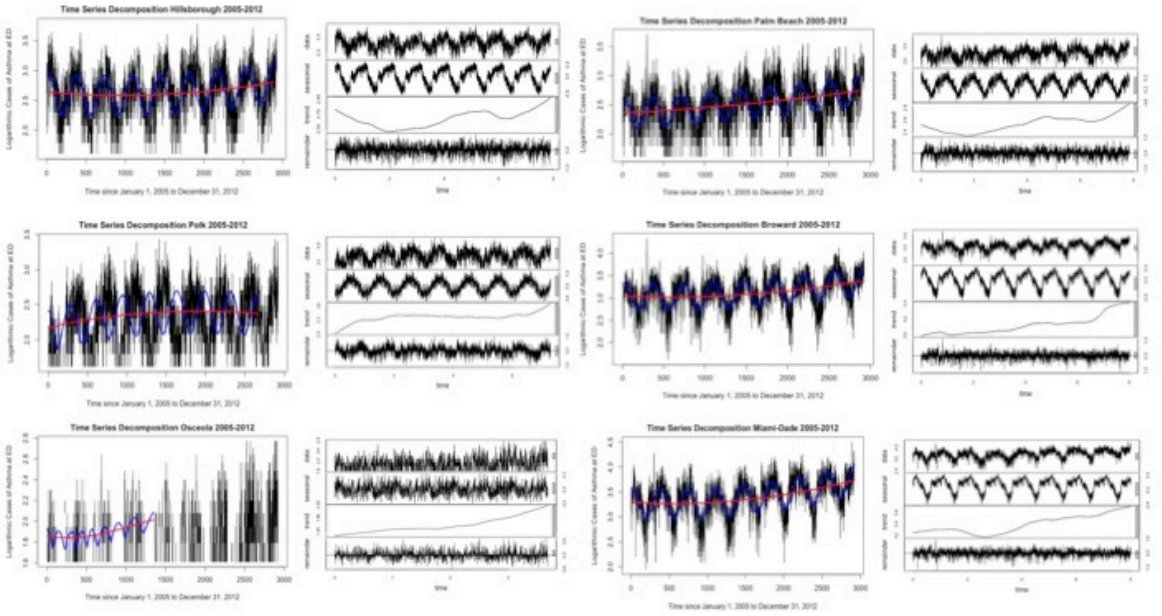

Fig. 4: Trend analysis of the time series of asthma cases. It was performed with software RStudio. Notice components, the seasonal and the overall trend towards higher values.

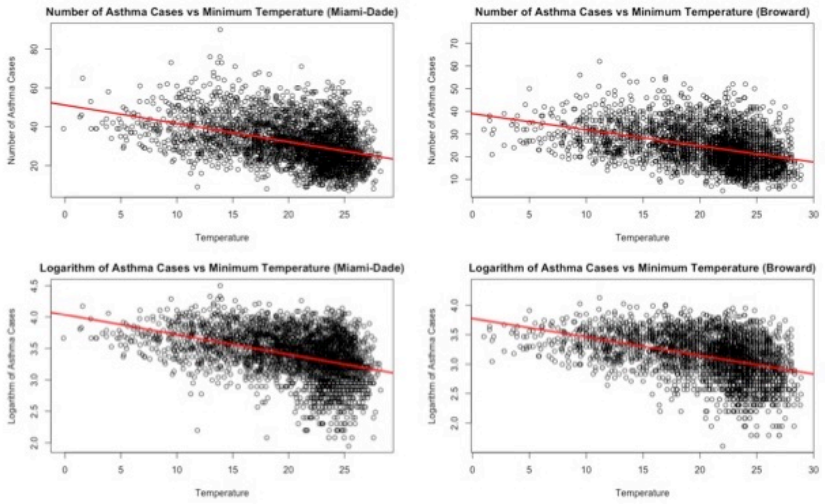

Fig. 5: Correlation analysis between the numbers of cases and minimum temperature using a linear and exponential model. In both cases, as the temperature increases, the number of cases decreases.

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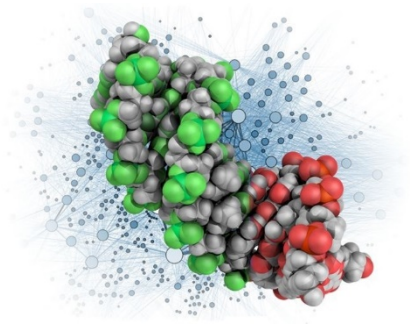

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In silico studies on the pH induced membrane insertion of pHLIP peptides

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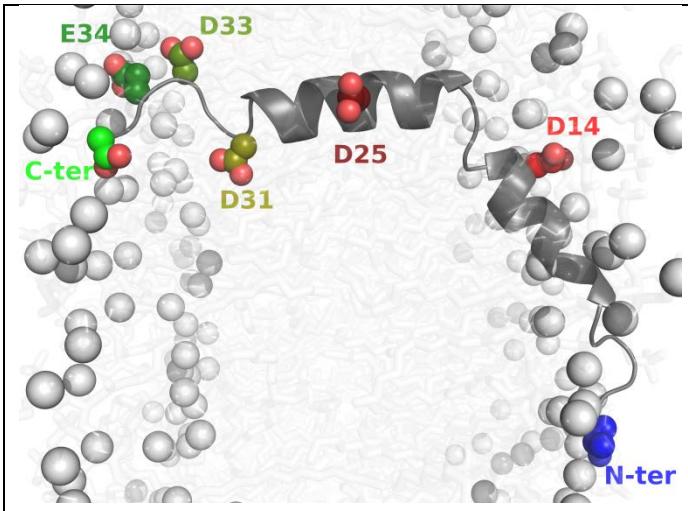
Graphical Abstract *(mandatory)*	**Abstract.** *(mandatory)*
	The pH (low) insertion peptide (pHLIP) belongs to a family of peptides originated from a segment of the transmembranar C helix of bacteriorhodopsin. The peptide has three major states: state I - soluble and unstructured; state II - adsorbed at the membrane surface and unstructured; state III - inserted in the bilayer as an α-helix at low pH values. One of the major applications of pHLIP befalls on its ability to insert into membrane cells with an acidic vicinity, such as tumoral cells, thus working as an efficient tumor-specific biomarker[1]. However, *wt*-pHLIP has a significant limitation, since it accumulates in the kidneys in considerable amounts due to their naturally acidic extracellular pH. This limitation led to a need for increased pHLIP specificity by delimiting the pH range of insertion, further strengthening its application as a



biomarker and possible drug-delivery system for inflammatory tissues.

The stochastic titration constant-pH molecular dynamics (CpHMD) method has been successfully used to sample protonation behaviour of titrable amino acids inserted in a lipid bilayer, presenting, however, an insufficient amount of data to extensively describe pK_a profiles[2]. The newly developed pH-replica exchange (pHRE) method, allows the exchange of pH values between replicas within a certain probability. This approach enhances the transitions between energy minima, improving the sampling of non-favorable protonation states, which leads to a better description of the pK_a profiles. This new method was applied to simulations of *wt*-pHLIP and L16H variants.

The pHRE simulations led to more detailed, accurate and consistent pK_a profiles and allowed the identification of Asp14 as the key residue whose protonation state triggers the insertion process. The calculated insertion pK_a value of this residue is in good agreement with the experimental insertion pK value for the *wt* sequence. Moreover, the simulations of L16H showed that this variant exhibits a second insertion pK_a, at lower pH, indicating that, below this value, the peptide would exit the membrane. These results were corroborated by new experimental data performed by our collaborators, Prof. Oleg Andreev in Rhode Island, USA.

We acknowledge financial support from FCT through projects UID/MULTI/00612/2013, PTDC/QEQ-COM/5904/2014 and grant SFRH/BPD/110491/2015.

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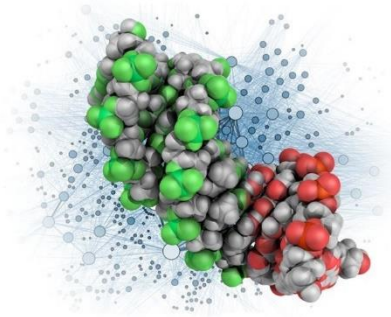

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Improving pK_a calculations of membrane inserting amino acids using replica exchange CpHMD simulations

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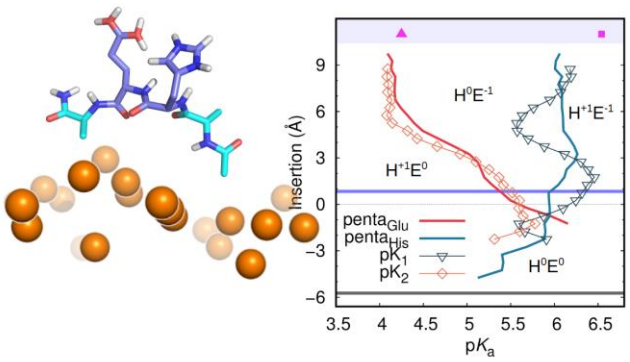
Graphical Abstract



Abstract.

pH is one of the most important solution parameters. It plays a major role in most biochemical processes by, among other, inducing protein conformational changes and influencing protein-lipid interactions. These systems have been modeled using constant-pH molecular dynamics (CpHMD) methods since they are able to correctly capture the conformational/protonation coupling. In a previous CpHMD study[1], we have shown that, upon membrane insertion, the titrable amino acids are prone to adopt a neutral state. In that work, CpHMD experienced difficulties sampling ionized conformations in inserted regions, since in the time scale of our simulations most residues retained their neutral state upon insertion/desolvation.

Enhanced sampling techniques are a widely used solution to deal with kinetic traps in molecular dynamics simulations. Since our sampling problems are related with protonation, we have implemented a pH-based replica exchange

	(pHRE)[2]. In this method, each simulation replica is assigned a unique pH value and attempts to exchange the simulated pH value of simulations pairs are periodically performed. The acceptance criterion is influenced by the exchanging simulations pH values and protonation states of the titrable sites. In this work, a more accurate description of the membrane influence on the pK_a profiles of titrable amino acids is provided by using the pHRE methodology, a newly developed method to calculate insertion, and more rigorous criteria to define the acceptable protonation sampling. Since in pHRE, due to replica mixing, all pH values sample similar insertion regions, a larger amount of inserted conformations in the ionized state are obtained. Our efficient pHRE results outperformed previous CpHMD ones, granting more sampling in less simulation time. In the future, pHRE will replace CpHMD as our go-to method to study pH-dependent phenomena.

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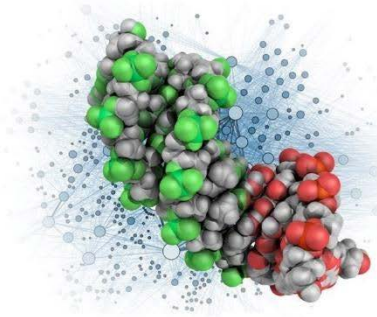

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"*In silico*" estimation of encapsulation-induced pK_a shifts in drugs.

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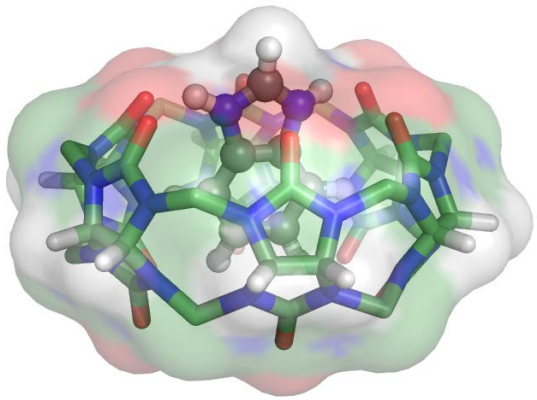
Graphical Abstract



Abstract

Molecular machines have recently been associated with the development of molecular carriers to enhance drug properties, such as solubility or bioavailability. One possible approach is the drug encapsulation by a host molecule, such as cucurbituril (CB) rings, modifying the environment of the guest molecule. CB rings are able to encapsulate guest molecules providing a hydrophobic cavity and several carbonyl groups that stabilize cationic hosts that interact with this region. This will result in significant pK_a shifts for drugs with titrable (cationic) groups that can be exploited in order to improve drug bioavailability, whether by enhancing their solubility, stabilizing their active form or by protecting them against external agents. This approach can be used for medical targeting, such as cancer therapy, by designing carriers that deliver guest molecules at specific conditions, knowing the target properties.

Computational tools are a powerful way to help the rational design of CB-guest complexes. In particular, the stochastic titrations constant-pH MD (CpHMD) method allows a molecular dynamics simulation to have the pH value as an external parameter and, consequently, obtain full titration curves and pK_a values. The main goal here is to develop a strategy to model benzimidazole (BZ) pK_a shifts, our «proof-of-

	concept» molecule, and then extrapolate this process to other host-guest complexes. BZ has a well-known shift of ~3.5 pK_a units when encapsulated by a CB ring and, with the refinement and fine tuning of this process, it is possible to elucidate the molecular details of these host-guest interactions. We acknowledge financial support from FCT through project UID/MULTI/00612/2013 and grant SFRH/BPD/110491/2015.

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Functional characterization of α_1 adrenergic receptor in the rat locus coeruleus in vitro

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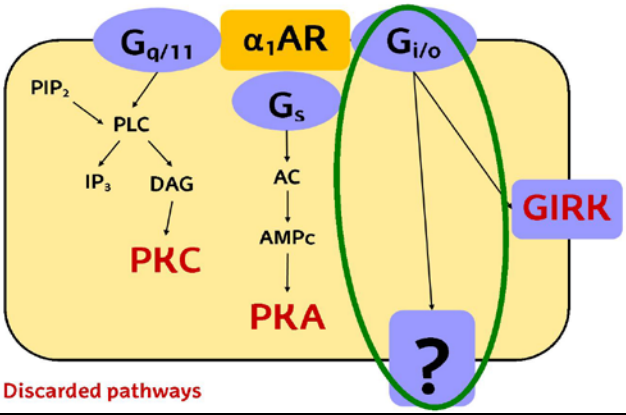
Abstract

α_1-adrenoceptor (α_1AR) is involved in the physiopathology of the central nervous system (CNS) and could constitute a therapeutic target for neurological disorders such as drug addiction or Alzheimer´s disease. α_1AR mainly couples to $G_{q/11}$ protein, which activation leads to stimulation of phospholipase C (PLC) and subsequent activation of protein kinase C (PKC). However, other G proteins (G_i, G_s) have also been described to be coupled to α_1AR receptors. The locus coeruleus (LC), the main noradrenergic nucleus in the CNS, has been shown to express α_1AR, but to date functional role of α_1AR in the adult rat brain LC remains unclear. The aim of this study was to characterize, by single-unit extracellular recordings of LC neurons, the role of α_1AR in the regulation of the firing rate (FR) of LC neurons in adult rat brain *in vitro*. For that purpose, we first characterize the effect of the $\alpha_{1/}\alpha_2$AR agonist noradrenaline (NA) in the presence and absence of the α_2AR antagonist RS 79948 (0.1 µM). Then, we investigated the signalling pathway involved in the effect of NA. Perfusion with NA (100 µM) inhibited the FR of LC neurons through activation of α_2AR. However, in the presence of the α_2-adrenoceptor (α_2AR) antagonist RS 79948 (0.1 µM) perfusion with NA increased the FR of NA neurons (stimulatory effect = 114%). The stimulatory effect of NA (100 µM) was blocked by the α_1AR antagonist WB 4101 (0.5 µM). Administration of the PKC inhibitor Go 6976 (1 µM), the G protein-coupled inwardly-rectifying potassium channel (GIRK) blocker $BaCl_2$ (300 µM) or PKA inhibitor H-89 (10 µM) failed to change the stimulatory effect of NA. However, NA (100 µM) induced stimulation was reduced by 64% in the presence of the $G_{i/o}$ protein inactivator pertussis toxin (PTX) (500 ng·ml^{-1}). In conclusion, α_1AR activation stimulates the FR of NA neurons in the adult rat LC through a signalling pathway that involves activation of the $G_{i/o}$ protein. It remains to be studied the mechanism by which $G_{i/o}$ proteins stimulates the FR of LC neurons via α_1AR activation.

Graphical Abstract



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Electrophysiological study of the effect of cannabidiol on the dorsal raphe nucleus serotonergic neurons

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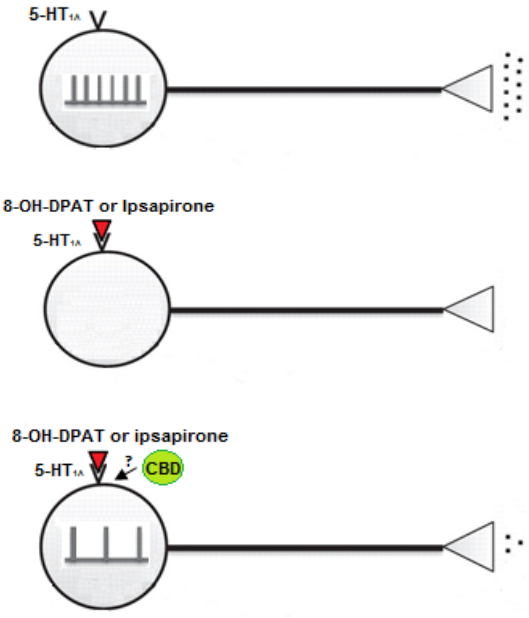
Keywords: cannabidiol, 5-HT$_{1A}$ receptor, raphe nucleus, electrophysiology

Graphical Abstract



Abstract

Cannabidiol (CBD) is the main non-psychoactive cannabinoid found in the *Cannabis* plant, which exerts several pharmacological effects including anxiolytic, antiemetic, antidepressant, antiepileptic and motor effects. *In vivo* evidence suggests that these pharmacological effects could be mediated by serotonergic 5-HT$_{1A}$ receptors. The dorsal raphe nucleus (DRN), which is the main serotonergic cluster in the brain, expresses 5-HT$_{1A}$ receptor and plays a key role in the regulation of different functions such as mood and anxiety. To date, the nuclei involved in the action of CBD and the mechanisms by which it regulates 5-HT$_{1A}$ receptor are still unknown. Therefore, the aim of this study was to characterize the effect of CBD on the firing rate of dorsal raphe 5-HT neurons and 5-HT$_{1A}$ receptor activation by single-unit extracellular electrophysiological recordings *in vitro*. Direct perfusion with CBD (30 μM) slightly but significantly reduced the firing activity of DRN 5-HT cells. In order to study the effect of CBD on 5-HT$_{1A}$ receptor activation, we applied the cannabinoid in the presence of two different 5-HT$_{1A}$ receptor agonists: 8-OH DPAT (10 nM) and ipsapirone (100 nM). Application of 8-OH-DPAT or ipsapirone completely inhibited the firing activity of DRN 5-HT cells. However, in the presence of CBD (30 μM) the inhibitory effects of 8-OH-DPAT and ipsapirone were reduced by 66% and 53%,

	respectively. Finally, to discard the possible role of CBD as a competitive 5-HT_{1A} receptor antagonist, we administrated CBD once the cells had been totally inhibited with ipsapirone. Perfusion with CBD (30 μM) failed to recover the firing activity of inhibited 5-HT cells, whereas 5-HT_{1A} antagonist WAY 100635 (30 nM) recovered the firing rate of all 5-HT cells. In conclusion, these results suggest that CBD regulates the activity of 5-HT_{1A} receptor in an indirect manner since it does not displace the agonist from the binding site.

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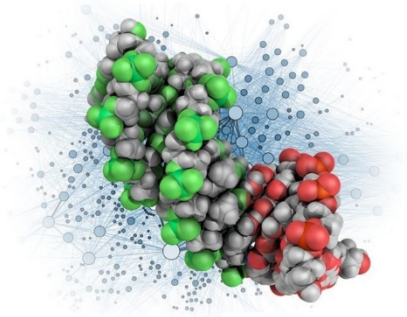

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Effect of protonation state on the interaction of Hoechst 33342 with lipid membranes – An experimental and computational study

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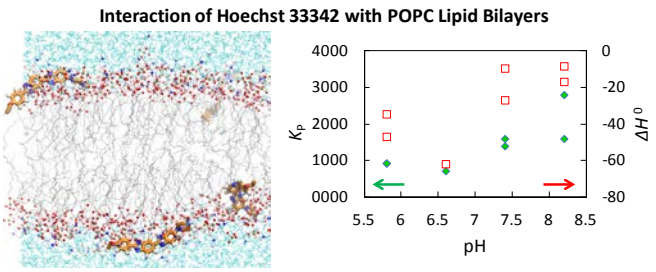

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Abstract. *(mandatory)*

<<Hoechst 33342 (H33342) is a fluorescent probe that stains the DNA of living cells, permeating through cell membranes.[1] However, the influence of the probe ionization state [2] in this process is poorly characterized. The knowledge of H33342 ionization state in lipid bilayers will help to predict and interpret its passive permeation through cell membranes. In this work we characterized the acid/base properties of the interaction of H33342 with POPC bilayers using an experimental and computational combined approach.

	H33342 pK_a values in aqueous solution of 6.4 and 11.1 were measured by its UV/Visible spectra at different pH. H33342 partition coefficient (K_p) to POPC bilayers at different pH was measured by isothermal titration calorimetry (ITC). An increase of K_p for higher pH values was obtained, indicating stronger interaction with membranes for the less charged or neutral forms of the probe. The enthalpy variation (ΔH^0) for the partition to the bilayer was negative at all pH values, with higher absolute values at low pH. This may indicate that when H33342 is more protonated, it adopts a more external position in the bilayer, being able to make favorable interactions in this membrane region. Detailed characterization of H33342-membrane interactions was also obtained through Molecular Dynamics (MD) simulations. This allowed to support experimental results by the calculation of membrane transverse location and preferential orientation of the H33342 in different protonation states, as well as possibility of hydrogen bonding between the probe and the membrane. We conclude that at physiological pH H33342 presents a high fraction of the neutral form while associated with POPC bilayers, justifying the fast permeation observed through cell membranes. Acknowledgements: This work was partially supported by the Portuguese "Fundação para a Ciência e a Tecnologia" (FCT) through projects 007630 UID/QUI/00313/2013 and PT2020_PTDC_DTP-FTO_2784_2014, cofunded by COMPETE2020-UE.>>

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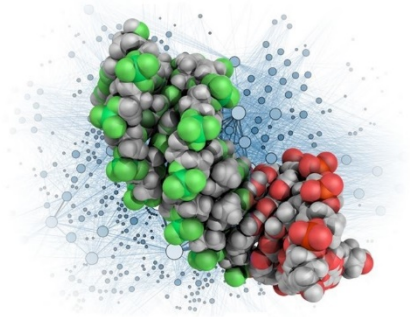

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Using big-data to understand the protein interface landscape

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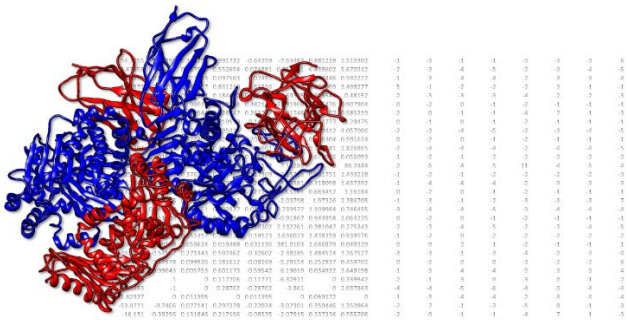
Graphical Abstract



Abstract.

Protein-protein interactions (PPIs) are the foundation of basic organism functions and understanding them is key in determining the importance of different proteins in a wide array of complex networks and processes [1]. The variety underlying PPIs is immense and some residues are more essential in interface stabilization than others [2]. Such is the case of hot-spots (HS), residues whose mutation to alanine is detrimental for the stability of the PPI, as opposed to null-spots (NS), which constitute the remaining interfacial residues [3]. Considering the complex landscape in protein interfaces, some patterns and characteristics arise when a high amount of data is considered, by minimizing the effect of less prevalent interactions and characteristics. In this work, the SpotOn pipeline [4] - developed by our group - custom scripts and conservation servers were used to determine structural features of interfacial residues and to classify them as HS and NS in the PPI4DOCK database [5], comprising over 1400 non-redundant complexes. This study allowed us to further understand the structural differences between HS and NS and will be available in a web-server in the near future.

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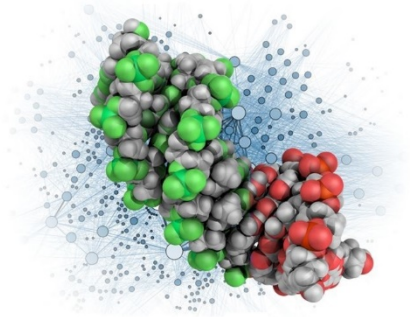

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Creating a valid *in silico* Dopamine D2-receptor model for small molecular docking studies

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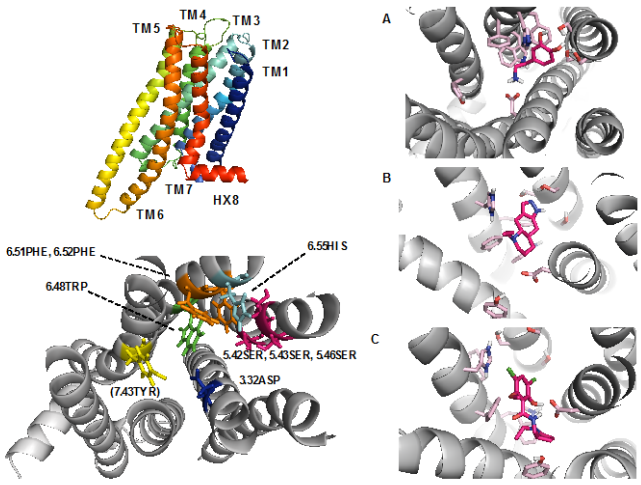
Graphical Abstract



A: Dopamine, B: Quinpirole, C: Raclopride

Abstract

Due to the clinical importance of the Dopamine D2-receptor (D2R) in several brain dysfunctions, the utilization of *in silico* models for drug development is a growing field of investigation. We provided a transparent and reproducible pipeline for creating a valid D2R model for small molecular docking studies. Furthermore, we suggested a binding pocket for the endogenous ligand of D2R, which was attained upon careful consideration of the available experimental data. Molecular docking studies with Dopamine, Quinpirole and Raclopride allowed also a better understanding of the binding pocket characteristics.

Introduction

Dopamine D2-receptor (D2R) is a member of G-Protein Coupled Receptors (GPCR) super-family, consisting in seven transmembranar helices (TM), three extracellular and three intracellular loops (1,2). D2R, along with D3R, are believed to have a more complex function within the dopamine receptor family as they are present in post- and pre-synaptic termina (3). In addition they exert a negative feedback loop in the presynaptic terminal to control firing rate of neurons (4). Along with this functional complexity, it has been shown that alterations in dopaminergic transmission in the brain are correlated to several dysfunctions such as Parkinson's disease and Schizophrenia (5). These distinctive characteristics make D2R an important candidate for drug targeting and in *silico* drug development (3,5). Although some molecular docking studies have been performed with D2R, there are still a lot of problems and open questions that need a new effort in order to be fully understood. First, there is much incongruence in the way new models are created as the use of different templates and modeling softwares makes it hard for a clear comparison between models attained in different research groups. Second, absolute scores of model evaluation programs are often not shown, making model-building not transparent and comparable enough. Another problem rises from the various binding pockets definitions found in literature. Here, we tried to overcome this issue by using a large set of experimental data from Floresca *et al* (6). We aimed to attain a reproducible pipeline to calculate and evaluate correctly GPCR-models in general, and to D2R in particular.

Materials and Methods

Modelling

The building of the D2R model was performed using MODELLER (7), with D3R complexed with Eticlopride, (Protein DataBank (8) ID 3PBL) as template (9). This crystallographic structure was chosen in accordance with a total sequence similarity of 68%, as calculated with BLAST (10). In addition each TM was then aligned to the TMs of the template, which numeration was obtained at the GPCRdatabase (11). The TMs were checked for sequence similarity and a average value of 77 % was attained for this more relevant and conserved helical bundle. Modelling was performed by specifying the lengths of the TMs and the perimembrane intracellular helix (HX8). Furthermore, disulphide bonds were considered in the pairs of unconserved cysteines at positions 79-154 and 249-251. Further loop refinement was performed when needed, in particular for the extracellular loop 2 (ECL2), since it is a long loop highly determinant for D2R's binding pocket access. Since it is known that the deletion of the intracellular loop (ICL3) middle residues does not affect ligand and G-protein binding (the contact points are normally the N- and C-termini of this loop), residues 214-254 from ICL3 were removed as well as the first 28 residues of the sequence, as there is no template for these regions. A dialanine linker was added to connect TM5 and TM6, which were modelled as helices up to the linker, making the intracellular extension of TM5 and TM6 similar to what is observed in the G-protein bound crystal structure of the β2 receptor (PDBid: 3SN6) (12). 2.50ASP was protonated as it known to interact with K+ binding site, which regulates by allostery the ions that regulate the function of the receptor. Furthermore, a part of the ICL2 (112-114) was set as alpha-helix in MODELLER protocol following the know structure-function experimental data available for this loop from the D3R (9).

Model evaluation

Although visual inspection immediately ruled out some trial models due to biological incoherence on particular and important structural features, selecting the best model was cumbersome. This was particularly true since D2R is a membrane protein, for which the more commonly available metrics are

less reliable. Discrete Optimized Protein Energy (DOPE) (13) and molecules' probability density functions (molpdf) are MODELLER's standard metrics for model assessment, based on the models' free energy and special occupation. These, however, did not allowed us to easily pick the best models, in part since they are mainly directed towards water soluble proteins. For this reason Protein Structure Analysis (ProSA) web service was additionally used for error recognition, in particular the z-score, which indicates overall model quality with respect to an energy distribution derived from random conformations (14). However, as the z-score was still not enough to choose the best model, we extended our analysis by using the online Protein Quality (ProQ) (15) prediction server. This is based on a neural network, the LGScore (16), to predict a p-value for the significance of a structural similarity match and the MaxSub (17) that identifies the largest subset of alpha carbons that superimposes with the template structure. Furthermore ProQ allows for the inclusion of secondary structure information (calculated by PSIPRED (18)) in order to further improve model quality assessment. Together with PSIPRED, ProQ can improve up to 15% its' prediction accuracy. Finally, Ballesteros and Weinstein (19) numbering system for class A GPCRs was applied. The numbering system determines helical numbering (for TM1-7 and HX8) depending on a previously determined most conserved residue in each of the helices, named residue x.50.

Docking Protocol

AutoDockTools, a package of MGLTools was used to perform ligand docking (17). Docking itself was performed using Autodock4.2 (version autodock 4.2.6, released in 2009) (21). D2R hydrogens were added and Kollman united atom charges were assigned. Hydrogens were also added to ligand and Gasteiger-Marsili was used to calculate charges (22). Before docking an energy grid was created using Autogrid (version autogrid 4.2.6, released 2009) with a box-size of 50 Å x 50 Å x 50 Å in dimension with a 0.375 Å-spacing. The grid center was set at 18.235, 17.556, 6.595. For each docking simulation 100 independent Lamarckian genetic algorithm (LGA) runs were performed with the number of energy evaluations set to 10.000.000, the population size set to 200 and the maximum number of generations set to 27.000 (23). Default settings were maintained for the rest of the parameters. Docked conformations within a RMSD of 2 Å were clustered. The most populated and lowest energy cluster was used for conformational binding analysis. According to Floresca *et al.* (6), the following residues are important for ligand binding, and were therefore treated as fully flexible during the docking process, along with all rotatable bonds of ligands: 3.32ASP (ASP86), 5.42SER (SER165), 5.43SER (SER166), 5.46SER (SER169), 6.48TRP (TRP236), 6.51PHE (PHE239), 6.52PHE (PHE240), 6.55HIS (HIS243) and 7.43TYR (TYR266). In this study, we docked Dopamine, the endogenous ligand, Quinpirole, a selective D2R/D3R-agonist and Raclopride, a selective D2R-antagonist. We calculated all distances between the center of mass of the ligand and the alpha-C-atom (Cα) of the flexible residues used in the docking for all the top conformations achieved with AutoDock4.2 in order to attain an initial evaluation of these models (21).

Results and Discussion

Due to the high degree of homology between the D2R and D3R (total similarity 68%), it was suitable to use the recently solved crystal structure of the D3R as template (9,24). The ClustalOmega alignment showed that the chosen TMs for the D2R model were conserved compared to D3R (similarity for TM1: 60%, for TM2: 92.86%, for TM3: 87.88%, for TM4: 68.18%, for TM5: 64.71%, for TM6: 70.59%, for TM7: 84.00% and for HX8: 92.31%). For the D2R model a DOPE-score of -39622.63 and z-score of -2.55 (MODELLER and ProSA-web) were attained. In addition, the model was evaluated with ProQ with and without a PSIPRED secondary prediction LGscore: 3.43/3.78 and MaxSub: 0.12

/0.57, where accurate scores were achieved. Although other D2R models were built, definite scores are not provided here. The calculated z-scores outcome is comparable to the z-score of the template structure (PDBid: 3pbl) (9), -2.37, concluding that the created D2R-model showed no faulty regions and was overall in line with the D3R crystal structure. The utilization of ProQ provides general scores categories for a batter comparison. The following classifications were defined for LGscore: "correct" scores >1.50, "good" scores >3 and "very good" scores >5.00. For MaxSub scores >0.1 are defined as "correct", >0.50 is "good" and scores >0.80 are considered "very good". According to that gradation the created D2R is "good" regarding the LGscore and "correct/good" regarding the MaxSub. Interestingly, the MaxSub improved when applying PSIPRED prediction. All in all, the created D2R model seems valid among various protein evaluation programs.

One of the challenges in building *in silico* models is the identification of the binding pocket. In the case of D2R there was enough experimental data to get a close idea about the 3D localization of the pocket. (6) For binding Dopamine, the following residues were considered as interacting residues: 3.32ASP, 5.42SER, 5.43SER, 5.46SER, 6.48TRP, 6.51PHE, 6.52PHE and 6.55HIS according to Floresca *et al*. (6). Additionally, these residues were considered also in others studies involving molecular docking, but in a different constellation, concluding that they are important for general ligand binding to the D2R (26,28,29). To compare the performance between the endogenous ligand, the selective D2R/D3R-agonist Quinpirole and the selective D2R-antagonist Raclopride, an additional conserved residue, 7.43TYR, was considered as part of the flexible residues in the docking procedure, since some ligands, especially antagonists, are believed to access a Secondary Binding Pocket (SBP) (30,31).

With 100 runs performed, AutoDock4.2 clusters similar docking conformations into groups with the same binding energies. The more conformations are found in one cluster, the higher the possibility of this specific docked position to be the closest to reality. Furthermore, the clusters are ranked by lowest binding energy. For Dopamine, the lowest binding energy achieved was -10.51 kcal/mol (22 conformations in this cluster). Moreover, this was the cluster with the most conformations sorted, concluding that the docking performance of Dopamine was reliable. For Quinpirole the lowest binding energy was -8.11 kcal/mol with 17 conformations counted in this cluster. Again, this was the cluster with the higher number of possible conformations. The selective antagonist Raclopride achieved 17 conformations in the cluster with the lowest binding energy of -8.72 kcal/mol. These conformations for all three ligands are shown in the graphical abstract. Regarding literature for similar results, an average docking score of -10.63 kcal/mol was achieved for Dopamine from Durdagi *et al.* (26) using the software Glide (32) and of -6.6 kcal/mol for Quinpirole in a study of Platania *et al* using AutoDock4.2 (27). For further evaluation, the distance between the center of mass of the ligand and the alpha-carbon (Cα) of each residue was measured. First of all, a strong interaction with 3.32ASP was found for Dopamine as well as for the other two ligands, since the average distance between the Cα-atom of 3.32ASP and the center of mass of the ligand was around 8.47 Å for Dopamine, 7.00 Å for Quinpirole and 6.57 Å for Raclopride. According to literature, the positive aspartic acid performs a salt-bridge with protonable amines of the ligands (26,27). We also observed a strong interaction between the by Floresca *et al.* (6) defined serine microdomain (5.42SER, 5.43SER and 5.46SER) and the catecholamine hydroxyl-groups of Dopamine verified by distances of 8.60 Å, 7.57 Å and 8.50 Å between these serines and the ligand. Compared to that, Quinpirole and Raclopride do not seem to interact directly with that microdomain as greater distances were attained: 9.40 Å, 9.14 Å and 9.97 Å and 10.03 Å, 9.37 Å, 10.60 Å, respectively. Other *in silico* docking studies with Dopamine hypothesized that the serine microdomain maintains a H-bonding network when the receptor is activated by its endogenous ligand (6). According to that, Raclopride should not interact with this microdomain at all, which was confirmed by the elevated distances obtained here.

Conclusions

Since a high-resolution crystal structure of D2R is not yet available, it is a common approach in rational drug design to use *in silico*-generated three-dimensional (3D). To attain an accurate 3D model to be used for molecular docking, it is fundamental to correctly define the disulphide-bridges and lengths of the TMs when utilizing the MODELLER-software. Furthermore, loop refinement is a helpful tool to improve the model afterwards. While incorrectly modelled loop positions at the TMs domains can be easily detected visually (e.g. if the loop is positioned into the TMs region), others errors or more problematic regions could be harder to detect. For that, the assessment of the created models with different protein validation programs like ProSA and ProQ is an important step to attain a better initial model for a particular system. Selecting reasonable flexible residues in the docking protocol is also a crucial step to achieve lower binding energies within the clustered conformations. Molecular docking results obtained here were similar to other studies in literature and makes us confident to have a reproducible pipeline to attain a 3D D2R model suitable for docking small ligands with different activation roles. In brief, this approach is a promising base for small molecular docking studies on the D2R.

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ALPHA-HELICAL AND BETA-SHEET MEMBRANE-MEMBRANE PROTEIN DIMERS: CENTRALIZING INFORMATION

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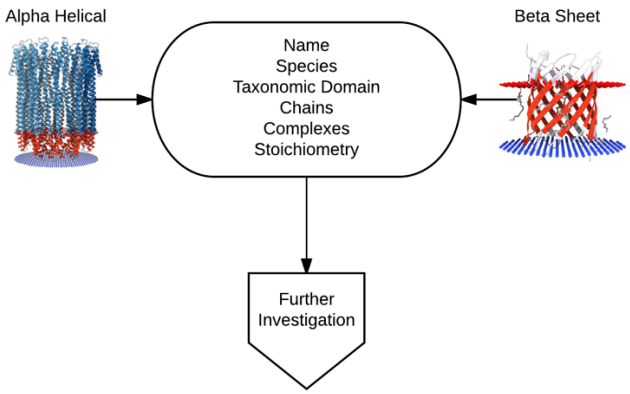
Graphical Abstract



Abstract.

Bioinformatics allows to automatically characterize a large number of proteins from numerous different databases[1], thus, uncovering new possible interactions between biomolecules in a huge set of individuals in a conscious and cost-efficient way[2]. Membrane proteins are indisputably important for the assurance of major processes in the cell, occupying approximately 25% of the whole cell genome[3]. In this work, some of the major features displayed at Protein Data Bank[4] (original species, chains and ligands, oligomer state, multimeric states, stoichiometry, among others) of membrane proteins listed in the Membrane Proteins with Known 3D Structure[5] database were registered together using manual and automated methods - some of these methods include the usage of python specific tools (like Selenium[6] and BioPython[7]). We aimed to construct a membrane-membrane dimers database that will serve as input for data-mining algorithms to unveiling new functional and evolutionary knowledge.

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Notes Towards a Network Approach to Gene Orientation

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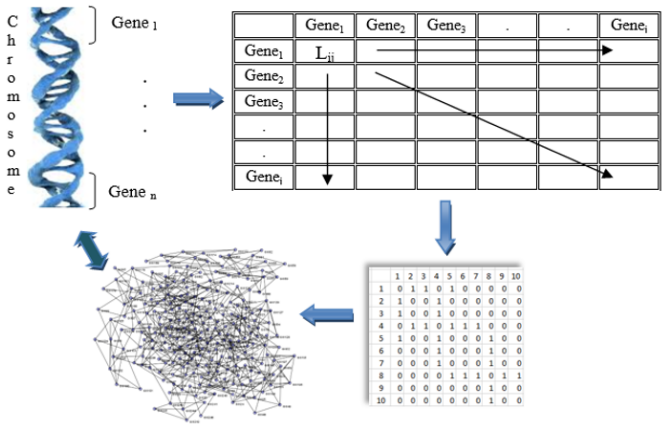
Graphical Abstract *(mandatory)*



Abstract. The distribution of the orientation genes in chromosomes is not random and may have function implications. In this work, we used complex networks to study the orientation of genes. We used as a case of study the 14 chromosomes of *Plasmodium falciparum*. We constructed the respective 14 networks with an average of 383 nodes (genes) and 1314 links (pairs of gene with inverse orientation). Node centralities of these nodes were used to study the structure of the network.

Keywords: Malaria; *Plasmodium sp.* proteome; Chromosome microstructure; Gene orientation; Complex Networks; Machine Learning

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Diyne formation from alkynes in the presence of palladium pincer complexes

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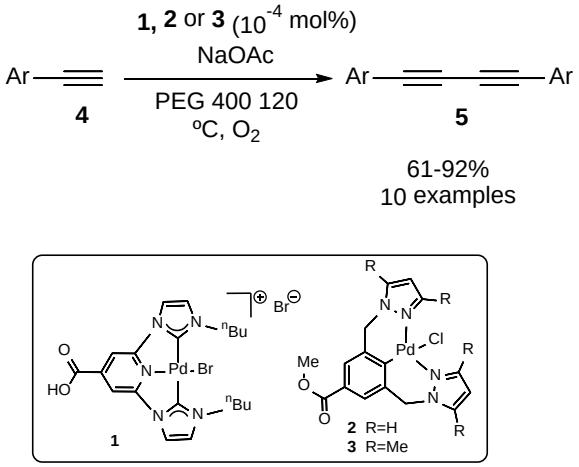
Graphical Abstract



Abstract.

1,3-Diynes are valuable building blocks for biologically active molecules and precursors of polymers, macrocycles and supramolecular structures. The combination of metal catalysts and molecular oxygen is considered a suitable protocol for the direct homocoupling of alkynes leading to diynes. Regarding the catalyst, palladium species have proved to be efficient and selective, but copper salts as co-catalysts and/or stoichiometric amounts of reoxidants are often needed. Palladium pincer complexes are presented as alternative palladium sources for the homocoupling of alkynes in an environmentally friendly solvent as polyethylene glycol, in the absence of any other co-catalyst and under aerobic conditions.

Introduction

1,3-Diyne compounds are important building blocks for many natural, pharmacological active products as well as for oligomers and polymers.[1] Back in the XIX century Carl Glaser[2] reported the synthesis of 1,3-diynes from terminal alkynes through an oxidative process mediated by copper(I) chloride exposed to air. Although classical Glaser conditions for the homocoupling of terminal alkynes is still used, other protocols can be found in the literature about this specific and useful reaction. Regarding the oxidant, even when molecular oxygen[3] is considerate as the most suitable one due to it availability, low cost and harmless by-product, other oxidants have been explored (I_2, $Na_2CO_3 \cdot 1.5\ H_2O$, Ag, chloroacetone, etc.).[4]

In relation to the catalyst and even when other metal have been tested in the afore mentioned transformation[5] palladium catalysed homocoupling of terminal alkynes is considerate the most suitable, but co-catalyst as copper and/or stoichiometric amounts of reoxidants are often required.[6] However, a

few examples about palladium solely mediated homocoupling of alkynes have been reported, but in all of the high amounts of catalytic loading are required.[7] For these reason and taking into account the experience of the group in palladium pincer complexes as catalytic species in organic transformations we envisioned that these kind of complexes could also be suitable in the above transformation and if it is possible in a green media.

Materials and Methods

A round bottom flask equipped with a magnetic stirrer bar was charged with the alkyne (1 mmol), NaOAc (8.0 mg, 0.1 mmol), pincer compound (**1**, **2** or **3**) (20 μL of a 5 x 10^{-5} M solution in PEG 400, 10^{-6} mmol) and PEG 400 (1mL per mmol of the substrate) at room temperature. The system was purged with molecular oxygen, and an oxygen-filled balloon (1-1.2 atm) was connected. The mixture was heated at 120ºC under continuous stirring for 24 hours. The reaction outcome was monitored by ^{1}H-NMR. Upon completion, the mixture was cooled to room temperature and water was added (50 mL aprox.). The resulting solution was acidified with HCl 1M (pH≈1-2) and extracted with Et_2O (4 x 6 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo to give a residue which was purified by flash column chromatography using hexane:ethyl acetate as eluent.

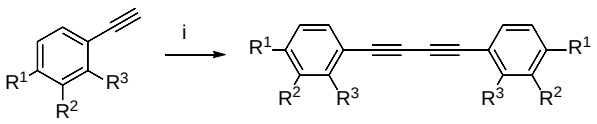
Results and Discussion

In order to improve the catalytic activity of some of the palladacycles prepared in the research group (CNC **1**, NCN **2** and NCN **3**)[8] there were applied in the homocoupling of terminal aromatic alkynes. Phenylacetylene (**4a**) was chosen as model substrate, after a variety of optimization assays in which a catalytic loading of 0.01 mol% of the pincer complexes 1-3 was employed and in which priority was given to environmentally friendly solvents (polyol solvents, water, etc) in order to the typical reaction medias (acetonitrile, DCM, DMF, etc). We decided to use **1**, **2** or **3**, O_2, NaOAc, PEG 400 and 120 ºC and decrease the catalytic loading down to 10^{-4} mol%. In order to verify if addition of a substoichiometric amount of a copper salt could improve results, phenylacetylene was again submitted to the above conditions adding 0.1 equiv. of $CuBr_2$ as co-catalyst, surprisingly no improvement was observed. Blank experiments also confirmed the crucial role of the palladium pincer complexes, since no product was detected when commercially available palladium source was employed or even when the pincer type ligands were tested.

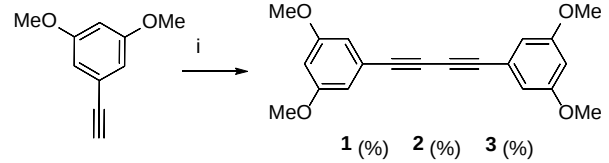
The afore mentioned optimized conditions were respectively applied to a number of aromatic acetylenes, proving good result regardless the nature of the substrate as summaries in Table 1. No steric hindrance was observed in the coupling of *ortho*-substituted aromatic acetylenes. Furthermore, different halogens as F, Cl or Br or relatively acidic protons were also tolerated obtaining good yields in all the cases. Regarding the catalyst, no clear trend or marked differences was observed for complexes **1**-**3**.

Finally, the recycling of the palladium pincer complexes was as well carried out using the homocoupling reaction of the model substrate 4a. To be able to reuse the reaction media it was necessary to replace the workup procedure with an extraction at low temperature (-78 ºC). With this small modification several runs were performed (4 times) in the same PEG media without observing any decrease in the product yield.

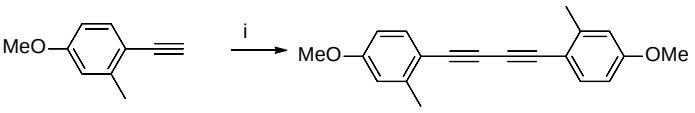
Table 1. Oxidative homocoupling of terminal alkynes in presence of palladium pincer complexes.



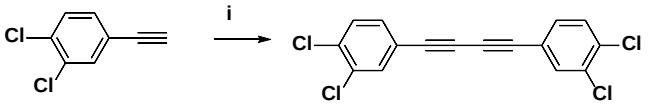
	1 (%)	**2** (%)	**3** (%)
R^1= R^2= R^3= H	83	83	81
R^1= Bu; R^2= R^3= H	62	90	87
R^1= Br; R^2= R^3= H	63	74	74
R^1= R^3= F; R^2= H	92	84	92
R^1= Et; R^2= R^3= H	50	50	61
R^1= OBu; R^2= R^3= H	71	66	74
R^1= R^3= H; R^2= OH	76	81	74



1 (%) **2** (%) **3** (%)
61 64 67

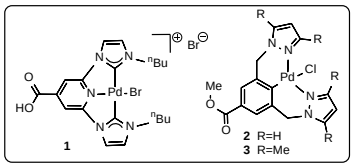


1 (%) **2** (%) **3** (%)
79 79 82



1 (%) **2** (%) **3** (%)
60 60 70

i: O_2 (1 atm), **1**, **2** or **3** (10^{-4} mol%), NaOAc, PEG-400, 120ºC, 24h.



Conclusions

To sum up, palladium pincer complexes constitute a highly active catalytic species for the aerobic homocoupling of aromatic terminal alkynes. In addition, the presence of metal traces in the final product has been thereby minimized to values below legal limits for food and drug products. Furthermore, it has been proven not only that the presented method was performed in a sustainable media but also that the catalysts can be effectively reused.

Acknowledgments

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Efficient preparation of carboxylic acids from alkynes

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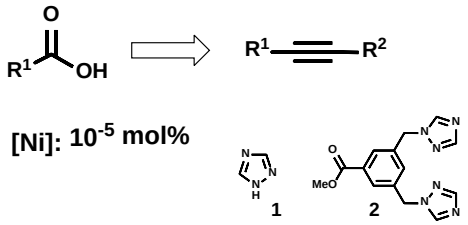
Graphical Abstract



Abstract.

The preparation of carboxylic acids from arylacetylenes has traditionally been carried out by ozonolysis or other harmful oxidants, alone or in presence of some metal catalysts. In spite of the safety, availability and environmental benignity of molecular oxygen, the selective cleavage of unsaturated hydrocarbons mediated by the latter oxidant has been rarely reported. Herein we wish to present the efficient preparation of carboxylic acid derivatives from alkynes employing molecular oxygen as the only oxidant and in the presence of a Ni(II) salt and 1,2,4-triazole ligands.

Introduction

Carboxylic acids are prepared from a variety of oxidative processes[1] including the cleavage of C-C triple bonds, traditionally performed by ozonolysis.[2] However, it requires specific equipments to avoid secondary reactions like explosions. In addition, ozone is considered to be toxic. To avoid the use of the latter oxidant and the necessity of specific equipments new alternatives have been developed. For instance, oxidants as oxone, H_2O_2, tert-butyl hydroperoxide or I(III) reagents[3] have been reported to provide carboxylic acids from alkynes, in most of the cases in stoichiometric amounts. The afore mentioned oxidants could work alone or in presence of a metal catalyst as W, Ru, Os, Fe and In among others.[4] Therefore, the use of molecular oxygen in these context or in any oxidative process avoid the use of harmful chemicals as there could be the mentioned oxidants.

Molecular oxygen is has been considerate as the most sustainable oxidant for many oxidative processes due to it safety, easy availability and low cost. However, to the best of knowledge there is an only example about the utility of dioxygen as oxidant in these kind of transformations. Itoh reported the photooxidative aerobic cleavage of alkynes employing carbon tetrabromide as catalyst under photoirradiation from a 400 W mercury lamp.[5] Following with the experience of the group in the aerobic oxidative processes[6] we envisioned to perform the preparation of carboxylic acids from alkynes

in presence of a nickel(II) salt and a 1,2,4-triazole derivative ligand as catalytic media under molecular oxygen atmosphere.

Materials and Methods

A round bottom flask equipped with a magnetic stirrer bar was charged with the alkyne (1 mmol), NaOAc (8.0 mg, 0.1 mmol), NiBr2 (20 μL of a 5 x 10-6M solution in PEG-400, 10-7 mmol), **1** or **2** (20 μL of a 5 x 10-6M solution in PEG-400, 10-7 mmol) and PEG 400 (1 mL) at room temperature. The system was purged with molecular oxygen, an oxygen-filled balloon (1-1.2 atm) was connected. The mixture was heated at 120 ºC under stirring for 48 h. The reaction outcome was monitored by ^{1}H-NMR. Upon completion, the mixture was cooled to room temperature and water was added (50 mL aprox.). The resulting solution was acidified with HCl 1M (pH≈1-2), extracted with Et2O (4 x 6 mL) and the combined organic layers were washed with brine, dried over anhydrous Na2SO4 and evaporated in vacuo to give a residue which was purified by flash column chromatography using hexane:ethyl acetate as eluent.

Results and Discussion

With the only intention of expanding our experience on the aerobic oxidative process, and following with the unexpected results obtained in the oxidation of 3-phenyl-2-propyn-1-ol and 1-phenyl-2-propyn-1-ol[6b] in presence of nickel(II) bromide and 1,2,4-triazole derivative ligand, in which instead of obtaining the corresponding oxidation product, 3-phenyl-2-propynoic acid and 1-phenyl-2-propyn-1-one respectively benzoic acid was observed, we decided to continue with the aerobic cleavage of alkynes. According with the initial assays carried out on the aerobic cleavage of phenylacetylene we decide to use $NiBr_2$, **1** or **2** and NaOAc in an envaironmentally friendly solvent as it is PEG 400 for a heating (120 ºC) during 48 hours under atmospheric pressure of O_2. After a decrease of the catalytic amount we decided to continue with an amount of 10^{-5} mol% of Ni and **1**/**2**. Blank experiments showed not only that the metal was essential to cleavage selectively phenylacetylene but also that 1,2,4-triazole derivative ligands **1** or **2** were necessary.

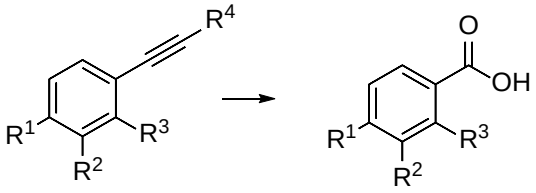
To test if other alkynes could also selectively cleavage to the corresponding carboxylic acid a variety of commercially available arylacetylenes were submitted under the optimized conditions as summaries in Table 1. As it can be appreciate many arylacetylenes were successfully cleavage to the corresponding benzoic acid with excellent results in most of the cases regardless the electronic and steric nature of the arylacetylene, with the only exception of the 1,4-diethynylbenzene.

In respect to the catalytic system Ni/**1** or Ni/**2** differences between both could be appreciated, being the catalytic system formed by $NiBr_2$ and tricoordinated ligand **2** the most efficient one. In some cases when the other catalytic system, Ni/**1**, was employed the oxidative cleavage failed to provide the corresponding oxidized products, a result that was not altered by lengthening reaction times or increasing catalytic loadings.

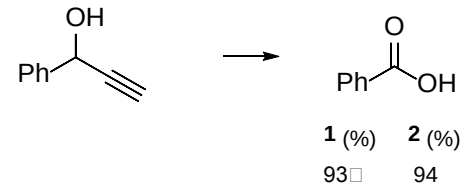
Even more, the novel protocol has not only been successfully applied to substrates bearing halogen (F, Br, Cl), alkyl, carboxy, keto and ester moieties, but also it was useful to carried out the aerobic cleavage of phenylacetylene in gram scale. In addition to the fact that oxygen mediates this transformation at atmospheric pressure, it should be also pointed that the reaction is conducted in an environmentally friendly solvent, PEG-400, widely used as a very convenient media for chemical and medicinal purposes.

The role of this solvent in the reported oxidative process might be related to its unusual coordinating properties, similar to those of crown ethers.[7]

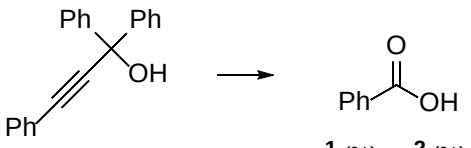
Table 1. Oxidative cleavage of alkynes.[a]



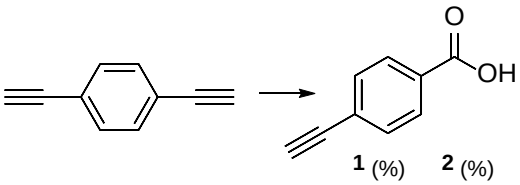
	1 (%)	**2** (%)
R^1= R^2= R^3= R^4= H	90	92
R^1= Bu; R^2= R^3= R^4= H	89	91
R^1= Br; R^2= R^3= R^4= H	-	79
R^1= R^3= F; R^2= R^4= H	68	71
R^1= OMe; R^2= R^4= H; R^3= Me	88	97
R^1= R^2= Cl; R^3= R^4= H	85	90
R^1= R^2= R^3= H; R^4= Ph	-	96
R^1= R^2= R^3= H; R^4= CH_2OH	80	94
R^1= R^2= R^3= H; R^4= Me	-	88
R^1= R^2= R^3= H; R^4= COOH	72	75
R^1= R^2= R^3= H; R^4= COOEt	60	90
R^1= R^2= R^3= H; R^4= Bz	82	86
R^1= COMe; R^2= R^3= H; R^4= Ph	34[c]	46[c]



1 (%) **2** (%)
93 94



1 (%) **2** (%)
50[b] 60[b]



1 (%) **2** (%)
20 23

[a] Reaction conditions: O_2 (1 atm), $NiBr_2$ (10^{-5} mol%), **1** or **2** (10^{-5} mol%), NaOAc, PEG-400, 120ºC, 48h; [b] Benzoic acid was also obtained (35% from **1** and 47% from **2**). [c] Benzophenone was also isolated (30% from **1** and 20% from **2**).

Conclusions

To sum up, the presented protocol not only showed to be suitable to carried out the selective aerobic oxidation of arylacetylenes to corresponding benzoic acids, but also to be respective with the environment, associated to the use of molecular oxygen as only oxidant and in an ecofriendly solvent as it is PEG 400. Even more, the use of infinitesimal amounts of metal/ligand system allow isolation of "*metal-free*" compounds for pharmaceutical uses. These features is close to those exhibited by oxidase enzymes.

Acknowledgments

This research was supported by the Basque Government (IT-774-13) and the Spanish Ministry of Economy and Competitiveness (CTQ2013-46970-P). G.U thanks the University of the Basque Country for a postdoctoral scholarship. Finally, technical and human support provided by SGIker of UPV/EHU is gratefully acknowledged.

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Prediction of RIFIN proteins with gene orientation network indices

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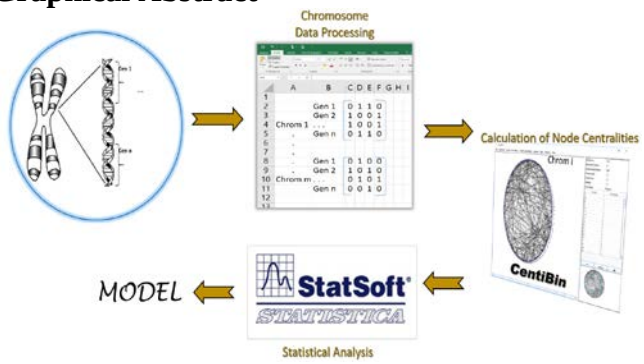
Graphical Abstract



Abstract. Gene orientation may have a direct influence on the expression of genes. In this work, we developed a Linear Discriminant Analysis (LDA) model able to predict RIFIN-like proteins of out of 5365 of *Plasmodium falciparum* proteins with Sensitivity and Specificity 70-80% in training and external validation series.

Keywords: Malaria; *Plasmodium sp.* proteome; Chromosome microstructure; Gene orientation; Complex Networks; Machine Learning

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A more efficient catalyst for the cycloisomerization of alkynoic acids

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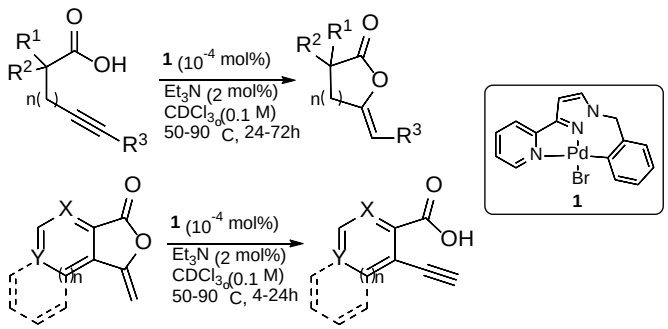
Graphical Abstract



Abstract.

A number of terminal and internal alkynoic acids with different substitution patterns have been efficiently cycloisomerized to the corresponding exocyclic enol lactones in the presence of low amounts of a non-symmetric NNC palladium complex and a catalytic amount of triethylamine.

Introduction

According to atom economy, the cycloisomerization of alkynoic acids is an advantageous option to synthesize γ or δ-alkyliden lactones, structures present in several natural products, biologically active compounds[1] and valuable synthetic intermediates.[2]. A number of transition metals have been employed as catalysts in this reaction.[3] However, relatively high catalyst loadings (10-0.01 mol%), prolonged reaction times and/or high temperatures are often required. In this context, the development of more efficient catalysts that overcome the above limitations has attracted much attentiont.[4] Herein we report the application of non-symmetrical NNC pincer complex **1** (Figure 1) to the cycloisomerization reaction of alkynoic acids.

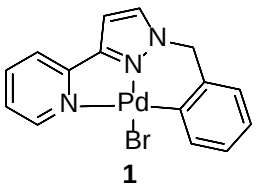


Figure 1. Structure of the NNC type palladium catalyst used in this study.

Materials and Methods

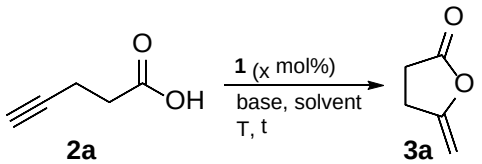
The alkynoic acid **2** (0.2 mmol), triethylamine (25 mL of a 0.16M solution in $CHCl_3$, $4x10^{-3}$ mmol), palladacycle 1 (50 mL of a $4x10^{-6}$M solution in $CHCl_3$, $2x10^{-7}$mmol) and $CDCl_3$ (2 mL) were placed in a screw-capped tube and heated in an oil bath at the indicated temperature for an appropriate time. The reaction mixture was subsequently filtered through a short plug of silica gel to remove triethylamine,

thus providing pure lactone **3**, or alternatively, purified by flash column chromatography using hexanes:EtOAc (7:3) in the referred cases. The progress of the reaction was monitored by ^{1}H NMR.

Results and Discussion

We began our research exploring the cycloisomerization of 4-pentynoic acid **2a** into 5-methylenedihydrofuran-2(3*H*)-one **3a**(Table 1). We used deuterated solvents in order to observe easily the formation of the target lactone in the reaction medium. We set the initial catalyst loading and reaction concentration at 10^{-2} mol% and 0.1 M. These initial assays shown that, on one hand, the presence of a base and, specifically, the presence of triethylamine was crucial to obtain the lactone (entry 1-6). Fortunately, a catalytic amount of this base was enough to achieve good results (entry 10). Moreover, moderate heating was necessary to obtain acceptable yields (entry 8 vs entry 5). On the other hand, deuterium chloroform shown to be a good solvent for this reaction since poor yields were obtained when dichloromethane was used (entry 8 vs 9). Finally, we probe that the use of palladium catalyst was indispensable to cycloisomerize the acid (entry 18,19). This catalyst shown to be highly efficient since just a 10^{-4}mol% provided the target lactone in excellent yield (entry 16, 17), although longer reaction times and higher temperatures were needed. Considering all studied parameters, we selected the conditions shown in Table 1, entry 14.

Table 1.Cycloisomerization of 4-pentynoic acid in the presence of palladium pincer complex **1**[a]



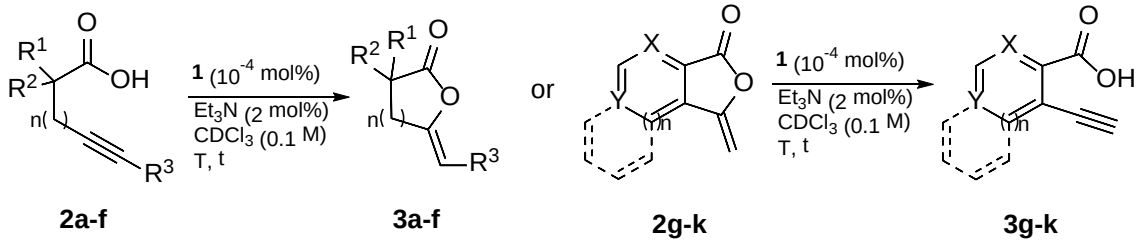
Entry	Solvent	**1** (mol%)	Base	T (ºC)	T (h)	Conversion (%)[b]
1	$CDCl_3$	10^{-2}	---	rt	2	---
2	CD_3COCD_3	10^{-2}	---	rt	2	---
3	$CDCl_3$	10^{-2}	K_2CO_3 (5 mol%)	rt	2	---
4	$CDCl_3$	10^{-2}	Et_3N (5 mol%)	rt	2	27
5	CD_3COCD_3	10^{-2}	Et_3N (5 mol%)	rt	2	---
6	$CDCl_3$	10^{-2}	KO-t-Bu (5 mol%)	rt	2	---
7	$CDCl_3$	10^{-2}	Et_3N (5 mol%)	90	12	99
8	$CDCl_3$	10^{-2}	Et_3N (5 mol%)	50	12	99
9	CH_2Cl_2	10^{-2}	Et_3N (5 mol%)	50	12	11
10	$CDCl_3$	10^{-2}	Et_3N (2mol%)	90	12	99
11	$CDCl_3$	10^{-2}	Et_3N (10mol%)	90	12	70
12	$CDCl_3$	10^{-2}	Et_3N (2mol%)	50	12	99
13	$CDCl_3$	10^{-3}	Et_3N (2 mol%)	50	24	99
14	$CDCl_3$	10^{-4}	Et_3N (2 mol%)	50	24	99
15	$CDCl_3$	10^{-5}	Et_3N (2 mol%)	50	24	83
16	$CDCl_3$	10^{-5}	Et_3N (2 mol%)	90	12	99
17	$CDCl_3$	10^{-4}	Et_3N (2 mol%)	50	12	72
18	$CDCl_3$	---	Et_3N (2 mol%)	50	24	---
19[c]	$CDCl_3$	10^{-4}	Et_3N (2 mol%)	50	24	---

[a]Reaction conditions: 4-pentynoic acid **x** (0.2 mmol), solvent (0.1M). [b]Conversion rate determined by ^{1}H NMR spectroscopy. [c]$Pd(OAc)_2$.

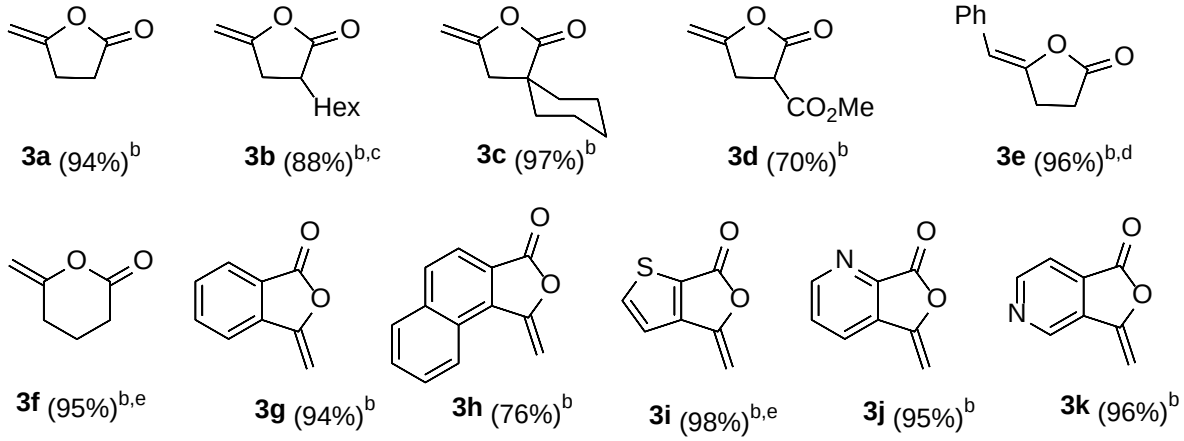
Employing mentioned conditions, we synthetized a series of enol lactones. As shown in Table 2, our protocol tolerated the presence of different substituents at the α-position to the carboxy group (compounds 3**b**-**3c**), although higher temperature were required. It is worth mentioning that benzylidenelactone**3e**was obtained with complete diastereoselectivity. On the other hand, rigid aromatic

and heteroaromatic 4-alkynoics acids (**acids 2g-2k**) were also cycloisomerized under optimized reaction conditions in excellent yields.

Table 2. Cycloisomerization of alkynoic acids in the presence of palladium complex **1**.[a]



2a-f **3a-f** **2g-k** **3g-k**



3a (94%)[b] **3b** (88%)[b,c] **3c** (97%)[b] **3d** (70%)[b] **3e** (96%)[b,d]

3f (95%)[b,e] **3g** (94%)[b] **3h** (76%)[b] **3i** (98%)[b,e] **3j** (95%)[b] **3k** (96%)[b]

[a] Reaction conditions: alkynoic acid **x** (0.2 mmol), palladium complex 1 (10^{-4}mol%), Et3N (2 mol%), $CDCl_3$ (2 mL), 50 °C, 24h. [b] Isolated yields. [c] 70 °C.[d] 72 h. [e] 90 °C. [f] 4h.

Conclusions

In conclusion, γ- alkylidene lactones can be easily obtained through the cycloisomerization of the corresponding acetylenic acid in the presence of very low amounts (10^{-4}mol%) of palladium pincer complex **1**. This procedure shows tolerance to a variety of functional groups on the α position of the acetylenic acids.

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Enaminoketone derivatives as key intermediates for the synthesis of 4-quinolones

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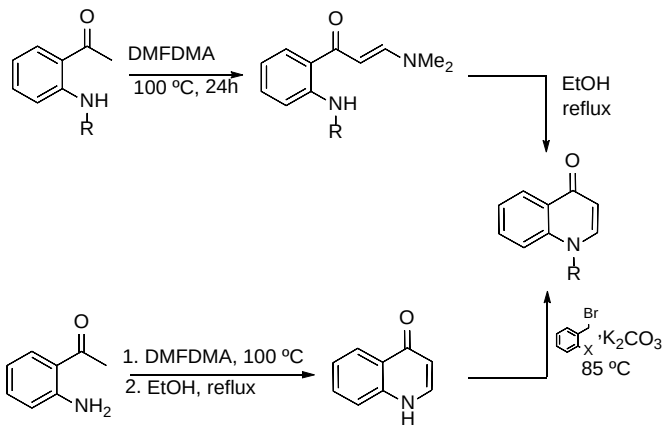
Graphical Abstract



Abstract

N-substituted 4-quinolones can be obtained in good yields *via* intramolecular amine exchage reaction from *N*-substituted (E)-1-(2-aminophenyl)-3-(dimethylamino)prop-2-en-1-ones or, alternatively, from (E)-1-(2-aminophenyl)-3-(dimethylamino)prop-2-en-1-one through intramolecular amine exchange/N-benzylation. Both strategies can lead to *N*-(2-halobenzyl)-4-quinolones, functionalized substrates which could undergo further transformations to provide interesting polyheterocycles.

Introduction

Many natural products and synthetic compounds with diverse pharmacological properties share quinolinone *core* structure (Scheme 1).[1] Quinolinone skeleton has been traditionally synthetized by methods that need harsh reaction conditions to effect final cyclization and show poor regioselectivity such as Gould–Jacobs reaction or Conrad-Limpach synthesis.[2]

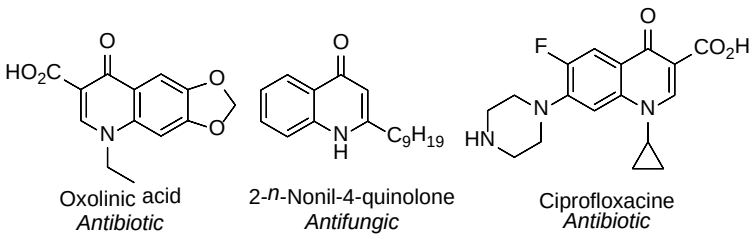
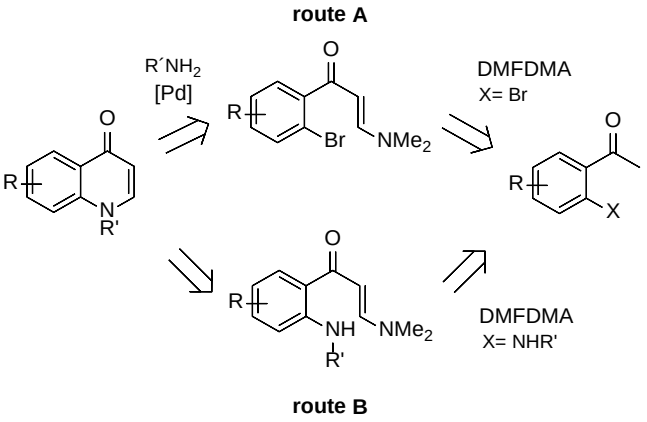


Figure 1: 4-Quinolinone containing compounds with pharmaceutical activity

Recently, synthetic strategies based on amine exchange of enaminoketones have allowed much milder reaction conditions and better regioselectivities.[3] Taking advantage of our previous experience in this research subject,[4] we designed two simple synthetic routes for the access to *N*-aryl and *N*-

benzylsubstituted 4-quinolones, potential precursors of more complex polyheterocyclic systems (Scheme 1). Both strategies were based on an amine exchange reaction, however, while in route A, the quinolinone ring would be provided by intramolecular *N*-arylation of the intermediate enaminoketone, in route B, the intramolecular amine exchange reaction would effect the cyclization itself.

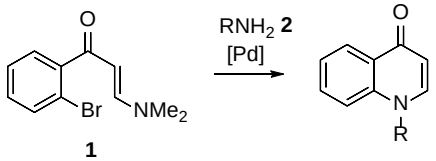


Scheme 1. Designed pathways to synthesize 4-quinolones from 1-aril-3-dimetilamino-2-propen-1-ones

Results and Discussion

We began our research by testing designed route A. After synthetizing required enaminoketone **1** by reaction of 2-bromophenylacetophenone with *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA), a number of reaction conditions was assayed in order to achieve the amine exchange/intramolecular *N*-arylation tandem sequence that would provide the desired 1-substituted-4-quinolinone (Table 1). Different palladium (0) and palladium (II) sources were tested in combination with phosphine-type quelating ligands , commonly used in *N*-arylation reactions, such as BINAP or DPPF. Regarding the base, we used NaO^tBu, Cs_2CO_3 or K_3PO_4 and aniline and benzylamine were the amines of choice in these assays.

Table 1. Selected assays for the reaction of dimethylamino-2-propen-1-one **1** with amines **2**



1

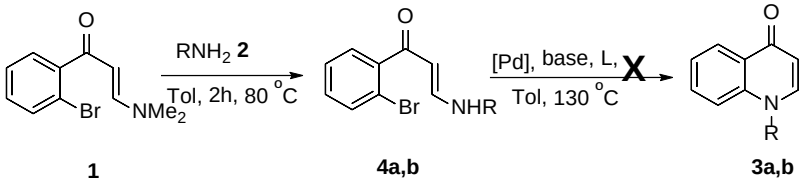
3a R= Bn
3b R= Ph

Entry	Reaction conditions	3a (%)[a]	3b (%)[a]
1	1.0 eq. **1**, 1.4 eq. **2**, 1.6 eq. K_2CO_3, 1.6 eq. NaO^tBu, 10% $Pd(PPh_3)_4$, 3 ml/mmol Tol, ,2h	traces	30%
2	1.0 eq. **1**, 1.4 eq. **2**, 0.08 eq. DPPF, 1.6 eq. NaO^tBu, 6% $Pd(dba)_3$, 3 ml/mmol Tol, 24h	20%	20%
3	1.0 eq. **1**, 2.0 eq. **2**, 1.6 eq. K_3PO_4, 0.08 eq. BINAP, 5% $Pd(OAc)_2$, 10 ml/mmol Tol, 4 ml/mmol H_2O, 5h	10%	13%
4	1.0 eq.**1**, 2.0 eq. **2**, 1.6 eq. K_3PO_4, 0.08 eq. BINAP, 5% $Pd(OAc)_2$, 10 ml/mmol Tol, 22h	27%	12%

[a] *Yield calculated by ^{1}H NMR employing 3,4,5-tricholoropyridine as internal standard.*

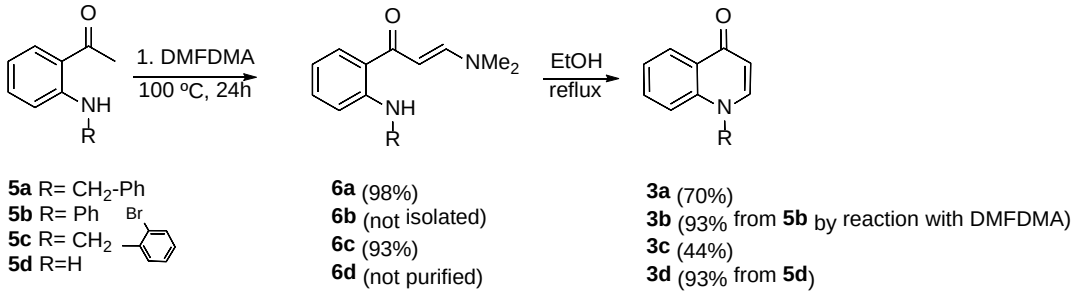
Unfortunately, only in few occasions 4-quinolinone was obtained and, in all cases, in poor yield (Table 1). The use of $Pd(PPh_3)_4$ as palladium source and a combination of K_2CO_3 and NaO^tBu provided the

N-phenyl-4-quinolinone **3b** in 30% yield. On the other hand, the best result for the *N*-benzyl-4-quinolinone **3a** was obtained when $Pd(OAc)_2$ was used in presence of BINAP and toluene as reaction medium. In order to improve the yields we tested the stepwise version but, unfortunately, in no case was the 4-quinolinone detected (Scheme 2).



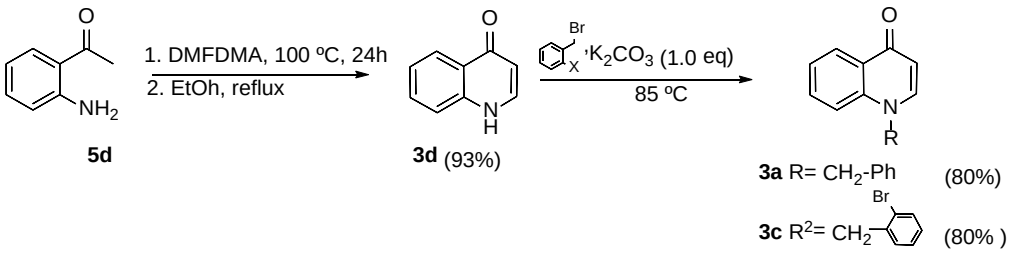
Scheme 2. Stepwise synthesis of 4-quinolinones fron enaminoketone **1**

Considering the poor results obtained, we decided to focus our efforts on the route B (Scheme 1). As usual, required enaminoketones were prepared from the corresponding 2-aminophenylacetophenone by reaction with DMFDMA. Surprisingly, in the case of *N*-phenylamine, the reaction with DMFDMA provided 1-phenylquinolin-4(1H)-one **3b** in an excellent yield. In the rest of the cases, the corresponding 4-quinolinone was obtained by refluxing in toluene. 4-quinolone **3d** was prepared in excellent overall yield from 2-aminoacetophenone. In the case of *N*-benzylaminofenil enaminoketone **3a** and *N*-phenyl enaminoketone **3c**, the corresponding quinolinones were obtained in lower overall yields, 69% and 41%, respectively.



Scheme 3. Synthesis of 1-substituted-4-quinolinones reaction from N-substituted enaminoketones **6** by intramolecular amine exchange

With the aim of improving global yields and, taking into account that the substituent in the nitrogen of the aniline precursor **6** seemed to have a negative effect on the intramolecular amine exchange reaction, we decided to introduce said substituent after carrying out the cyclization. (Scheme 4). The aminomethynelation/amine exchage/N-alkylation sequence allowed us to improve the previous results and we obtained quinolinones **4a** and **4c** in 74% overall yield.



Scheme 4.Synthesis of quinolinones **3a** and **3c** by N-alkylation of 4-quinolinone **3d**

Conclusions

The sequence amine exchange/intramolecular *N*-arylation has provided the N-substituted 4-quinolones with modest yields so far and, therefore, further research is needed in order to improve the results.

On the other hand, N-substituted 4-quinolones can be obtained in good yields *via* intramolecular amine exchage reaction from N-substituted (E)-1-(2-aminophenyl)-3-(dimethylamino)prop-2-en-1-ones or, alternatively, from (E)-1-(2-aminophenyl)-3-(dimethylamino)prop-2-en-1-one via intramolecular amine exchange/N-benzylation. Both strategies can lead to *N*-(2-halobenzyl)-4-quinolones, functionalizated substrates which could undergo further transformations to provide interesting polyheterocycles.

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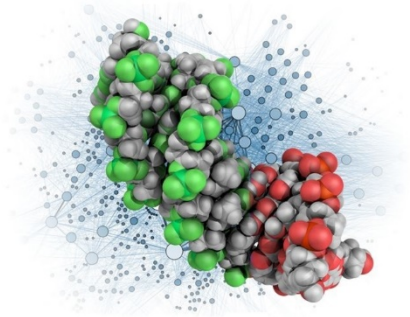
Acknowledgments

We thank Basque Government (IT-774-13) and the Spanish Ministry of Economy and Competitiveness (CTQ2013-46970-P) for financial support. Finally, technical and human support provided by SGIker of UPV/EHU is gratefully acknowledged..



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Mapping the conformational and structural regulators involved in the inhibition of the human 20S proteasome inhibitors

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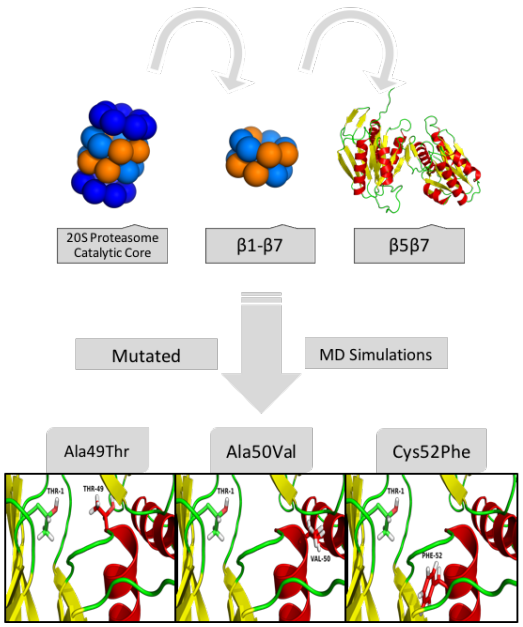

Abstract. *(mandatory)*

The Ubiquitin Proteasome Pathway (UPP) plays a pivotal role in intracellular protein degradation and turnover in eukaryotic cells.[1] Therefore, modulation of the UPP emerged as a rational therapeutic approach in cancer, neurodegenerative diseases (Alzheimer, Parkinson), inflammatory pathologies (arthritis, psoriasis, asthma, colitis), organ transplant, infective diseases (malaria), among others.[2]

During the last two decades academia and pharmaceutical industry made huge efforts to develop natural and synthetic proteasome inhibitors (PI). In 2003 FDA approved the pioneering dipeptidyl boronic acid derivative PI bortezomib for the treatment of refractory multiple myeloma (MM) and subsequently frontline therapy for MM. However, despite the enormous potential of PI, their use is still limited to certain types of blood cancer and shows severe side effects, dose limiting toxicity, peripheral neuropathy, limited activity in solid tumour and innate or acquired drug resistance.[3]

In this work, we have used Molecular Dynamics (MD) simulations to perform the first conformational and structural characterization of the human native 20S proteasome structure[4]. We focused our analysis on the three catalytic subunits well known for their proteolytic activity (β1, β2 and β5) and we further extended our study to additional MD simulations of three different point mutations in the β5 catalytic subunit, with recognized importance in PI's resistance: Ala49Thr, Ala50Val and Cys52Phe. Hopefully, our studies will be able to shed the light on the structural key determinants that regulate the observed PI's resistance in

	the different mutations, and ultimately use the acquired knowledge in the development of new alternative and efficient proteasome inhibitors.

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IMPORTANCE OF COACHING AND MENTORING TOOLS FOR TRAINING BUSINESS LEADERS

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Abstract

Due to commercial globalization, the market goes through constant changes, which increasingly demands that company servers keep up with these changes, along with organizations. The business team leadership is of paramount importance for the employees' team progress and, because it is not an easy task, it must be performed by suitably competent professionals. Based on this context, this study proposed to approach the theoretical aspects, through a bibliographical review of the coaching and mentoring tools, which are extremely relevant for the new leaders' formation and the teams' management within companies.

Introduction

Leadership, when well executed, has an extreme relevance to the progress of an individuals group, for through it, it is possible to conduct the energy application in people, guiding the leaders' steps and synchronizing their efforts. Because it has a real impact, leadership represents the fundamental indicator of a company's potential, since it is not limited only to financial results, which indicate only where the company has been. A strong leadership makes a business organization even better in many ways.

Nowadays, what is expected of a collaborator at work is different from what was expected a few years ago. Due to the market dynamism, brought about by an economic, political and technological globalization, societies undergo major structural changes, which emphatically affect the modes of organization and the business decisions. These organizational variations require individuals change processes involved in the work, mainly at the behavioral level. However the organizations, driven by the changes, need a precious elasticity to adapt to the new market demands [2].

Within this context, companies need to become organizational learning systems where collective work (in a team) is prioritized, and employees can improve their autonomy sense and self-actualization, so that the limited individuals' feelings are excluded. In this landscape, coaching and mentoring become established as high importance tools for behavioral self-correction and learning within companies [3].

Coaching and *mentoring* can inspire and empower employees develop commitment, increase productivity, increase talent, and promote success. They are now essential modern managerial practice elements. However, many companies have not yet established these techniques applicability schemes, and by not developing them, they also fail to capture the experience and personal employees' knowledge [4, 5].

Although the effectiveness of coaching and mentoring techniques is well established [6], there are still few studies available in the literature that clearly show the

subject, which makes an objective approach to the subject extremely relevant. Thus, this study had as objective to address the importance of *coaching* and *mentoring* from the main aspects directed to the teams leadership.

Coaching

The term *coaching* is originated from the word *coach*, meaning a transport, a vehicle to transport people from one place to another and it was used for the first time where, today is Hungary [7, 8]. In fact, the word continues to convey that same meaning in the present day. The *coach* is literally a vehicle that transports a person or a group of people from one source to another desired destination. *Coaching* is a methodology that has been used for several years by countries such as France and the United States, and is currently at its peak in several other countries, such as Brazil and Spain; and consists in the creation of new paradigms with the purpose of giving innovative results for a certain need. It can be said that through the use of *coaching* there is the possibility of increasing our potential and realizing what skills, tools and resources we can use to overcome obstacles [9].

Already in the 80's, in the United States, the word *coaching* concept came to represent a professional area within organizations. At that time the economy went through a deep development within the services section with the break in the production molds and an increase of the social and economic complexity. The information phenomenon overcame the industrial era and managers came to realize that knowledge would have a higher value at the financial results origin [10].

The main focus of coaching is to contribute about the individual's progress so that he or she solves their problems and translates what they have learned into positive and meaningful outcomes for themselves and the team they lead. In this way, his knowledge is extended to his work group and, consequently, to the organizational collectivity [11].

Coaching has expanded in recent years, both in Brazil and in the world. In a recent survey, it was shown that 85% of European companies and 95% of those located in the UK use *coaching* as a tool for professional development. From the Fortune 500 companies, 40% use *coaching*, of which 99% stated that the tool can bring concrete benefits to individuals and companies, while 96% said that *coaching* is an effective way to promote entrepreneurial learning [12].

Due to this breadth of *coaching* in recent years, the theme has attracted the researchers' interest from different areas and places in the world, which is justified because, among other facts, *coaching*, as a practice of professional orientation, is moving a growing industry of consultants who offer training and qualify professionals as coaches. And this has drawn attention, because although the international scientific production on the subject is increasing, mainly from the year 2000, many researchers still question the validity of the tools [13].

Nowack and Wime (1999) propose *coaching* as an alternative so that the executive with few leadership skills understands their failures, works out the questions and improves their leadership habits. Initially, one must ascertain the applicability of *coaching* in the organization. This task can be guided by the elaboration of a project or plan of action, where the company needs are identified, the individual role in the organizational context, its strengths, the areas that need to be improved and the specific issues that must be faced. With the *coaching* applicability positive response, one starts with the execution of the process itself [14].

In a study published by Goldsmith, Lyons and Freas (2000) [15], some factors considered strategic in the corporate environment, responsible for the current and future progress of several organizations, have been identified. Goldsmith, Lyons, and Freas (2000) [15] present *coaching* through a broad vision that unites the individual ambitions, work teams, and organization. Among the factors that the authors put as strategic points, team leadership is included, which conceptually is very different from that traditional boss. Goldsmith, Lyons, and Freas (2000) [15], in bringing leadership as strategy, emphasizes that the *coach* should respect people as individuals and not merely as gears of a machine, and lead people to success by uniting the way in which they want to work the way they have to work, with a directive and delegation to the activities. These are contexts that give *coaching* visibility and strategic status.

Mentoring

Like *coaching*, *mentoring* is also being applied in large, medium and small organizations to form new leaders. Literature records that the origins of the term mentor date back to Homer's odyssey and originated in the legendary Trojan War when Odysseus, King of Ithaca, went to the front lines and conferred the his family care on the figure of the a slave named Mentor, who worked as a teacher and adviser to his son Telemachus. Therefore, the word mentor would serve to designate a counselor, friend, teacher, and wise man [16].

Unlike the vocational guidance processes, which can be used in different contexts and whose beneficiaries are citizens of any age and with any need for vocational support, *mentoring* processes generally have their applicability restricted universe to professionals already inserted in the work world. In these processes, the mentor functions can either be performed by an external consultant or by an experienced professional belonging to the company's staff [16].

Mentoring, like so many other important business management tools, is also closely related to organizations' needs to meet global challenges in quality terms, productivity and competitiveness. This posture necessarily requires large doses of training and all employees' development at different organizational levels in which they operate, especially in the formation of a new leader [17].

However, *mentoring* is not only limited to the internal environment of an organization, but can also extend outwards. External *mentoring* can mean a business-

to-business relationship and include learning and sharing information between companies and other partners. These relationships can be critical to the company's business success and become a vital resource to help communities. External *mentoring* can also be a manifestation or extension of the organization's social responsibility [17]

Unlike *coaching*, *mentoring* is a process in which the *mentee*, or participant in the process, learns about the organization culture where it is embedded. A more senior leader of this organization decides to be a leader of the new leader or new employee, who can be appointed as a high potential leader. The mentor's job is to prepare him for promotion or increasing his responsibility by making fine adjustments to behavioral characteristics or performance, increasing his exposure to other areas of the organization so that other peers recognize this performance promise. *Mentoring* is not a tool applied to correct immediate problems, because to correct these types of problems, be it behavior or performance, the most appropriate method is *coaching* [19].

Silva (2010) states that *mentoring* methods can be presented in two ways: formal or informal. Informal modes appear spontaneously due to similar interests or interests between two individuals. On the other hand, formal processes are usually developed and administered by a previously defined mentor [16].

Conclusions

This study presented some important aspects of *coaching* and *mentoring* methods for the new leaders formation. It was observed that, although the present work focuses on the formation of the new leader, it is evident that the techniques presented here can be applied in several ways, including by the leaders (already trained) in personal management.

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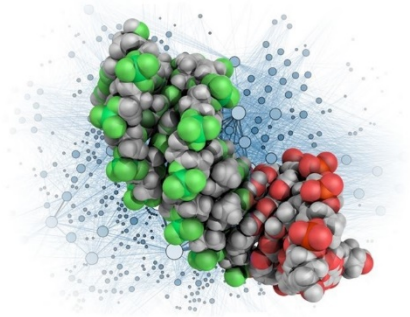

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Characterization of the membrane permeability of different proteasome inhibitors using molecular dynamics methods

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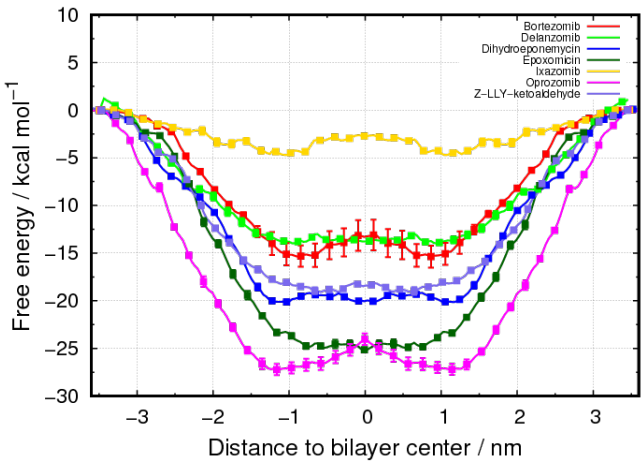
Graphical Abstract *(mandatory)*



Abstract. *(mandatory)*

Protein degradation is a key function developed by organisms to remove damaged and abnormal proteins, preventing their accumulation, and serving at the same time to regulate cellular processes by removing enzymes and regulatory proteins that are no longer needed.[1] This regulatory process can be achieved through two independent pathways: proteolysis in lysosome, or a ubiquitin-dependent process targeting unwanted proteins to proteasome. Due to its shattering function, proteasome has constituted an important therapeutic target to the control of different diseases such as malaria, cancer, multiple sclerosis, psoriasis, among others.[2] Since this protein can be found both on the cell cytoplasm and nucleus, inhibitors developed to target it, must be able to cross the membrane lipidic barrier. Until now, it is unclear if transport involves simple passive diffusion or occurs via a yet unidentified transport system. In both scenarios, associations with the cell wall and the membrane are to be expected. Modeling the interaction of different inhibitors derivatives with the cell wall is not feasible because of its complicated and variable structure. However, it is possible to model and compare the interactions of different proven proteasome inhibitors with a lipid bilayer.

In this work, by using restrained (Potential of Mean Force - PMF) and unrestrained Molecular Dynamics simulations at

	the water/membrane interface, we have evaluated the membrane permeability rates of different proteasome inhibitors (available on the market and identified in our lab) and their configurational and positional preference in this mixed medium. Our results will allow us to compare the trafficking of the evaluated compounds through the cell membrane and to relate it with the proteasome inhibition efficiency. *Acknowledgements:* We thank the Fundação para a Ciência e a Tecnologia for financial support through PTDC/QEQ-MED/7042/2014, UID/DTP/04138/2013, and SAICTPAC/0019/2015.

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The Effect of Fictional Literature on Empathy in Children

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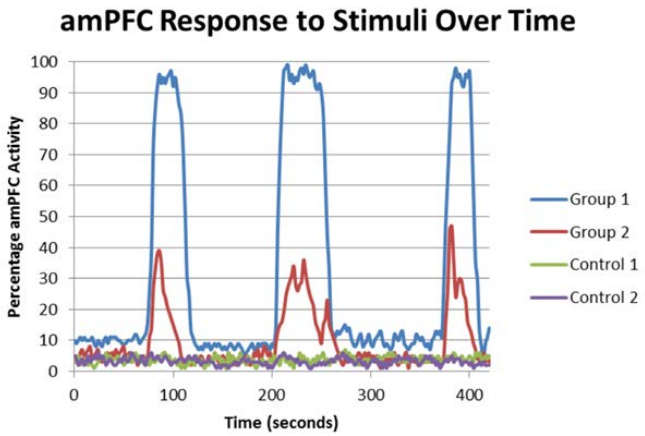
Graphical Abstract *(mandatory)*



Abstract

The purpose of this paper is to identify the correlation between reading fictional literature and an increased level of empathy in children. Using an fMRI machine this paper studies the level of empathy that children who read significantly more or significantly less than one another experience while listening to Hans Christian Andersen's *The Ugly Duckling*. The findings concluded that the anterior medial prefrontal cortex in children who read significantly more every week is more active than in children who do not. Children who read significantly more are also more likely to detect a situation for which empathy is the proper response and in turn respond empathetically. The results of this study hold significance for the education system, which in the past 15 years has shifted toward teaching for the purpose of standardized testing scores. As this study shows reading fictional literature increases learning outcomes in children that benefits them into adulthood, in turn, benefitting society as a whole. Therefore, the education system needs to focus on teaching fictional literature accompanied by empathy-based discussion, rather than how to take standardized tests. This will increase learning outcomes in children and benefit society through the development of empathetical adults.

Note: This paper was an assignment for a nursing school General Education Capstone course. The student writer did not conduct a real study; she rather simulated a study to demonstrate writing/research skills, creativity, scientific knowledge, and an understanding of how to generate and analyze data. The corresponding author is the student's instructor, who guided the student on each section of the

	scientific paper, providing feedback on how to "conduct" the study and on how to revise the writing.

Introduction

Literature Review: Scientific

Empathy is a multidimensional concept found in humans that allows them to cognitively participate in other people's feelings or processes of their mind (Butler, Carlin, & Nickerson, 2009). Empathy develops throughout childhood and, as outlined by Hoffman (1984), progresses through four distinct developmental stages. The first stage is infancy, in which infants do not have the capacity to distinguish themselves from others. Hoffman says, that while infants are not yet developmentally mature enough to empathize, hearing another infant cry will induce crying. The ability to empathize is innate and therefore, precursors such as this can be seen throughout life. The second developmental phase that Hoffman discusses forms when the child is able to recognize him or herself as physically different from others. From ages two to three, children learn that other people also experience feelings that are distinct from their own. As they develop the ability to speak and experience higher cognition thoughts, children also develop the ability to empathize with people who are not present; this is known as perspective taking (Hoffman, 1984). The last level of empathetic development occurs in late childhood, at which point children are able to empathize with people who are not present or who they do not know about ongoing problems (Cress 1998).

Society refers to people who do not develop empathy as psychopaths. According to Anderson and Kiehl (2011) due to their lack of empathy psychopaths commit a disproportionate amount of violent crimes that create significant emotional and financial burden on society. Aharon-Peretz, Harari, Levkovitz, and Shamay-Tsoory (2010) theorize that an impairment in the emotional aspect of theory of mind in psychopaths' accounts for their impaired social behavior. Aharon-Peretz, *et al.* hypothesize that the manifestation of a deficient emotional theory of mind is associated with a dysfunctional orbitofrontal cortex (OFC). In order to test this hypothesis, they compared the theory of mind abilities of criminal offenders who were diagnosed with antisocial personality disorder and high psychopathy features to subjects with localized OFC lesions. The results of this study showed that subjects with psychopathy and those with frontal lobe, specifically OFC, lesions were both impaired in their emotional theory of mind abilities (Aharon- Peretz, *et al.*, 2010). This shows that there is a distinct correlation between brain dysfunction and the lack of empathy that is found in psychopaths. Anderson and Kiehl (2010) also concluded that there is a set of brain regions that are consistently found in correlation to psychopathy. These regions include the OFC, amygdala, and anterior cingulate and ad acent limbic structures (Anderson & Keihl, 2010).

Using technology such as functional magnetic resonance imaging (fMRI), scientists are able to examine the neurological mechanisms behind empathetic responses, giving people further insight into the development and formation of empathy. A study by Nijhof and Willems (2015) localized the cortical motor network and mentalizing network using neuroimaging. Their goal was to examine the response of both networks during an empathetic stimulus. The stimulus used in this study was fictional literature, which has historically been linked to a mentalizing response by the brain. Mentalizing is the brain's ability to understand the thoughts, feelings, and intent of others, i.e. empathy (Nijhof & Willems, 2015). A study by Wallentin and colleagues, that observed both sensori-motor stimulation and mentalizing during the reading of "The Ugly Duckling", found that the visual cortex, for observing visual stimulus, and the section of the brain related to mentalizing were both activated (Nijhof & Willems, 2015). This indicates that not only do people hear literature and mentalize it verbally but they also "experience" it in their minds, indicated by the activation of the visual cortex while performing a verbal activity (Nijhof & Willems, 2015). Therefore, fictional literature can create a scenario that readers experience in a similar way to a scenario they visually observe. Nijhof and Willems' study found that there was a significant response by the anterior medial prefrontal cortex (amPFC) in

reaction to listening to mentalizing- related literature. The right temporoparietal junction (rTPJ), used for mentalizing, and the amygdala, used in emotional learning, were also observed but showed no significant results in this study (Nijhof & Willems, 2015). In 2003 Frith and Frith used neuroimaging to reveal the three-system complex involved in both implicit and explicit mentalizing: the medial prefrontal cortex (MPFC), the temporal poles and the posterior superior temporal sulcus (STS). More importantly, Frith and Frith theorized about the uses of each system individually based on their study. They found that the amPFC is the mechanism that distinguishes between mental state information reception and physical state information reception. The STS is the region involved in the awareness of agency, and the temporal poles in accessing social scripts of past schemas (Frith & Frith, 2003).

Many theories have been proposed for how certain actions, such as reading fictional literature, increase empathy (Beierl, 2012). Simulation theory is the theory that by transposing oneself into the minds of another person one is able to imagine oneself as that person. This would be the equivalent of a naturalist observation study, by putting oneself in another context. By integrating the thoughts of another person into one's own cognition one can experience their thoughts and feelings. This is a necessary aspect of empathy (Beierl, 2012). Schatzki states the occurrence of three major intellectual operations involved in stimulation: reading, inferring, and imagining. By "reading", Schatzki is referring to the act of understanding the mentality of another person, primarily his or her emotions. Inferring is the act of considering specific facts about a person, then drawing conclusions based on those facts. Finally, imagining is when someone considers how the other person sees his or her situation and then determines how he or she will react (Beierl, 2012). Fictional literature plays a key role in the development of empathy through the theory of stimulation. When reading, people follow the same three steps outlined by Schatzi. In doing so they gain ethical agency in the character and the story that helps us to formulate a new ethical point of view (Cress,1998).

The neurological basis behind the internalization of literature begins the moment the reader receives the information (Beierl, 2012). Iser explains that the aesthetic response, which occurs after beginning to read, is when the reader adjusts his or her own focus and relies on perceptive faculties in order to comprehend the literature. The reader then formulates the meaning of the text based off his or her own imaginative process, therefore arousing motor neurons to generate an empathetic response in order to comprehend the literature from the perspective of the narrator (Beierl, 2012). A study performed by S.A. Lee and colleagues investigated the relationship between certain personality traits and the reading of fictional literature. The study involved having subjects self-identify how much they engaged in fictional literature by a multiple-questionnaire. They then showed the subjects photos of another person's "eye region" and asked a question regarding his or her mental state. Lee and colleagues found that there was a correlation between the amount of fictional literature the subjects engaged in and their ability to properly grade the mentality of the person in the photo (Beierl, 2012).

Literature Review: Cultural

From a cultural standpoint, the ability to properly grade the mentality of a person is important in the development of empathy because to empathize, one must know the other persons' thoughts and feelings, and react accordingly. In order to properly empathize people must be able to understand a person's unique life experiences and decipher how they feel about a situation on the basis of their experiences. Recent research supports fictional literature as a beneficial way to gain new "experiences" in order to positively enhance an ability to understand others. Through simulation, literature gives one the opportunity to immerse oneself in the fictional world of the protagonist (Beierl, 2012). Not only does this give the reader the ability to "see" what the protagonist sees, but also gives them the opportunity to experience what the protagonist experiences from his or her point of view, likely giving readers ethical agency in a new context.

Encouraging reading in children has become increasingly more important for motives other than promoting academic intelligence, as research on the benefits of fictional literature continues to be published. Not only has research linked fictional literature to an increase in empathy, it has also shown that empathetic reading mediates pro-social behavior and a decrease in prejudice. Pro-social behaviors include cooperation, sharing, and other altruistic acts (Feshbach & Feshbach, 2009). The development of empathy encourages pro-social behavior because without the ability to understand how people feel,

think, and react, we are ineffective communicators and cannot fulfill one of human's most innate behaviors as social beings. As Beirel (2012) put it, "There are people who have the capacity to imagine themselves as someone else, [and] there are people who have no such capacity (when the lack is extreme, we call them psychopaths)" (p.34).

The ability to imagine themselves as someone else is also what makes children good readers to begin with. When children are capable of this kind of projection they have a better chance of experiencing what the characters do similarly to the way they experience events that occur in their own lives. This means that they will also feel what the characters describe their feelings to be more realistically. This not only makes reading more enjoyable for children, but it also helps them develop empathy from what they read. For parents who feel that their children are not innately drawn to books or naturally good readers methods are available to help their children become good readers. Former first lady Laura Bush said "As parents the most important thing we can do is read to our children early and often. Reading is the path to success in school and life. When children learn to love books, they learn to love reading" (Spellings, 2000). The best way to encourage children to become readers is to talk and listen to them and to read together with them (Spelling, 2000). This can be made easier by finding books on the topics that interest the child and will peak their creativity and imagination. The more immersed a child is in book the more they will gain from its content. Reading not only benefits the children who learn to read but also society.

Society considers empathy the norm not only because it is innate, but because it encourages people to take a societal approach that benefits the growth of humanity. Prejudice is a problem that often hounds society and is the result of a lack of empathy. An individual who understands the thoughts and feelings of a person who is socially or racially different than themselves is able to empathize with the experiences of this person. A study performed by Doyle and Aboud (1995) showed that children who participate in role-playing activities show an increased level of empathy and decreased level of social prejudice (Feshbach & Feshbach, 2009). The use of role-playing, referred to as "reflection-projection in reverse" by Butler and colleagues, is said to increase empathy because when people "play" another person, what they believe they know about the other persons abilities affects the way in which the person perceives his/her own abilities (Buysse, et al., 2009). This effectively changes the "role-players" perception even after the role is over. Keen states that the reader of fictional literature feels empathy for and with the protagonist and connects to aspects of the fictional world he/she is reading about, despite being able to relate to the historical, cultural, social or economic situation (Beierl, 2012).

Fictional literature is composed in a way that does not tell the reader the "lesson" or purpose of the story, but instead offers the reader an opportunity to decode the meaning of what they have just read (Beierl, 2012). According to Iser, this idea is called "wandering" or "moving". It is the idea that the reader cannot comprehend a text in its entirety at any one time. Instead, when reading people must take in what they can and when finished, come out with a combination of insights. Iser claims that these gaps, which he/she calls "prose" gaps, are a lack of frame by the author that allows the reader to insert agency each time he reads. Each time the text is read it can be interpreted differently, allowing the reader to combine his/her ideas with the author's and "reformulate" the text to be his own (Beierl, 2012).

This topic is also discussed by Holland who also presents literature as an experience for the reader that is shrouded in the layers of meaning contained in one text. For Holland uncovering these layers is a psychoanalytic experience of transforming the fantasy of fiction into the conscious mind of the reader decipherable through conventional interpretation (Beierl, 2012). According to this psychoanalytic view, the associations people make when reading texts are fueled by their conscious mind and the way they interpret texts reflects this. Dewey offers readers the opposite perspective. People do not shape the literature they read to fit their current state but rather the literature shapes them. According to Dewey, reading is the equivalent of experiencing. When reading, people combine their old experiences with the new experiences in the text. Dewey is implying that people's entire thought process is reshaped through their learned experience (Beierl, 2012). The brain is shaped by experiences every day, says Maryanne Wolf. In order to comprehend literature, one must immerse oneself in the text by having agency in the protagonist. By doing so, one is able to gain empathetic agency based off learned literary experience.

Materials and Methods

- A "Reading Frequency" survey (Supplemental Image 1)
- Volunteers age 7-10 (boys and girls of any socioeconomic background)
- fMRI machine (3T whole body scanner with standard head coil)
- Desktop to display fMRI results
- Body pads to secure subject into machine
- Head pad with adjustable plastic strap that secures across the forehead
- Conjoining rooms connected by a glass window allowing researcher to observe subjects
- Speaker attached at head of fMRI machine
- Microphone (in adjoining room) connecting to speaker attached to head of fMRI machine
- Microphone attached to fMRI machine
- Speaker (in adjoining room) connecting to microphone attached to fMRI machine
- *The Ugly Duckling* by Hans Christian Andersen
- The "Empathetical Response" survey (Supplemental Image 2)
- Note: the subject should not have any metal on their persons and there should be no metal in the fMRI machine being as it will interfere with the machine; speakers and microphones should be placed directly outside of the head of the machine

First, we had the guardians of volunteers (age 7-10) complete the reading frequency survey. Based on survey results: we placed three subjects who read 6 or more hours per week into experimental group 1, three subjects who read 6 or more hours per week into control group 1, three subjects who read 3 or fewer hours per week into experimental group 2, and three subjects who read 3 or fewer hours per week into control group 2. We excluded all other volunteers who did not fall into any of these categories.

We then placed the subjects, from the Experimental groups (1 and 2), individually into the fMRI machine beginning with Experimental group 1 and proceeding in randomized order. We secured the subject using body pads to prevent any body movement and a fitted head pad with an adjustable forehead strap to immobilize the head. Once the subject was secure we ran a preliminary microphone/speaker check to assure the subject could hear exactly what was being said to them. The researchers then left the room and proceeded to the adjoining room*. We used the Echo Planar Imaging method (EPI) to collect 2-dimensional transverse images of the brain at resolution 3.4x3.4x4 mm^3 with repetition time TR=2s/volume throughout the experiment. After the subject was secure we began by taking a preliminary image of the subject's brain prior to any stimuli. Next, we had one of our researchers** read *The Ugly Duckling* to the Experimental group subjects through the speaker/microphone setup connected to the fMRI. Throughout the story, in 2 second increments, images were ttaken of the subject's brain. Immediately after the story was finished being read, while the subject was still in the fMRI machine, the subject was read the "Empathetical Response" survey by the researcher and asked to verbalize their answers. Their responses were recorded. The subject was then removed from the fMRI machine and allowed to go.

Next, the Control groups (1 and 2) were individually placed into the fMRI machine beginning with Control group 1 and proceeding in randomized order. The subjects were secured into the machine in the same manner as the Experimental group subjects. The researchers then ran a preliminary microphone/speaker check to assure if necessary the subjects could communicate with them. The researchers left the room and proceeded to the adjoining room*. We used the same fMRI settings that had been used for the Experimental groups to again take 2-dimensional transverse images of the subject's brains. We ran the fMRI machine for 7 minutes taking images in 2 second increments while the room remained completely silent with no stimulus. The subject was then removed from the fMRI machine and allowed to go.

*Note: No one other than the subject was allowed in the room while the fMRI machine was running. Researchers observed from a conjoined room.

**Note: The researcher who read *The Ugly Duckling* remained the same throughout the study in order to generate consistent results.

Results

Experimental group one participant one (E1P1) showed increased anterior medial prefrontal cortex (amPFC) activity at minutes 1.10-1.70, 3.30-4.0, and 6.10-6.50. The activity response was characterized by an 0.86-0.97 increase in blood flow to the amPFC during the time ranges of increased activity. In all cases the response was prefaced by increased cerebellum activity lasting from 1.30-3.60 seconds. It should also be noted that participant one experienced increased limbic activity prior to all amPFC episodes lasting between 0.30-0.56 seconds. Experimental group one participant 2 (E1P2) showed similar results with increased amPFC activity at minutes 1.20-1.50, 3.40-4.40,6.20-6.60. The response was characterized by an 0.87-0.97 increase in blood flow to the amPFC during the time of increased activity. E1P2 experienced increased cerebellum activity prior to all events lasting from 0.6-1.0 seconds. E1P2 also experienced increased limbic activity prior to all amPFC episodes lasting between 0.32-0.54 seconds. Experimental group one participant 3 (E1P3) showed increased amPFC activity at minutes 1.25-1.80, 3.60-4.00, and 6.21-6.90. The activity response for minutes 1.25-1.80 and 6.21-6.90 showed an average increased response of 0.87-0.94. For minutes 3.60-4.00 the activity response peaked at 0.75 increased blood flow to the amPFC. The amPFC response during minutes 1.25-1.80 and 6.21-6.90 was prefaced by a peak in cerebellum activity lasting 1.41-3.23 seconds. The peak at 3.60-4.00 seconds was prefaced by a cerebellum response lasting 0.78-1.20 seconds. E1P3 showed increased limbic activity in relation to all amPFC activity peaks. See Figure 1.

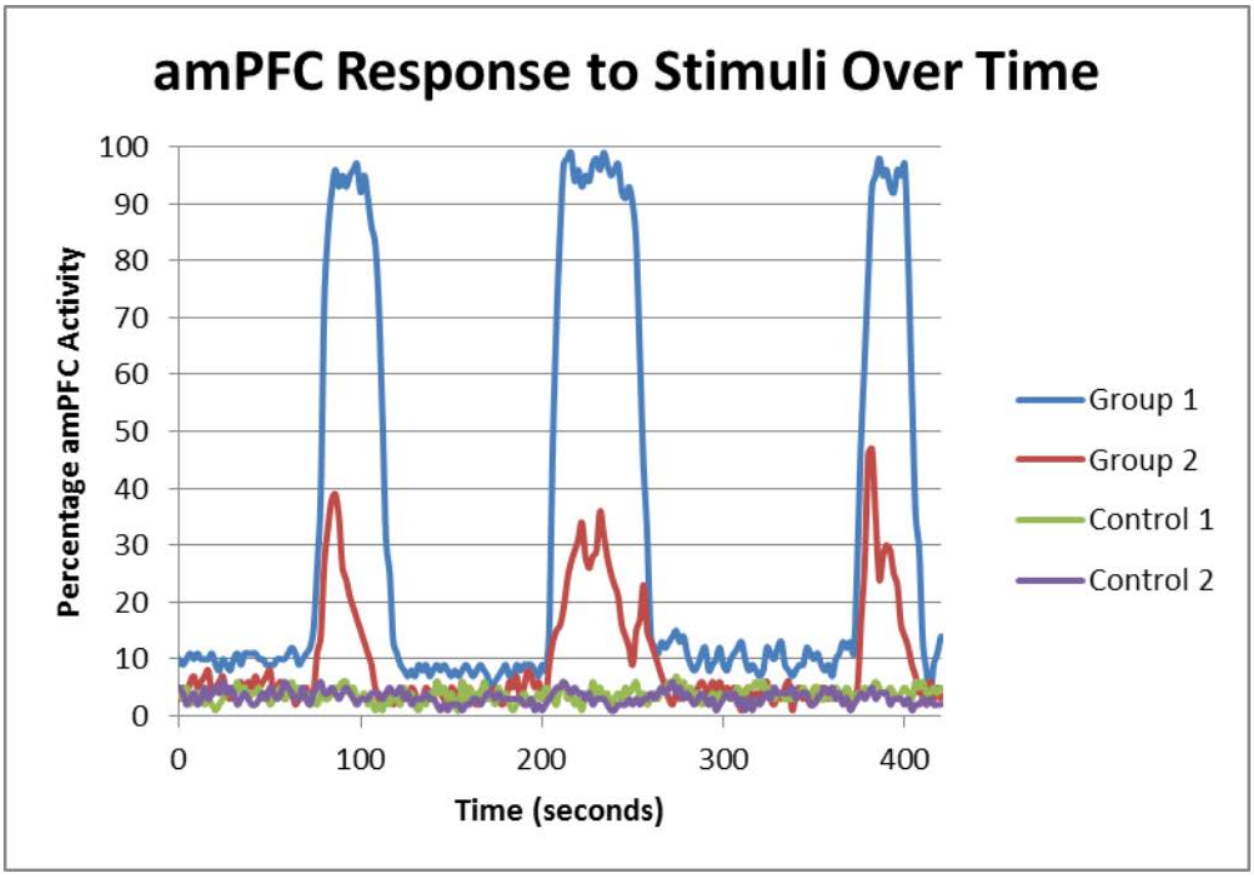


Figure 1: amPFC Response to Stimuli Over Time

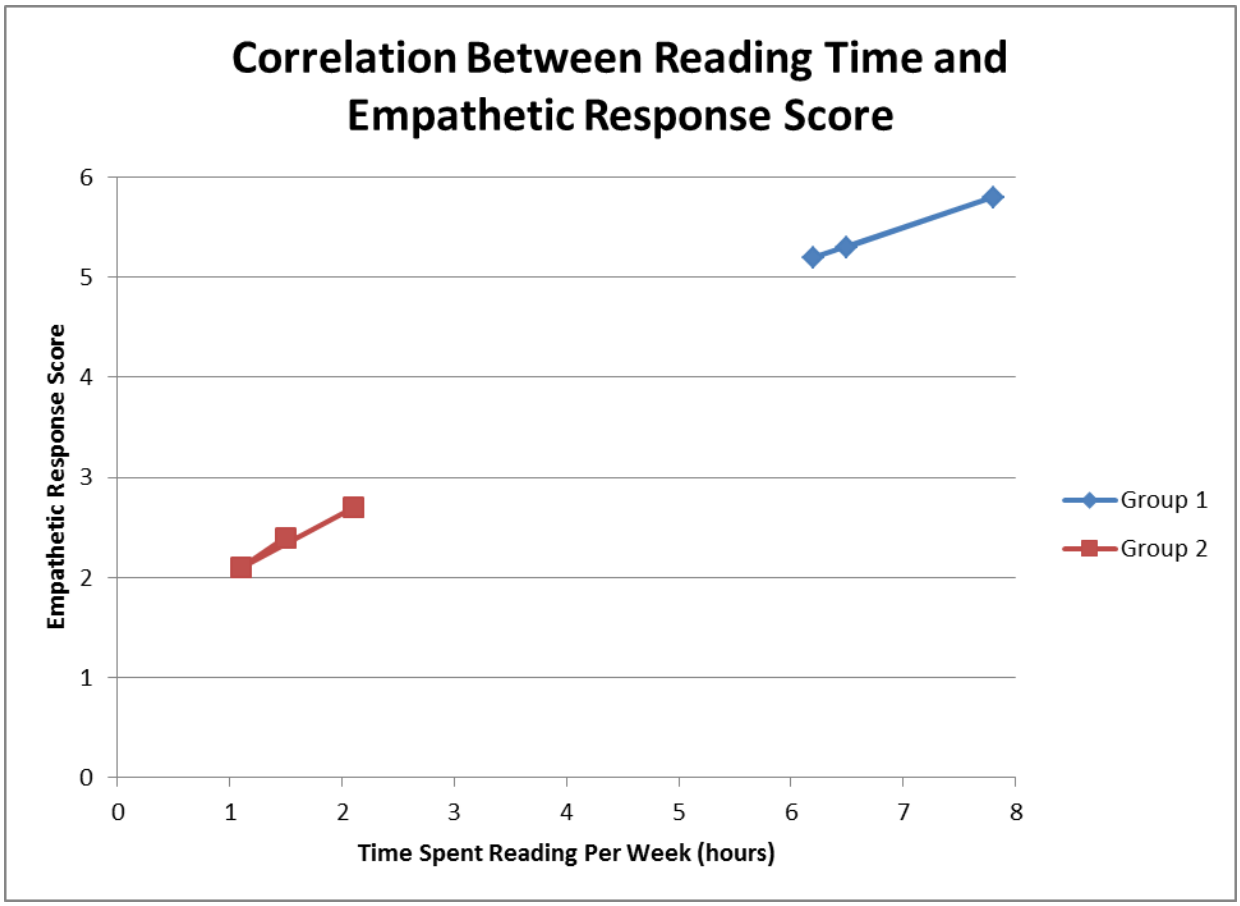
Experimental group two participant one (E2P1) showed increase amPFC activity minutes 1.23-1.80, 3.10-3.98, and 6.04-6.45. The activity response was characterized by a 0.25-0.37 increase in blood flow to the amPFC during the time ranges of increased activity. In all cases the response was

prefaced by increased cerebellum activity lasting 0.34-1.20 seconds. There was no increased limbic activity worth noting. Experimental group two participant 2 (E2P2) showed increased amPFC activity minutes 1.30-1.83, 3.15-3.76, and 6.08-6.32. The response was characterized by a 0.20-0.31 increase in blood flow to the amPFC. E2P3 showed cerebellum activity lasting 0.10-0.23 prior to activity in minutes 1.30-1.83 and 6.08-6.32 but not during minutes 3.15-3.76. There was not notable limbic activity during or prior to peak amPFC. Experimental group 2 participant 3 (E2P3) showed increased amPFC activity minutes 1.31-1.41 and 6.21-6.35. The responses were characterized by a 0.15-0.23 increase in blood flow to the amPFC. E2P3 experienced increased cerebellum activity prior to both events lasting 0.20-0.45 seconds. There was no notable increased limbic activity. See Figure 1.

The participants reading frequency was determined using the "Reading Frequency" survey (see Appendix) given to volunteers prior to participation. The average results for each group are as follows: Experiment group 1: 420 minutes/ week; Control group 1: 400 minutes/ week; Experimental group 2: 140 minutes/ week; Control group 2: 125 minutes/ week. See Figure 2.

The average score of the Empathetic Response survey for Experimental group 1 is 5.33 (5,5, and 6). The average score of the Empathetic Response survey for Experimental group 2 is 2.33 (2,2, and 3). See Figure 2.

Figure 2: The Correlation Between Time and Empathetic Response Score



Conclusions

The result of this study holds significance for the education system as a whole and for individual families with school age children. According to Staff (2011) the annual spending on public education in the United States was $806.9 billion, that is $7,743 per child. This amount is a colossal considering that the United States is third to last in math test scores and fourth to last in science test scores (Staff, 2011). When it comes to literacy of children age 15 and up the United States has a 99% literacy rate (Staff, 2011). The current curriculum in the United States focuses largely on how to score well on standardized tests rather than improving learning outcomes. These tests are used to judge

children, teachers and schools in order to delegate spending. Not only is this form of teaching not retained by students, it is not beneficial to their learning outcomes or development. Never before have standardized testing scores had such a prominent role in education (Staff, 2011). According to Khon (2000) the United States is one of the few countries that gives formal exams such as this to children as young as six "despite the fact that almost all experts in early childhood education condemn this practice" (p.1).

The research from this study shows that reading fictional literature is positively correlated to increased empathy in children. Rather than require schools to comply to standardized testing, the government needs to implement a curriculum that supports children's development into autonomous adults. This can be done by adding fictional literature, and empathy focused discussion, into elementary and middle school curriculum. This will not only increase empathy later in life but also help children understand one another thus promoting prosocial behavior. Until this change occurs parents are encouraged to incorporate fictional literature into their children's everyday life through bed time stories or adding it to suggested reading lists. According Margaret Spellings of the U.S. Department of Education (2000), "Other than helping your child grow up healthy and happy, the most important thing that you can do for them is to help them develop their reading skills. It is no exaggeration to say that how well children learn to read affects directly not only how successful they are in school by how well they do throughout their lives" (p. 4).

While this study revealed a large gap in the current public education system in providing children with the education needed to support a healthy development of empathy and into adulthood more supplemental research must be done. This study revealed that reading fictional literature for six or more hours per week increased an empathetical response in children but it was unable to supply a mechanism for this occurrence. Further studies must be done in order to find what aspects of fictional literature specifically induce an increased empathetic response. This can be done by studying how children react to different aspects of the stories. For example, does empathy peak at the part of a story when the character feels guilt for their actions, when they help another character solve a problem, etc. A study such as this would provide more information as to why this phenomenon occurs.

Bullying in the United State is an epidemic. According to Barbara Coloroso (2008) 86 percent of children ages twelve to fifteen are bullied in school "making bullying more prevalent than smoking, alcohol, drugs, or sex among the same age group" (Gourneau, 2012, p. 117). The result of bullying is low self-esteem, body dysmorphic disorders, and adolescent and childhood suicide. A lack of empathy and inability to judge the harm that one inflicts on others is grossly contributing to the epidemic. By teaching children empathy from a young age through literature and empathy-based discussion it is possible to decrease these effects by increasing children's awareness of others feelings. By doing so schools will become a safe environment for all students to foster their education and grow into empathetic adults. This begins by encouraging parents to expose their children to fictional literature and promote reading at home.

In 2000, and again in 2002 and 2005, the U.S Department of Education released a booklet titled *Helping Your Child Become a Reader*. The purpose of this booklet is to teach parents how to foster a curious mind in children and increase interest in reading. In order to do so, Spelling urges parents to promote children's language skills and encourage interest in reading by talking with and listening to one's child, reading with ones' child, helping one's child to learn about books and print, encouraging early writing efforts, and preparing one's child to be successful in school (Spelling, 2000). According to Spelling reading books with children is one of the most relevant activities parents can encourage to promote their children to becomes readers. The government and media need to release more information such as this pamphlet by the Department of Education in order to teach parents about the positive effects of reading as a whole and the positive effects on empathy of reading fictional literature. The government also needs to change the curriculum in public schools to focus less on teaching in order to achieve standardized testing scores and more on education. This involves incorporating more literature and empathy-based discussion. As this study has shown, this will increase empathy in children who partake, and in turn have a positive impact on the development of society.

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Supplemental Images

READING FREQUENCY Survey

Supplemental Image 1

Volunteer #:

This survey is to be completed by parents/guardians of volunteers. Please circle the answers below that correspond to your child's reading habits.

1. How many hours per week does your child read at home for school? 1 or less 2-5 6 or more

2. How many hours per week does your child read at home for pleasure? 1 or less 2-5 6 or more

3. How many hours total does your child read per week?

(add answers for question 1 and 2)	1 or less	2-5	6 or more
4. Does your child read mostly fictional or non-fictional literature?	Fictional		Non-Fictional

EMPATHETICAL RESPONSE Survey

Supplemental Image 2

Subject #

Group #

1. How does the Ugly Duckling feel after his brothers and sisters abandon him?
    a. Indifferent
    b. Sad
    c. Angry
    d. Happy

2. How did it make you feel after the Ugly Duckling's brothers and sisters abandoned him?
    a. Indifferent
    b. Sad
    c. Angry
    d. Happy

3. How does the Ugly Duckling feel after he finds his new family?
    a. Indifferent
    b. Sad
    c. Angry
    d. Happy

4. How did it make you feel after the Ugly Duckling found his family?
    a. Indifferent
    b. Sad
    c. Angry
    d. Happy

5. Do you think the Ugly Duckling's brothers and sisters had a reason to be mean to him?
    a. Yes
    b. No

Tell the subject that the Ugly Duckling's siblings made fun of him because someone had also made fun of them and hurt their feelings.

6. Do you think that the Ugly Duckling should forgive his brothers and sister?
    a. Yes
    b. No

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Factors That Influence Conservatism vs. Liberalism

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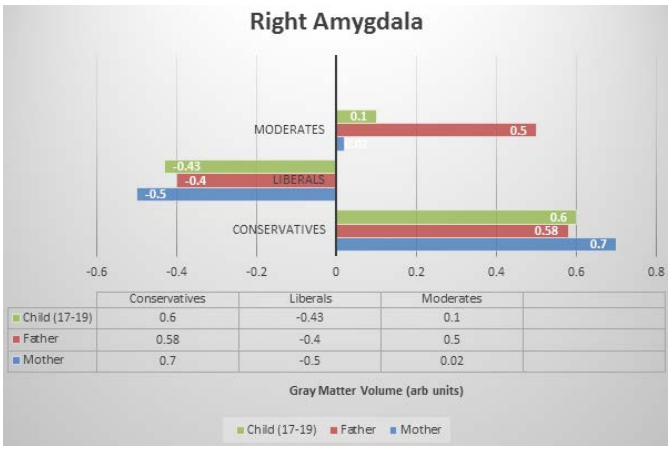
Graphical Abstract



Abstract

Recent studies have shown that genetics have been linked to the influence of conservatism and liberalism in the United States. Evidence has shown that conservatives present more brain matter volume within the anterior cingulate cortex, and liberals present more brain matter volume within the right amygdala. Despite other social and scientific factors that may influence conservatism and liberalism, such as morals, culture, demographics, personality traits, etc., the study conducted determines a correlation between brain structures and conservatism and liberalism. The study conducted consisted of brain studies using MRI evaluations of conservative, liberal and moderate families. These families include a mother, father, and child between the ages of 17 and 19. Results suggest that conservatism and liberalism is influenced by the brain structures of individuals. These results also indicate that conservative and liberal parents have strong influences on the brain structures of their teenage children.

Note: This paper was an assignment for a nursing school General Education Capstone course. The student writer did not conduct a real study; she rather simulated a study to demonstrate writing/research skills, creativity, scientific knowledge, and an understanding of how to generate and analyze data. The corresponding author is the student's instructor, who guided the student on each section of the scientific paper, providing feedback on how to "conduct" the study and on how to revise the writing.

Introduction

Literature Review: Scientific

Liberals and conservatives show cognitive differences (Anyaso, 2016). Neuroscientific and genetic studies have shown that conservatives have more structured and perpetual cognitive styles while liberals are less structured and more flexible (Anyaso, 2016). MRI scans have been used to detect gray matter in certain areas of the brain that show different results in conservatives and liberals (Feilden, Firth, Kanai, & Rees, 2011). The excess gray matter that is found in the part of the brain in liberals is associated with understanding complexity (Feilden, et al., 2011). The area of the brain where fear is processed is found to be bigger in size in conservatives (Feilden, et al., 2011). High amounts of gray matter in the brain are found within the anterior cingulate cortex in liberals, and high amounts of gray matter in the brain are found within the amygdala in conservatives (Feilden, et al., 2011). To support that gray matter is present in different parts of the brain in conservatives and liberals, MRI scans were recorded from 90 young adults who reported their political stances (Feilden, et al., 2011). Results showing greater amounts of gray matter in parts of the brain in conservatives and liberals also show increased brain activities in those areas and can link to different cognitive styles (Feilden, et al., 2011).

The study of brain structures, and the study of brain activity are two different concepts (Feilden, et al., 2011). There are greater brain activities in the areas associated with excessive gray matter within conservatives and liberals. (Feilden, et al., 2011). Not only do liberals show high amounts of brain matter within the anterior cingulate cortex, but they also show occurring responses within the anterior cingulate cortex (Feilden, et al., 2011). The right amygdala, left insula, right entorhinal cortex, and anterior cingulate cortex are the four brain regions that are associated with conscious decision-making (Dawes, et al., 2013). These four brain regions have proven differences between conservatives and liberals when it comes to their physiological responses (Dawes, et al., 2013). Liberals show obvious activity within the left insula (Dawes, et al., 2013). Being able to correlate ideological processes with physiological responses will explain differences in behavior (Amodio, 2011). To determine different brain functions in conservatives and liberals, 82 candidates were chosen from public voter records, and preformed a risk-taking task during functional imaging (Dawes, et al., 2013). During the study, the risk-taking behavior of conservatives and liberals did not differ, but their brain activity did (Dawes, et al., 2013). Conservatives showed increased brain activity in the right amygdala, and liberals showed increased brain activity in the left insula (Dawes, et al.,2013). These results suggest that conservatives and liberals possess different cognitive responses when dealing with similar situations (Dawes, et al., 2013). Liberals and conservatives show differences in brain activity when they are dealing with risks, and threatening situations (Dawes, et al., 2013). Studies have shown that liberals use their anterior cingulate cortex when dealing with conflict (Feilden et al., 2011). Conservatives show more aggression than liberals when involved in threatening situations, this is one reason why they show occurring responses within the amygdala of the brain as it is responsible for emotion and survival instincts (Feilden, et al., 2011). Liberals show different psychological characteristics than conservatives when reacting to situations as they make impulsive and uncertain decisions while conservatives express greater aggression (Dawes, et al., 2013)

Political attitudes are influenced by genetics and its connection with environmental factors (Feilden, et al., 2011). Genes determine physiology and biological mechanisms that can influence the behaviors, attitudes that link to the preferences of a conservative or liberal (McDermott, 2013). Studies using functional magnetic resonance imaging were used to record brain activity of the conservative and liberal participants responding to disgusting images (Feilden, et al., 2011). Measuring a person's strength to revolting images can predict their political ideology (Feilden, et al., 2011). When a disgusting image is presented, neural responses show a person's reaction, and can dictate information about that person such as political ideology (Feilden et al., 2011). During the study, participants were exposed to several disgusting pictures as well as neutral and pleasing images while their brains were being scanned (Feilden, et al., 2011). Conservatives have more magnified responses to the disgusting images in comparison to liberals (Feilden, et al., 2011). After the brain scan, participants were given a political ideology survey in which the responses were 95 to 98 percent accurate based on their reactions to the disgusting images (Feilden, et al.,2011). These results suggest that neural responses are

genetic and can be passed down from our ancestors that are associated with protection against environmental threats (Feilden, et al., 2011). Dopamine is released in the brain from a gene, the presence or lack thereof can influence conservatism or liberalism (Physorg, 2015). Dopamine plays a role in behavior (Physorg, 2015). Political preference and beliefs may be linked to genetics due to research studies that study fraternal and identical twins (Morin, 2013). Studies involving twins are practices that are used to test nature vs. nurture controversies (Morin, 2013). Using twins to conduct research regarding whether genetics are linked to political preference is presumed to be constant, because with twins, all variables are the same, such as upbringing, culture, morals (Morin, 2013).

It is human nature to be ideologic (Amodio & Jost, 2011). To support that ideologic is human nature, social cognition and neuroscience studies are conducted (Amodio & Jost, 2001). Political ideology is a form of comfort and security (Amodio & Jost, 2011). People naturally affiliate themselves with a group such as social and political groups to better cope with life situations, it becomes a need (Amodio & Jost, 2011). The differences between conservatives and liberals is their psychological preferences towards uncertainty, threat, and conformity (Amodio & Jost, 2011). Neural responses indicate that political ideology is linked to cognitive and emotional processes (Amodio & Jost, 2011). The choice between conservatism and liberalism is partially influenced by a person's interests, and needs (Amodio & Jost, 2011). A person leaning toward conservatism more likely holds a need for coping with uncertainty and threat (Amodio & Jost, 2011). A person leaning toward liberalism has low needs in managing uncertainty and threat, and are uncertain with social changes (Amodio & Jost, 2011).

Literature Review: Cultural

Conservatives and liberals differ in the sources they choose to rely on for news and information about politics and government (Gottfried, Kiley, Matsa, & Mitchell, 2014). Strong liberals rely on an array of news sources such as CNN, MSNBC, NRP, and NYT, as opposed to strong conservatives who mainly rely on one main source for information which is Fox News (Gottfried, et al., 2014). The news media, social media, and conversations between friends and family are the different ways in which people get information regarding government and politics (Gottfried, et al., 2014). Conservatives and liberals are politically divergent when it comes to the patterns of social media sharing (Duggan, & Smith, 2016). Social media influences political discussions and opinions which highly impacts the conservative and liberal parties (Duggan, & Smith, 2016). Based on a Pew Research Center survey of partisanship and political animosity, statistics have shown that 61 percent of Americans find that they have less in common when they talk to others on social media regarding politics (Duggan, & Smith, 2016). Out of the 61 percent, 36 percent of those people find themselves being influenced to agree with political opinions that they initially disagreed with (Duggan, & Smith, 2016).

Trusting and distrusting in different media outlets influences conservatism and liberalism (Gottfried, et al., 2014). The Media is biased, journalists and editors dictate what type of news they want to cover, they interpret information and topics in a way that they want to represent it; as a result, this influences conservatives and liberals to rely on the source outlets that they believe are true or right (Beder, 2004). Research has shown that liberals choose to get their information on government and politics from more reliable sources as opposed to conservatives (Gottfried, et al., 2014).

Conservatives and liberals have different moral judgements, and disagree on an array of moral issues (Cushman, Hannikainen, & Miller, in press). Liberals value care and fairness as opposed to conservatives who place greater values in loyalty, authority, sanctity, and purity (Cushman, et al., in press). The moral foundations theory hypothesizes that there are differences in moral values among conservatives and liberals that influences their political views. (Graham, Haidt, & Noek, 2009). Conservatism and liberalism is influenced by moral concerns about what is right and wrong (Day, Downing, Fiske, & Trail 2014). To prove that moral foundations can influence conservatism or liberalism, if one's moral foundations were altered, then that person's political attitudes could possibly result in at least two outcomes; they could be persuaded in another direction, or ingrained in their original political ideology (Day, et al., 2014). It is questioned whether conservatism or liberalism is chosen first and moral concerns follow that belief, or whether moral concerns are initially instilled and

that plays a role in one's preference in being conservative or liberal, or whether it is a mixture of both (Graham, et al., 2009).

Statistics have shown an increasing rate of educated college students becoming liberal over the past ten years (Kurtzleben, 2016). Going to college allows Americans to become more aware and educated on social issues such as gender equity (Kurtzleben, 2016). Attending college allows a student to grow mentally, socially, and individually (Suls, 2016). College curriculum has veered away from traditional values due to the social issues and controversies that we deal with in the 21st century (Suls, 2016). College campuses and classes are made up of a variety of social differences giving college students the opportunity to be more open to different attitudes and perceptions (Suls, 2016).

Conservativism and liberalism could be influenced by religious practices (Davis, & Ritter, n.d.). During campaigns, the governors openly accept religion, they travel to different churches, perform speeches, and link their political views with religion beliefs to persuade (Davis, & Ritter, n.d.). Religion has impacted U.S. politics (Davis, & Ritter, n.d.). Based on U.S. history, during the 70's, America experienced governmental hardships, entering the 1980s, the Reagan Revolution incorporated Christian Right and witnessed growth and change (Davis, & Ritter, n.d.). Starting in the 20th and 21st centuries, campaigns purposely involved religion and churches so that candidates can accumulate more votes (Davis, & Ritter, n.d). This later influenced other religious groups to stand up and fight for rights that they believed were right and wrong based on their religion such as abortion, marriage and prayer in public schools (Davis, & Ritter, n.d.). These public issues and moral beliefs are what shape conservatism and liberalism (Davis, & Ritter, n.d.). Religious groups base their political ideology on what God would think is right (Davis, & Ritter, n.d.). The bible is filled with laws, commandments, and judgements that are incorporated in the government and cause social issues, controversy, and opinions (Davis, & Ritter, n.d). The difference between liberals and conservatives is that conservatives are stricter while liberals tend to be more lenient, this also applies the perceptions of religion (DiDonato, 2015). Conservatives are more likely than liberals to judge immoral and moral behaviors (DiDonato, 2015). Liberals are more likely to accept forgiveness in religion (DiDonato, 2015). These conclusions were made by surveying a liberal clergy and a conservative clergy about political perception (DiDonato, 2015).

A Cognitive Reflective Test is used to test whether conservative and liberals process information differently (Deppe, Gonzalez, Hibbing, Jacobs, Neiman, Pahlke, & Smith, 2015). The Cognitive Reflective Test is a three-question test that measures individual differences using instinctive but invalid responses and reflective, and correct responses for each item (Deppe, et al., 2015). The CRT is a reliable test that predicts cognitive biases (Kahan, 2013). The Cognitive Reflective Test is associated with psychological traits, values, and beliefs (Deppe, et al., 2015). The Cognitive Reflective Test shows a correlation between thinking styles and political orientations with social attitudes (Deppe, et al., 2015). The correlation between reflection and intuition, and political attitudes may be more resistant to manipulation (Deppe, et al., 2015). The Cognitive Reflective Test concludes that conservatives are less reflective, and liberals are more reflective (Deppe, et al., 2015). Studies using the CRT have shown that conservatives are more intuitive than liberals (Deppe, et al., 2015). This indicates that conservatives rely on heuristics associated with implicit reasoning (Deppe, et al., 2015). Public issues on policies is associated with heuristic driven information processing (Kahan, 2013).

Materials and Methods

Brain structures are likely to explain conservatism and liberalism, and conservative or liberal parents are likely to influence political orientation and brain structures on teenage children. The study involved 60 families in total, and were broken down into three groups; 20 liberal families, 20 conservative families, and 20 moderate families. Each family consisted of a mother, a father, and a teenage child between the ages of 17-19. This study is made up of 180 participants in total.

The moderate family group was the control group. All individuals in each group were given an MRI scan that measured the volumes of matter in the anterior cingulate cortex, and the right amygdala. This research was conducted to see whether teenage children's brain structures and political orientations are influenced by their parent's conservative or liberal associations. The moderate group is

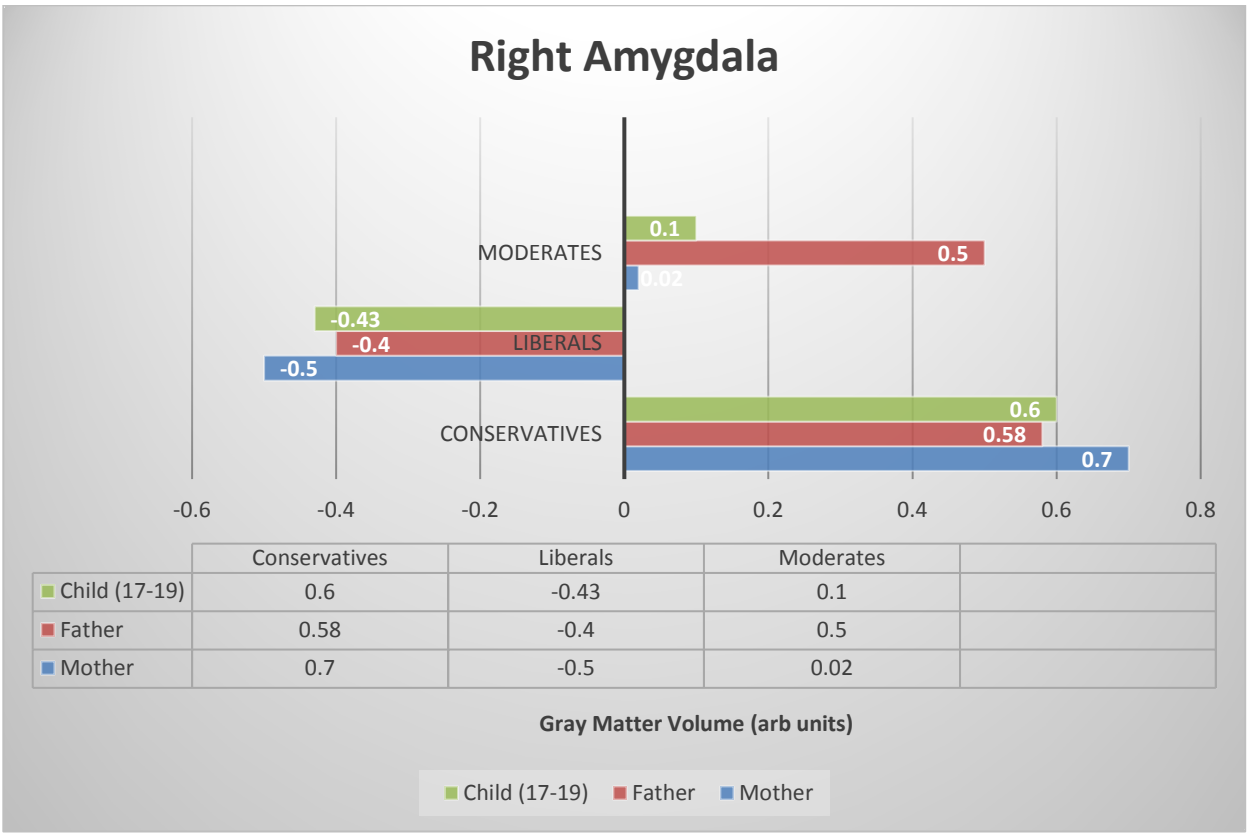
set to compare whether there is a difference in liberal and conservative children vs the influence that moderate parents have on their children.

These families were volunteers and were thoroughly evaluated before being selected. Each parent had to take three separate evaluations that determined their strong political orientations. The first evaluation was based on a 10 question ideologic consistency scale. These 10 questions were strategically chosen as they offer mostly conservative or mostly liberal views regarding political issues and beliefs. These questions had a traditional "left/right" association with conservatism vs liberalism. The second evaluation was a self-report questionnaire which asked each participant what political orientation they associate themselves with. This simple self-report questionnaire has been used in previous genetic studies dealing with political values, and is a reliable measure of political orientation. The third evaluation consisted of a morals foundations questionnaire which evaluated each parents' moral judgement which also correlates with political ideology. This three-step evaluation was placed to make sure that the participants were sure to have strong political views of conservatism, liberalism, or moderate. Participants were also demographically and culturally diverse.

Magnetic resonance imaging is a safe and painless test that provides detailed pictures of organs and structures within the body. This study examines the brain structures of participants, specifically focusing on the right amygdala and anterior cingulate cortex of the brain as those two regions of the brain mainly correlates with conservatives and liberals. MRIs were obtained with a 1.5-T Siemens Sonata MRI scanner. This scan produced high resolution images of the brain. A voxel-based morphometry analyses was used to measure the difference of gray matter within the anterior cingulate cortex and right amygdala between the conservative, liberal, and moderate groups.

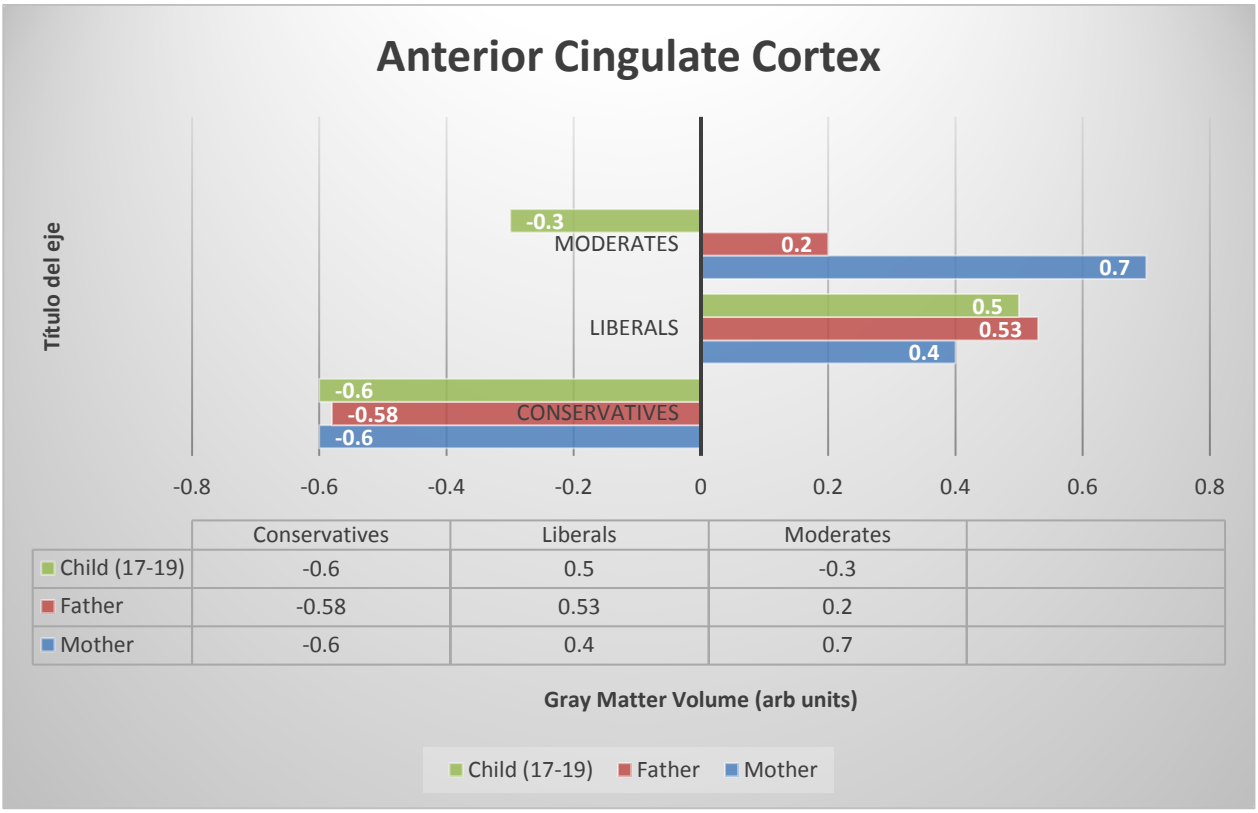
All participants underwent pre-scanning paperwork and safety protocol. Once the MRI scans were conducted, the participant's brains were segmented into gray matter based on the intensity at each voxel. The VBM analyses was used to warp each participant's image of gray matter into a space to average them together. Two bar graphs were made based on the MRIs, and VBM analyses to show the relations of brain structures between the groups by showing the gray matter values on an arbitrary unit scale.

Results



	Conservatives	Liberals	Moderates	
Child (17-19)	0.6	-0.43	0.1	
Father	0.58	-0.4	0.5	
Mother	0.7	-0.5	0.02	

This graph displays the amount of gray matter volume that is obtained in the right amygdala of the brain for each political group. Each political group is divided into the average amounts of gray matter volume within the mothers, fathers, and children of each political party. The bars on the graph measure the arbitrary units of gray matter volume. The moderate children on average had a value of 0.1 arb unit gray matter volume in the right amygdala, the moderate fathers on average had a value of 0.5 arb unit gray matter volume in the right amygdala, and the moderate mothers on average had a value of 0.02 arb unit gray matter volume in the right amygdala. The liberal children on average had a value of -0.43 arb unit gray matter volume in the right amygdala, the liberal mothers on average had a value of -0.4 arb unit gray matter volume in the right amygdala, and the liberal fathers on average had a value of -0.5 arb unit gray matter volume in the right amygdala. The conservative children on average had a value of 0.6 arb unit gray matter volume in the right amygdala, the conservative mothers on average had a value of 0.7 arb unit gray matter volume in the right amygdala, and the conservative fathers on average had a value of 0.58 arb unit gray matter volume in the right amygdala.



The graph above represents the amount of gray matter volume that is obtained in the anterior cingulate cortex of the brain for each political group. Each political group is divided into the average amount of gray matter volume within the mothers, fathers, and children of each political party. The bars on the graph measure the arbitrary units of gray matter volume. The moderate children on average had a value of -0.3 arb unit gray matter volume in the anterior cingulate cortex, the moderate mothers on average had a value of 0.7 arb unit gray matter volume in the anterior cingulate cortex, and the moderate fathers on average had a value of 0.2 arb unit gray matter volume in the anterior cingulate cortex. The liberal children on average had a value of 0.5 arb unit gray matter volume in the anterior cingulate cortex, the liberal mothers on average had a value of 0.4 arb unit gray matter volume in the anterior cingulate cortex, and the liberal fathers on average had a value of 0.53 arb unit gray matter volume in the anterior cingulate cortex. The conservative children on average had a value of -0.6 arb unit gray matter volume in the anterior cingulate cortex, the conservative mothers on average had a value of -0.6 arb unit gray matter volume in the anterior cingulate cortex, and the conservative fathers on average had a value of -0.58 arb unit gray matter volume in the anterior cingulate cortex.

Conclusions

Brain structure is one of the main factors that influence conservatism and liberalism. Based on the study, there was a correlation with the brain structures of the liberal and conservative families. Liberal parents who presented brain matter within the anterior cingulate cortex also had children who presented the same volumes of matter within the same region. Conservative parents who presented brain matter within the right amygdala also had children who presented the same volumes of matter within the same region. However, there was a significant difference in the MRI results with the moderate families. Moderates did not show any correlation in the volumes of matter that was found in their brains, and the MRI results also showed no correlation between the moderate parents and their children.

These results indicate that conservatism and liberalism is influenced by the brain structures of individuals. These results also indicate that conservative and liberal parents have strong influences on the brain structures of their teenage children. The significant evidence presented implies that excess brain matter volumes in different regions of the brain in individuals influences conservatism and liberalism. Conservative and liberal views are so closely related to brain structures and genetics that these same brain structures can be passed down from parents to teenage children as the results imply. Conservatives are associated with brain matter volumes within the anterior cingulate cortex, and liberals are associated with excess brain matter volumes within the right amygdala.

Many factors can influence conservatism vs liberalism. Due to these factors, there were limitations on the current brain study on the political families. To support the theory that conservative and liberal parents influence the brain structures of their teenage children, other studies regarding the relationships of brain structure and families of other political ideologies may strengthen the hypothesis. The results of other associated political parties can better support that brain structures and genetics in fact play a role in politics. The current brain study on political families could also be strengthened by studying and comparing the brain activities of conservative and liberal families during similar reactions or when put in similar life situations.

Some current social issues that are associated with conservative and liberal affiliation include the influence that social media and news outlets have on one's personal opinions and choices on political views. The perceptions that the population receive on political views influence their way of thinking and decision making which then impacts their upbringing, morals and brain structures. There is a lot of power and control involving conservatism and liberalism. The majority political parties have more power than minority political parties. This gives them the ability to control the government and political issues that they agree with. Social media outlets, and the government also control issues and events that are presented to the general population. Social media and news outlets are also biased, and this influences conservatism vs liberalism. There are also ethical issues that are associated with conservative and liberal affiliation. Morals, beliefs and the moral judgements that people possess can also influence conservativism and liberalism. There are social issues such as marriage and abortion laws that are controversial, individuals support their beliefs based on moral judgements which correlates with the different beliefs and views that conservatives and liberals have.

Currently, there are no specific laws that govern political parties, which include conservatism and liberalism. However, there should be laws that govern ways in which political issues are presented to the public, there should not be a filter or block that prevents the public from knowing certain information regarding government and politics. These limitations allow conservatives and liberals to associate themselves with their political ideologies without sufficient knowledge. Voting and affiliating with a political party should be governed and regulated. Individuals should be assessed and required to have knowledge of what is going on with the government, political issues, and differences in political parties to avoid social and behavioral factors that may influence conservatism and liberalism.

To help raise awareness about political affiliation, information regarding conservatism and liberalism should be shared with the public, classes should be taken and required as citizens to inform the public on the different factors that may influence political choices, as well as being aware of the different behaviors that may influence conservatism and liberalism. The government has a lot of power to control some of the factors that may influence conservatism and liberalism. The government has the power to control the way the public perceives political ideology. Some social factors such as

demographics, social media, and behavioral and moral judgements can all be regulated and become less of an influence on conservatism and liberalism.

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diPGA: Treatment of Type 2 Diabetes Mellitus with Adiponectin (ADN)

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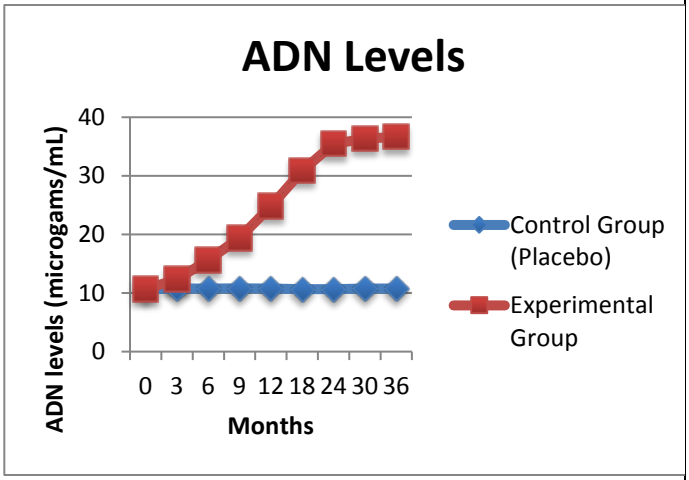
Graphical Abstract *(mandatory)*



Abstract

Adiponectin (ADN) is a protein hormone secreted by adipose tissues. Correlations have been discovered between low ADN plasma levels and insulin resistance with increased fasting glucose levels. In order to be diagnosed with T2D, individuals will be given an A1C Hemoglobin test, where a result of 6.5% or higher on two separate occasions constitutes diagnoses. Recent trends depict diabetes, specifically T2D, as a world wide epidemic. Researchers have found genetic markers as factors contributing to the acquisition of T2D, particularly in the Hispanic demographic. The gene associated with diabetes among Hispanics is a specific locus, known as SLC16A11, found in the 17th chromosome of the human genome. The pathogenesis of SLC16A11alters the carrier's ability to metabolize lipids and increases their intracellular triacylglycerol levels. The locus was traced back to ancestral mating among Homo sapiens and Neanderthals. A gene therapy medication known as diPGA, was constructed replicating the SLC16A11 locus and attaching ADN and IGF-1 onto receptor sites, and tested among Hispanic subjects throughout Miami-Dade County. Subjects were given the medication to observe if increasing ADN levels in individuals would have an adverse affect on fasting glucose levels (A1C%). Results illustrated that Experimental Groups receiving diPGA showed a significant upward spike in ADN levels, rising steadily and stabilizing during a span of 36 months. Adversely, a negative correlation was noted in reference to fasting glucose levels (A1C%), showing steady decrease and stabilization towards the cessation of the 36-month trial. The

	results of this clinical trial raise implications that can positively impact the scientific, medical, and Hispanic communities worldwide if successfully replicated in future studies. **Note:** This paper was an assignment for a nursing school General Education Capstone course. The student writer did not conduct a real study; he rather simulated a study to demonstrate writing/research skills, creativity, scientific knowledge, and an understanding of how to generate and analyze data. The corresponding author is the student's instructor, who guided the student on each section of the scientific paper, providing feedback on how to "conduct" the study and on how to revise the writing.

Introduction

Literature Review

Type 2 Diabetes (T2D), has become a disease epidemic. As stated in 2015 by the American Diabetes Association (2017), approximately 30 million Americans have been diagnosed with diabetes and there are approximately 1.5 million new cases of diabetes each year. Diabetes is the seventh leading cause of death in the United States, killing approximately 80,000 Americans annually as of 2015, and it costs Americans an excess of $240 billion annually (American Diabetes Association, 2017). There are two types of diabetes that affect the majority of the population, and can often be confused: Type 1 and Type 2 diabetes. The Diabetes Teaching Center (n.d) at the University of California, San Francisco defines Type 2 Diabetes as a dually defective disease in which the body has resistance to insulin in combination with the inability of insulin production. T2D is the most common form of diabetes, making up 80 – 90% of the diabetic population (Diabetes Teaching Center at UCSF, n.d).

In reference to pathogenesis, Copstead and Banasik (2013) characterize T2D by a relative systemic lack of insulin. The body's tissues become hyperglycemic due to insulin resistance, increasing the amount of excess glucose in the bloodstream (Copstead & Banasik, 2013). This resistance causes tissues to biologically need more insulin than normally required to consume glucose and remain homeostatic (Copstead & Banasik, 2013). Both the liver and gastrointestinal tract are responsible for releasing glucose into the blood stream so it can systemically spread throughout the body (Copstead & Banasik, 2013). In particular, the liver is responsible for gluconeogenesis, which converts consumed sugar from foods and synthesizes it into glucose for the body to use as energy (Copstead & Banasik, 2013).

Thereafter, the body, specifically the pancreas (organ responsible for insulin production) begins to compensate for the insulin resistance by creating larger quantities of insulin, hyperinsulimia; decomposition begins to occur when the Beta cells in the pancreas become dysfunctional which causes the pancreas to not produce enough insulin to metabolize the body's glucose levels and overcome the insulin resistance (Copstead & Banasik, 2013). The insulin deficiency over a period of time leads to the acquisition of diabetes and glucose toxicity, thus granting the need of some sort of exogenous diabetic treatment (Copstead & Banasik, 2013).

The correlation between Type 2 diabetes and dietary/lifestyle habits are well known, especially in association with high caloric Westernized eating habits consisting of high consumptions of red and/or processed meats, dairy products, high-sugar products, eggs, and refined grains (Zhao et al., 2017). Additionally, these Westernized eating habits increase risk factors such as Body Mass Index

(BMI), physical inactivity, and waist /extremity circumference (Zhao et al, 2017). In their research Zhao et al. (2017) concluded that there is a significant association between family history (FHD) and T2D, where offspring of T2D diagnosed parents are more likely to suffer from the disease than others that do not (Zhao et al., 2017). There have been over 40 genetic risk variants that have recently been validated, thus showing a strong correlation between diagnoses and genetics passed on from genetic donors (Anderson et al., 2013).

In order to be diagnosed with T2D, subjects will first be given a Glycated hemoglobin (A1C%) test, which indicates the mean blood glucose level over the past 60 – 90 days by measuring the percentage of blood glucose attached to hemoglobin (Mayo Clinic, 2017). A diagnosis of T2D warrants an A1C % level of greater than or equal to 6.5% on two separate testing occasions (Mayo Clinic, 2017). The most common treatment for T2D is healthy dieting, adequate physical activity, and oral medication administration (Mayo Clinic, 2017). Metformin is the most commonly prescribed medication for T2D and is typically considered as the first-line drug (Stuart, 2017). Stuart (2017) mentions that medication improves insulin sensitivity by inhibiting hepatic function, in turn regulating glucose uptake of the liver and skeletal muscle system.

Adiponectin (ADN) is a protein hormone secreted by adipose tissue that circulates the blood in different molecular weighted forms (Kogan, et al., 2013). Structurally speaking, ADN is a protein consisting of 244 amino acids and can be distinguished by its N-terminal variance region, collagenous domain, and C-terminal globular domain (Orrù, et al., 2017). ADN is also known as an insulin-sensitizing adipokine that improves insulin sensitivity by stimulating glucose uptake and inhibiting gluconeogenesis (Mei, Jeong-Sook, & Chang-Seon, 2010). One of the crucial functions of Adiponectin is metabolizing glucose and lipids, while also demonstrating protective effects against insulin resistance and inflammation (Orrù et al., 2017). Additionally, ADN makes better usage of lipids and simple carbohydrates (Orrù et al., 2017). Adiponectin is prevalently abundant in plasma (normal range: 5- 30 μ/ mL), however according to Hotta et al. (2000), plasma levels of ADN are significantly lower in patients with T2D (as cited by Mei et al., 2010).

ADN levels are 1.5 to 2 times higher in women than in men (Kogan et al., 2013). Furthermore, a linkage has been found between decreased ADN levels and insulin resistance, obesity, and other metabolic disorders (Orrù et al., 2017). Obese people have higher amounts of adipose tissues throughout the body, however they maintain low concentrations of AND throughout the abundant tissues; this is caused by inflammation of the adipose tissue, arbitrated by necrotic tumor factor alpha (suppressor of ADN) (Orrù et al., 2017). Moreover, after weight loss, adipose tissue functionality begins to improve and ADN levels begin to systemically increase across the body (Orrù et al., 2017).

According to Yamauchi et al. (2001), ADN causes decreased resistance among mice by lowering amounts of triglycerides located in muscle and the liver by increasing molecular expression of the molecules involved with fatty-acid combustion and energy dissipation (As cited by Mei et al, 2010). Furthermore, a study of Yamauchi et al. (2002) concluded that ADN activates 5'-AMP activated protein kinase (AMPK), which stimulates both glucose utilization and fatty-acid oxidation (As cited by Mei et al., 2010). ADN also prevents the progression of atherosclerosis in mice (Mei et al., 2010). It has been difficult to successfully deliver the ADN protein to targets by direct administration, thus researchers have diverted to genetic therapy as an alternative approach to ADN administration in mice (Mei et al., 2010).

Insulin-like growth factor 1 (IGF-1) is a protein produced by the liver consisting of 70 amino acids and has various functions throughout the body, specifically muscle growth and insulin-like activities (Orrù et al., 2017). Recent studies provide evidence that shows ADN, IGF-1, and insulin share common pathways, demonstrating an interconnection to obesity and other metabolic diseases such as T2D (Orrù et al., 2017).

In a study conducted by Harvard geneticist David Altshuler (n.d), evidence uncovered that humans acquired the diabetic gene mutation from Neanderthals, which is the first noted disease linkage to *Homo sapiens'* ancestry (Doucleff, 2013). In the research Altshuler and his team tested DNA sequences of approximately 8,000 subjects that lived in Latin American countries and found a mixture of European genetic markers among the subjects (Doucleff, 2013). Moreover, many known genes already associated with diabetes were identified in the subjects; however another gene, which increased diabetes diagnoses of 20%, was identified. (Doucleff, 2013).

The SIGMA Type 2 Diabetes Research Consortium (2014), analyzed approximately 9 million single nucleotide polymorphisms (SNP) of more than 8,000 Hispanic tests subjects, which were divided in two groups consisting of (1) diabetic and (2) non-diabetic (control). Among the sample, specific loci were identified in correlation with T2D (SIGMA, 2014). The loci identified was the solute carrier known as SLC16A11, which is a monocarboxylic acid transporter gene located in the 17th chromosome (SIGMA, 2014). The haplotype SLC16A11 carries four amino acid substitutes, its messenger RNA is located in the liver, and its protein is localized in the endoplasmic reticulum of cells (SIGMA, 2014).

The SLC16A11 gene was analyzed, and its genome sequence was traced back to archaic DNA of Neanderthals, suggesting that this genetic mutation is what increases the chances of Type 2 Diabetes in the Hispanic population (SIGMA, 2014). In reference to its Pathophysiology the gene SLC16A11 alters lipid metabolisms and increases the amount of intracellular triacylglycerol levels in the body (SIGMA, 2014).

In order to deliver to administer ADN, researchers constructed a cultured plasmid DNA encoded from a mouse by inserting the cDNA clone of ADN into a pVAX1 vector, naming it pVAX/ADN (Mei et al., 2010). According to the GenBank accession no. AF304466, RNA was extracted using Trizol and reverse transcriptase-polymerase chain reaction was performed in order to retrieve the full length of the mouse DNA (as cited by Mei et al., 2010). The cDNA clones were gel purified in 1% agar gels that contained 0.5 micrograms/mL ethidium bromide, and then inserted into T- vector, cut with EcoR 1, and re-inserted pVAX site resulting in the construction of pVAX/ADN strand (Mei et al., 2010). The Construction was then prepared using Endo Free Qiagen kit in order to remove all of the subsequent bacterial endotoxins (Mei et al., 2010).

According to an article published by the Centers for Disease Control and Prevention (2017), the current expectancy of adults to acquire T2D in the U.S is approximately 50%. Moreover, the CDC (2017) states that Hispanics have a greater risk of developing diabetes compared to non-Hispanics. Additionally, Hispanics have a 50% greater chance of dying from diabetes than Caucasian males are (Centers for Disease Control and Prevention, 2017). As previously mentioned, family history of diabetes (FHD) causes diabetes, however occasionally it is not only because of genetic relation. (CDC, 2017) There is an environmental factor that affects certain habits that increase risks; these habits are taught and/or absorbed family members (CDC, 2017).

The Pew Research Center displays a graph showing statistical data stating that 21.9% of Hispanics are living in poverty, and 61.4% of Hispanics have high school equivalent education or less (Flores, López, & Radford, 2017). The risk of T2D increases substantially in populations with low education and low socioeconomic position (SEP)(Dalsgaard, 2015). This can be caused by life style factors, accessibility to health care, and psychological burden, and stresses of poverty (Dalsgaard, 2015). Some well-known risk factors associated with Type 2 Diabetes and SEP are smoking, physical inactivity, and obesity (Dalsgaard, 2015). Agardh concluded that there is a 41% higher risk for T2D and people of low SEP in comparison to individuals with high SEP (as cited by Dalsgaard, 2015.)

The California Department of Health Services (2005), claims that in contrast to other ethnic groups in the state, Latinos have the highest rates of obesity; approximately 7 out of every 10 Hispanic adults (Latino Coalition for A Healthy California, 2006). According to the Surgeon General's Call to Action (2001), the cause of the obesity epidemic is excessive caloric intake in relation to unhealthy dietary habits along with inadequate physical activity (LCHC, 2006). Individual dietary habits and physical inactivity are shaped by external factors encountered in the physical environments of communities (LCHC, 2006). Typically the Latino/Hispanic populations live disproportionately among communities that embolden unhealthy dietary choices and dishearten individuals to not acquire adequate physical activity (LCHC, 2006). Such communities statistically have higher fast food outlets, convenient stores, small grocery outlets, and limited places where constituents can safely and/or consistently get sufficient exercise (LCHC, 2006). Along with understanding the biological and genetic factors of T2D, it is paramount to understand the coinciding health issues, culture, resources, and environmental structure of the Hispanic population (LCHC, 2006).

As previously mentioned, one of the barriers Hispanics face nationally is inadequate physical inactivity. Nationally Hispanics report low levels of physical activity (Bautista, et al., 2011). Engagement in physical activity is widely recognized to have numerous health benefits and regular

participation has been known to improve mental health and decrease risks of chronic health conditions, such as T2D and obesity (Bautista et al., 2011). According to the U.S Department of Human Health Services (2007), the minimum requirements for adults is at least 150 minutes of moderate-intensity physical activity per week or 75 minutes of high-intensity exercise per week (as stated by Bautista et al., 2011).

Hispanics are the largest minority group inhabiting the United States, approximately 15% of the population, and yet the National Health Interview Survey (2008) found that Hispanic subgroups all had lower leisure and physical activity time in comparison to Caucasians (Bautista et al., 2011). The Hispanic demographics have a higher frequency of diabetes, which has brought on the notion of promoting physical regular physical activity (Bautista et al., 2011).

Hispanics also face a dietary barrier and have societal constraints in finding food: many Hispanics inhabit low-income neighborhoods where health food outlets are scarce (LCHC, 2006). Typically Hispanics in low-income areas have limited choices and have predominantly more liquor stores and mini-markets at their disposable; which is stocked with high caloric options and have limited access to fresh produce and healthy alternatives (LCHC, 2006). The California Nutrition Network (2005) claims that 52% constituents of predominantly low-income areas are one-half mile away from a convenient store/mini market (as cited by LCHC, 2006). Additionally, fast food restaurants tend to cluster low-income areas and 64% of Hispanics say its too difficult get fresh fruits and vegetables at work (LCHC, 2006).

Materials and Methods

A partnership was acquired with local endocrinologists in order to randomize test subjects for the clinical trials conducted. Patients of these specialists were asked if they would like to participate in a clinical trial testing a new diabetic treatment awaiting human trials before FDA approval. The partnered physicians were blinded and not aware of the specifics of the clinical trial to ensure any formation of biases with possible subjects. The outlined eligibility criteria for the clinical trial are as follows: (1) Subject must be between the ages of 18 – 55, (2) have a diagnoses of T2D, (3) currently taking Metformin, (4) must be of Hispanic Origin, and (5) must be a resident of Miami-Dade County.

This resulted in the acquisition 3,318 potential participants. Subjects were then given a secondary preliminary screening in which their diet and exercise regimens were analyzed. Only subjects meeting the criteria of 100 or more minutes of weekly exercise, and dietary habits consistent with that suggested by their perspective endocrinologists were chosen, which in turn narrowed the sample size to 2,103 subjects. The 2,103 tests subjects were scheduled throughout different testing sites over the span of 10 days to undergo preliminary blood examinations. Subjects were advised to fast for 8 hours prior to undergoing blood sample draw. The intention of the blood exam was to analyze subjects fasting glucose levels (A1C %), Adiponectin levels, and DNA examination of the SLC16A11 loci located in the 17^{th} chromosome. Only 46% (n = 968) of the subjects tested positive for all 3 criteria and moved to the administration phase of diPGA. Of the 968, 492 were female and 476 were male.

The new medication being tested is known as diPGA (Diabetes Please Go Away) and contains an artificially replicated version of the subjects SLC16A11 loci: which consists of the loci along with Adiponectin (ADN) and insulin like growth factor 1 (IGF-1) attached to genetically modified enzymatic receptor sites. In order to achieve this, SLC16A11 was isolated and genetically engineered into a vector (SLAC). Plasmid encoded ADN and IGF-1 were constructed by inserting their cloned DNA copies into the SLAC vector using the EcoR 1 restriction enzyme. The vector was then cultured in Dulbecco's Modified Eagle's Medium. The RNA was extracted using Trizol and reverse transcriptase was performed to retrieve the strand. The cloned ADN and IGF-1 was reinserted to the SLAC vector, cut with the EcoR 1 enzyme and reinserted into the Eco R 1 site of the SLAC vector, creating diPGA, the AND/IGF-1 genetically modified into SLC16A11 locus. The diPGA was then purified using the Endo Free Qiagen Kit to ensure the removal of any harmful bactericides or endotoxins.

The test subjects were then administered diPGA intravenously as an infusion on a weight based dose at 0.5 μ/kg/min over 1 hour. The infusions were given twice a week to each subject on the same

allotted days. Subjects were instructed to continue on their regular Metformin medication throughout the duration of the Clinical Trial. The Control Group was given a B12 vitamin shot in substitution for the treatment. Experimental Groups were also given the B12 vitamin shots along with diPGA in order to accurately differentiate results between the groups.

Subjects were divided into 2 groups: (1) Control Group and (2) Experimental Group. The Sample consisted of 492 female and 476 male subjects (n = 968) and were advised that at any point in time they can remove themselves from the study. They were also advised to seek emergency medical care if any adverse reactions occurred. Possible side effects were and not limited to tachycardia, fever, nausea, vomiting, dizziness, and hypoglycemia. Throughout the clinical trial, 13% (117) of the remaining subjects extracted themselves from the study (n = 851; 433 females and 428 males), in which observational data of the subjects were excluded from the results. The final groups included in the results were: (1) Control group [n = 425; 217 females and 208 males], (2) Experimental Group [n = 426; 216 females and 210 males]. Results were organized and recorded using Microsoft Excel. The mean average of each dependent variable was used to create marked line graphs to demonstrate trends.

Subjects fasting glucose (A1C%) and ADN levels were tested at 3-month intervals for the first year, and 6-month intervals the following two years. They were advised to fast and discontinue Metformin 8 hours prior to testing. In order to obtain the fasting glucose levels of the subjects, the industry standard A1C Hemoglobin test was used to avoid any skewed results. The A1C test shows average levels of blood sugar over a corresponding period of 3 months. Blood was drawn using standard blood drawing procedures and were collected in the Lavender, or Purple, top test vials which are interiorly coated with EDTA K2, and required at least 1 mL of blood for testing. A HemoPoint H2 Analyzer was used to result the samples. The A1C results will be reported as percentages.

In order to test for ADN levels, the ADN Serum test was administered. The specimens were placed in in a Gel- barrier tube referred to as a SST, or Tiger Top, which requires 0.3 – 10.0 mL of serum. Upon collection of sample, tubes were gently inverted 5 times and allowed to clot for 30 minutes. Samples were then centrifuged at 1300 rcf for 10 minutes. Serum samples were stored in 2°C to 8°C and transported the same day to the laboratory testing facility. Turn around time for samples ranged from 7 to 10 days.

Results

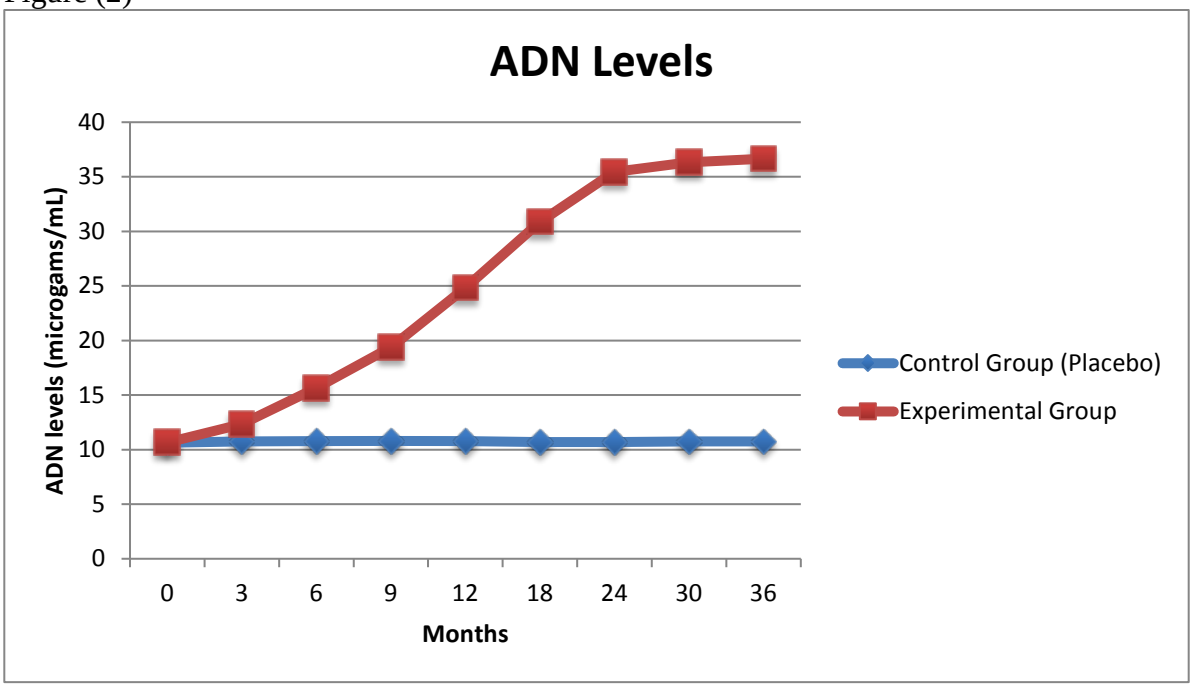
In order to effectively display fasting glucose levels, A1C hemoglobin levels were used in its place. Figure (1) displays a reference guide for A1C hemoglobin levels and there mean corresponding glucose levels. Levels were color categorized to display severity levels: **Red (**severely elevated levels. Involves Risks of serious health complications such heart attack, stroke, blindness, end stage renal failure, and necrosis), **Yellow (**Elevated and Poorly controlled levels), and **Green (**Normal Levels). An A1C Diabetes test above 5.9% is considered Pre-Diabetic. To be diagnosed with Diabetes, the minimum A1C level is 6.5% or above. An individual already diagnosed with Diabetes, an A1C of 7.0% or below is considered an adequate level.

Figure (1)

Severity	A1C Levels	Glucose Levels
Severly Elevated	13	380
	12	345
	11	310
	10	275
Elevated	9	240
	8	205
	7	170
Normal Levels	6	135
	5	100
	4	65

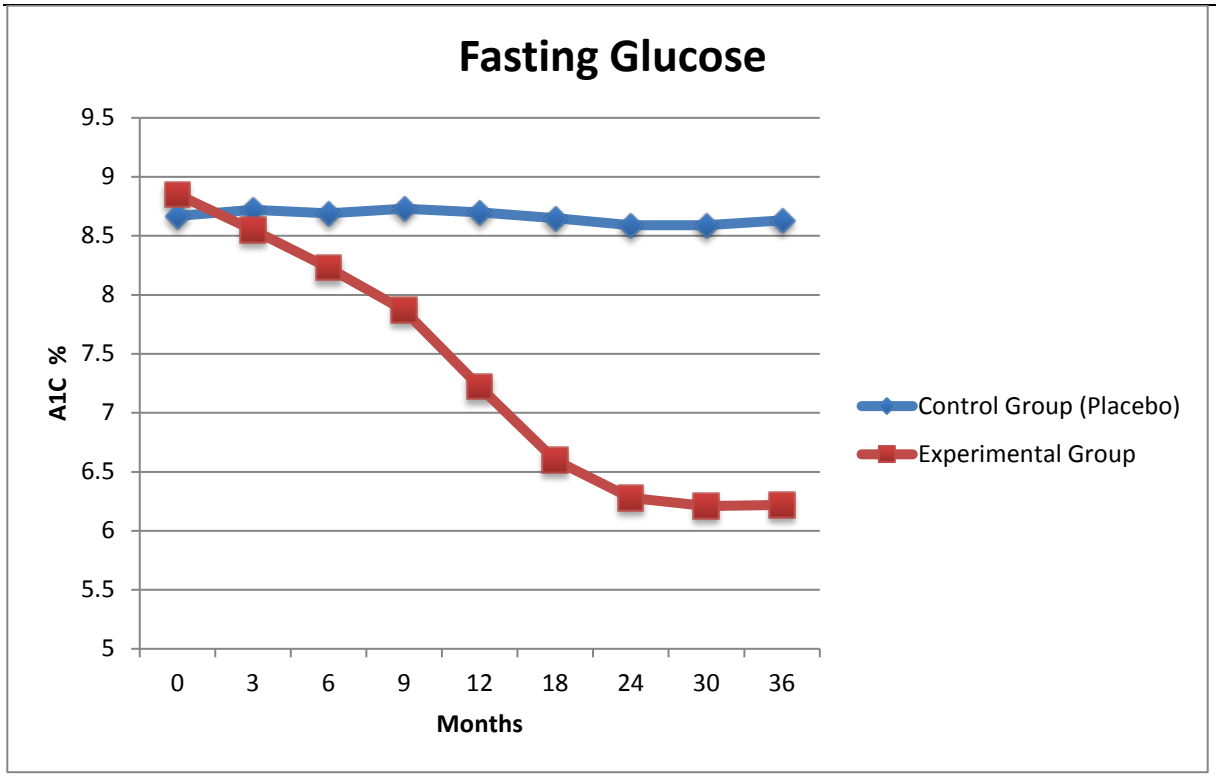
The ADN levels are displayed in Figure (2). The initial results are represented by the Value "0 Months" on the x-axis. The initial sample mean ADN levels of the Control Group (n = 425) was 10.63 μ /ml. The Control group demonstrated insignificant changes (less that 5% of mean) throughout the 36-month trial period. The Experimental Group (n = 426) had an initial sample mean of 10.65 μ/ml. The percentage increase [(new value – initial value) /new value] formula was used to acquire the percentage increase of each interval and is displayed next to its corresponding value. The values are followed; 3 months = 12.34 μ/ml (16%); 6 months = 15.66 μ/ml (47%), 9 months = 19.38 μ/ml (82%); 12 months = 24.82 μ/ml (133%); 18 months = 30.88 μ/ml (190%); 24 months = 35.44 μ/ml (233%); 30 months = 36.32 μ/ml (241%); 36 months = 36.66 (244%).

Figure (2)



The AIC hemoglobin (fasting glucose levels) are displayed in Figure (3). The initial sample mean of the Control Group (n = 425) was 8.67% at 0 months. The Control group demonstrated insignificant changes (less that 5% of initial result) throughout the 36-month trial period. The Experimental Group (n = 426) had an initial sample mean of 8.85% at 0 months. The values are as followed; 3 months = 8.55% (-3%); 6 months = 8.23% (-7%); 9 months = 7.87% (-11%); 12 months = 7.22% (-18%); 18 months = 6.6% (-25%); 24 months = 6.28% (-29%); 30 months = 6.21% (-30%); 36 months = 6.22% (-30%).

Figure (3)



Data was also categorized by gender (female and male) in order to determine any differentiation of results between the sexes. Figure (4) illustrates ADN levels of the Control Groups and Experimental Groups for both sexes. The Female Control Group (n = 217) displayed an initial mean 11.34 µ/ml and the Male Control Groups (n = 208) displayed an initial mean of 9.92 µ/ml. The two control groups did not show any significant levels of changes (less than 5% of initial result) throughout the trial. The initial mean of the Female Experimental Group (n = 216) was 11.35 µ/ml at 0 months. The results are as followed; 3 months = 14.67 µ/ml (29%); 6 months = 18.84 µ/ml (66%); 9 months = 21.22 µ/ml (87%); 12 months = 27.87 µ/ml (146%); 18 months = 35.88 µ/ml (216%); 24 months = 36.23 µ/ml (219%); 30 months = 36.44 µ/ml (222%); 36 months = 37.09 µ/ml (227%). The initial mean of the Male Experimental Group (n = 210) was 9.95 µ/ml. The results are as followed; 3 months = 10.01 µ/ml (1%); 6 months = 12.47 µ/ml (25%); 9 months = 17.53 µ/ml (76%); 12 months = 21.76 µ/ml (119%); 18 months = 25.88 µ/ml (160%); 24 months = 34.64 µ/ml (248%); 30 months = 36.08 µ/ml (263%); 36 months = 36.22µ/ml (264%).

Figure (4)

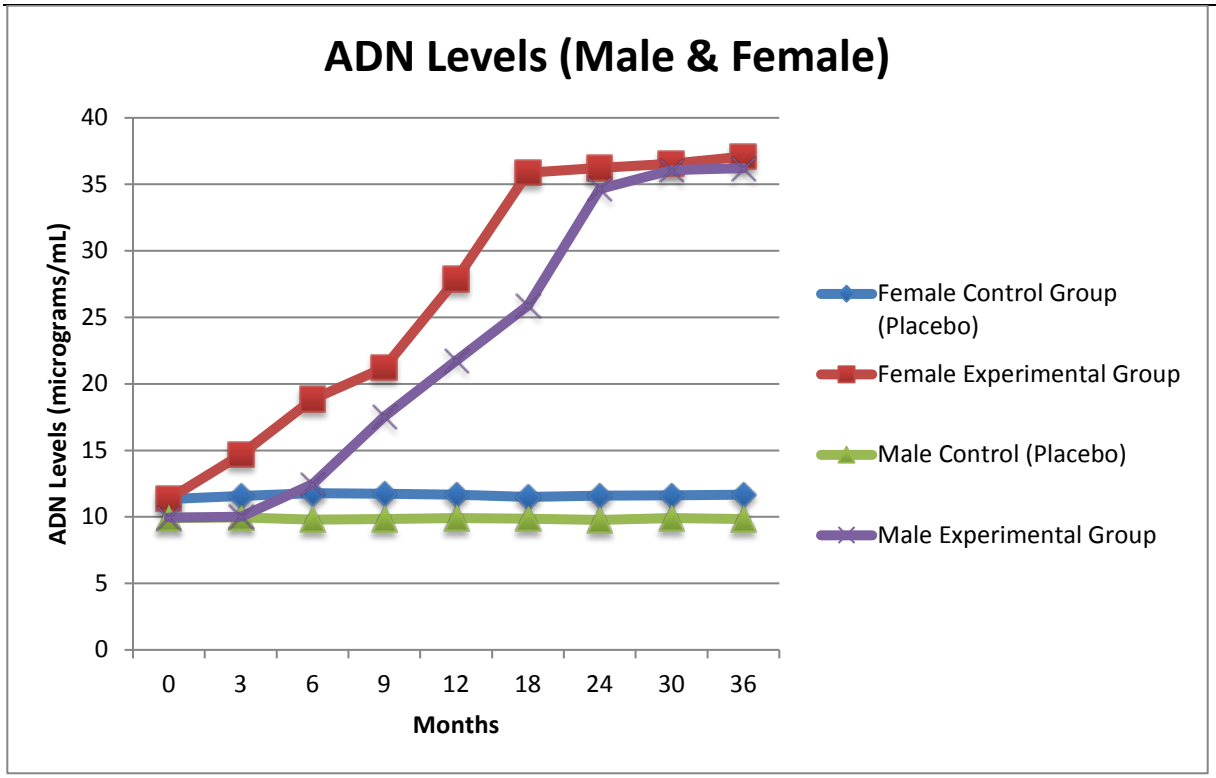
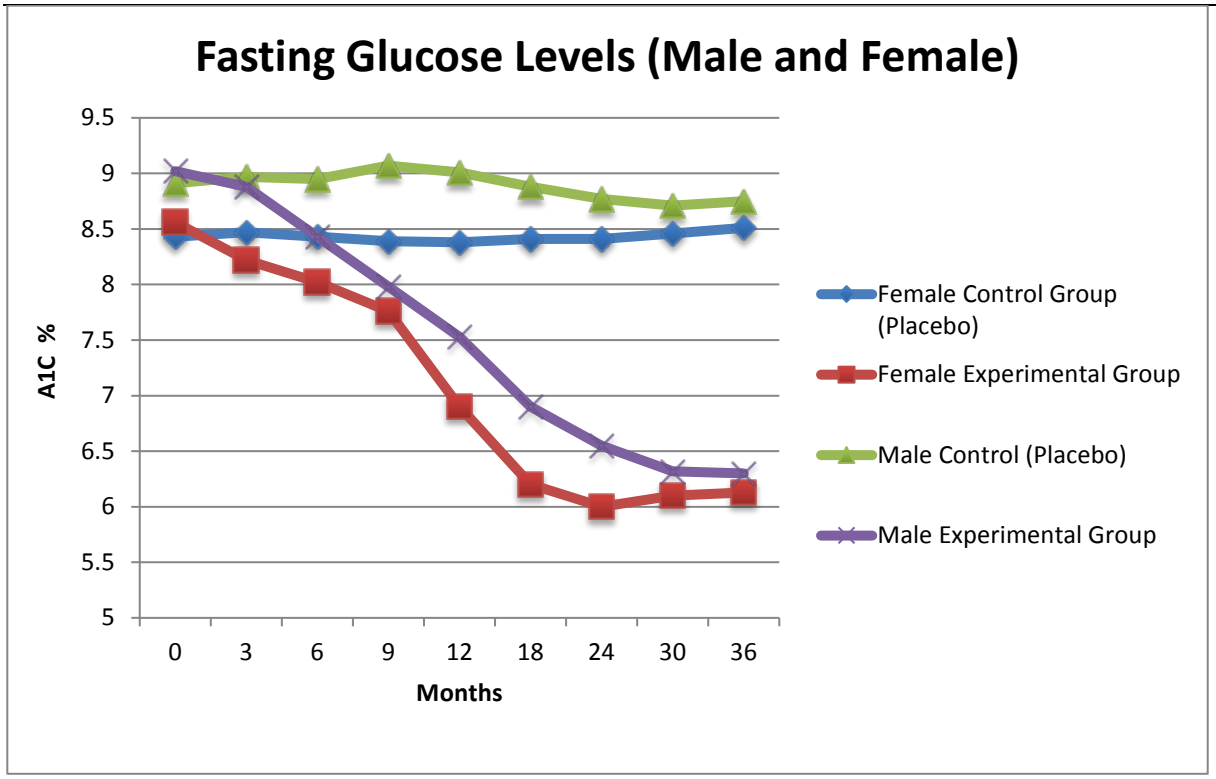


Figure (5) illustrates Fasting glucose levels as A1C % of the Control Groups and Experimental Groups for both sexes. The Female Control Group (n = 217) displayed an initial mean of 8.43% and the Male Control Groups (n = 208) displayed an initial mean of 8.91%. The two control groups did not show any significant levels of changes (less than 5% of initial result) throughout the trial. The initial mean of the Female Experimental Group (n = 216) was 8.56% at 0 months. The results are as followed; 3 months = 8.22% (-4%); 6 months = 8.02% (-6%); 9 months = 7.76% (-9%); 12 months = 6.9% (-19%); 18 months = 6.2% (-0.28%); 24 months = 6.0% (-30%); 30 months = 6.10% (-29%); 36 months = 6.13% (-28%). The initial mean of the Male Experimental Group (n = 210) was 9.02%. The results are as followed; 3 months = 8.88% (-2%); 6 months = 8.43% (-7%); 9 months = 7.98% (-12%); 12 months = 7.53% (17%); 18 months = 6.9% (-24%); 24 months = 6.55% (-27%); 30 months = 6.32% (-30%); 36 months = 6.30% (-30%).

Figure (5)



Conclusions

The results throughout this clinical trial have suggested a substantial significance among groups receiving treatments. The Control Group, displayed by Figure (2), had no significant changes (less than 5%) throughout the trial by displaying a plateauing trend throughout the graph. However, the Experimental group illustrates a 244% mean ADN level increase (36.66 **μ**/ml) in comparison to the initial mean level of 10.65 **μ**/ml. The results display a correlation between diPGA administration and rising ADN levels, where ADN levels rise steadily and begin to plateau at the end of the trial.

Additionally, in Figure (3), the Control Group had an initial mean A1C Hemoglobin level of 8.67% and the Experiential Group had an initial mean A1C Hemoglobin level of 8.85%, which according to Figure (1) translates to blood glucose levels between 205-240mg/dL. The Control Groups show an insignificant change throughout the clinical trial (less than 5%), and displays no trends. However, the Experimental Group showed a decrease of 30% from initial mean fasting glucose levels (A1C%) at the cessation of the trial, from 8.85% to 6.22%, which translates to an ending mean blood glucose level of 135-170 mg/dL (normal levels to borderline elevated as per Figure 1). The difference between ending and initial mean glucose levels is 70 mg/dL (29 -34% decrease).

The data in Figure (4) demonstrates the ADN levels, of the subjects subcategorized by the subject's sex. Among the Control Group, the initial mean ADN levels for females were 11.34 μ/ml and males were 9.92 μ/ml. Neither Control group displayed any trends with significant meaning (less than 5%) and remained horizontal throughout the trial. In reference to the Experimental Group, females displayed an initial mean ADN level of 11.35 μ/ml that increased 226% to 37.09 μ/ml, while male ADN levels were 9.95 μ/ml, which increased 264% to 36.22 μ/ml. The data suggests that females initially had higher ADN levels than males, however both sexes reacted to diPGA significantly. Males showed a higher increase in percentage (41%) than females.

The data in Figure (5) demonstrates the A1C% levels of the subjects subcategorized by the subject's sex. Among the sexes of the Control Group, the initial mean A1C% levels for females were 8.43% and males were 8.91%. Neither Control groups displayed any trends with significant meaning (less than 5%) and remained horizontal throughout the trial. In reference to the sexes of the Experimental Group, females displayed an initial mean A1C% level of 8.56 A1C%, which decreased 26% to 6.13 A1C% whereas male A1C% levels decreased 30% from 9.02 A1C% to 6.3 A1C% The data suggests that both sexes initially had relatively similar fasting glucose levels (A1C%), and both sexes reacted to diPGA significantly by falling between 6%-7% A1C Hemoglobin % level, which as

shown in Figure (1), indicates normal levels. Moreover, this suggests that the subjects are no longer considered to be Type 2 Diabetics due to the fact that A1C% criteria for diagnoses are no longer met.

The data collected suggests a negative correlation between the experimental groups: as ADN levels increased over time and stabilized, fasting glucose levels adversely decreased and stabilized. The results can have an astounding impact on the scientific community due to the genetic methods of treatment for the disease. The construction and results of the diPGA trials will hopefully cause a shift in medicine from treating diagnoses to curing diagnoses, particularly on the genetic level. The Hispanics of Miami-Dade County should also reap the benefits of this medication, which provides the first signs of curing of genetically predisposed T2D among their demographics. Their participation provided a pivotal impact on the Hispanic community and continued research and development may provide an end to the pandemic disease affecting the Hispanic demographic worldwide.

In order to test validity, accuracy, and relevance other geneticists should replicate the diPGA trials in order to compare. Moreover, a future study will be replicated in Hispanic demographics in other geographical areas for comparison of results. Further implications include testing the Fasting Glucose levels of subjects who have already undergone the trial without taking their Metformin medication. Also, a post trial should be conducted where ADN and A1C% levels are checked periodically to observe if any changes occur to the subjects once the cessation of diPGA has occurred. Furthermore, genetic therapy clinical trials for different demographics with T2D should be conducted by constructing diPGA based on a specific's demographic gene. The suggestions above could possibly strengthen the findings of this clinical trial and fill any gaps the FDA may have prior to allowing implementation of diPGA treatments nationwide.

The trial conducted was designed in accordance with standard ethical guidelines. The FDA was consulted and asked to perform an independent review prior to commencing the trials in order to ensure practices met certain ethical guidelines and found the risk-benefit ratio were acceptable. The research displayed scientific validity because an attainable and answerable question/ goal was used throughout. Subject selection was unbiased and fair, except for qualifying criteria. Subjects were not chosen based on vulnerability, socioeconomic position, or other unrelated prejudices to the study.

All subjects provided informed consent and were aware of the possible side effects of diPGA, and advised to seek emergency medical attention if specific reaction occurred throughout the trial. DiPGA's benefactors provided an emergency medical fund to compensate any medical issues caused by the trial. Additionally, the privacy of all subjects was maintained throughout the trial and HIPAA Laws were not violated throughout the duration of the trial. Subjects were given the results to demonstrate the significance the study provided.

Possible social issues that can present after diPGA approval are poverty, education, lack of access to affordable health care, and language barriers. As previously stated, an large portion of the Hispanic demographic in the United States live in poverty, and may not be able to afford the medication even if it were available on the market. Even if healthcare is available, insurance companies may not cover the medication due to contractual agreements or profits with current market medications. Therefore, legislation should be set forth by politicians to allot funding and subsidies for diPGA so that the Hispanic demographic can be able to receive treatment if they do not have the means to do so themselves. Implementation of this legislation should promote diPGA to the pharmaceutical and healthcare markets and incentivize the usage to both parties.

Awareness can be made by partnerships with physicians groups, government agencies, public health agencies, hospitals, and other community advocates promoting the implications of the medication. The most important aspect of awareness would be educating the public on the existence of the SLC16A11 loci (diabetic gene), and demonstrate that there can possibility a cure for the genetic deficiency.

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International Journal of Molecular Sciences

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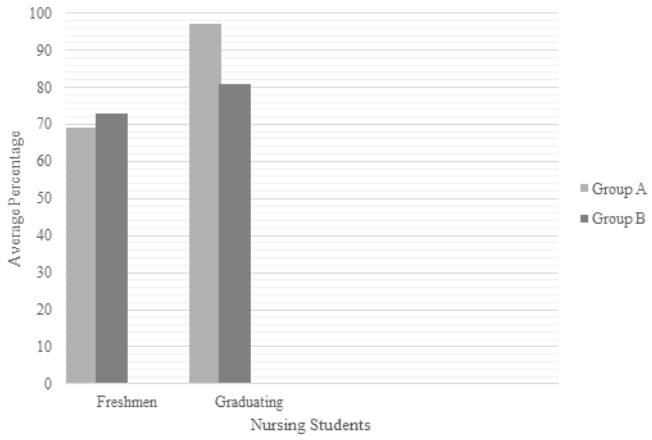
Graphical Abstract



Abstract

As the world becomes more diverse, effectiveness of interaction and empathy continue to decrease. In the nursing career, it is important to keep an open-mind and an empathetic heart to execute the maximum quality of care. Fiction literature has proven to engender prosocial skills along with empathy and cognitive participation. FMRIs have shown significant correlations between literary pieces and neural activity in parts of the brain associated with empathy. Because nursing students have shown tremendous decrease in empathy approaching their graduation date, this study tests the effectiveness of fiction literature to prove the enhancement of empathy in the nursing students. This research included an equal number of female and male participants entering the nursing program at West Coast University. Subjects were evaluated on empathy before entering the program, then once more as they were about to graduate. The participants were divided into two groups; one of them received a fiction literature course that focused on empathy and the other did not. The group that was enrolled in the fiction literature course excelled remarkably compared to the group who was not stimulated through fiction literature. Because fiction literature allows nursing students (through guided imagery and other literary tools), the group with the additional course comprehended empathy and embraced it better than the control group.

Note: This paper was an assignment for a nursing school General Education Capstone course. The student writer did not conduct a real study; she rather simulated a study to demonstrate writing/research skills, creativity, scientific knowledge, and an understanding of

	how to generate and analyze data. The corresponding author is the student's instructor, who guided the student on each section of the scientific paper, providing feedback on how to "conduct" the study and on how to revise the writing.

Introduction

Literature Review

One of the major issues nurse educators are facing is the decline in empathy in nursing students as they get closer to graduating. As exams get harder, study sessions become longer, and the board exam gets closer, nursing students are likely to prioritize empathy as last on their list. A quality so crucial to the nursing career should be improved continuously, and considered before beginning such an empathy demanding occupation (Atherton and Kyle, 2014). Atherton and Kyle (2014) disclose that empathy has no single definition and is not to be confused with sympathy, but can be described as the ability to understand another person emotionally followed by actions. Medical professionals who exhibit empathy are more likely to have an accurate diagnosis and effective treatment plan through honest communication (Stepien and Bernstein, 2006). When patients feel sincere interest in their care from the healthcare professional they are more likely to give more information, making it easier to diagnose the signs and symptoms and implement a treatment plan (Stepien and Bernstein, 2006). Patients are more likely to be compliant with medical advice when they trust the healthcare provider because that personal relationship is present. Not only is empathy imperative between a nurse and patient, but essential between colleagues in the workplace. Viggiani, Charlesworth, Hutchinson, and Faria (2005), agree that introducing empathy in the workplace makes for a compelling team of care, improving social interactions, and providing effective therapy. Many nursing programs have tried to approach this issue by implementing an additional literature course. Literature allows the reader to use imagination to develop a deeper understanding of a patient's situation and emotions (McAllister, Lasater, Stone and Levett-Jones, 2015), thus, creating a personal relationship with the patient and increasing empathy. McAllister, Lasater, Stone and Levett-Jones (2015) also reveal that by reading fiction literature, nurses are able to gain new perspective, imagining themselves as the patient in different scenarios. As critical thinking and knowledge develops in these future healthcare professionals, empathy and effective communication increases.

Literature can affect a person in a plethora of ways, one of those being empathy production. Using an fMRI (functional magnetic resonance imaging) researchers are able to create images of the brain and its neural activity while a literary piece is read or heard by a participant. Nijhof and Willems (2015) believe that the neurocognitive mechanisms underlying fiction comprehension are unclear and conducted a similar study to better understand the relationship between the literature and its impact on the brain. In the study, Nijhof and Willems used eighteen participants to listen to literary pieces using MR-compatible earphones while lying in the MRI scanner. Their results showed neural activation while the participants listened to excerpts from literary stories, discovering evidence that engagement with fiction renders people more empathic. An fMRI is the ideal neural imaging equipment for this research type because it measures the brain activity by detecting changes while simulation is conducted. Another fMRI study conducted by Larence, Shaw, Giampietro, Surguladze, and Brammer (2006) identifies regions of the brain correlated with empathy. The premotor cortex/precentral gyrus, inferior frontal gyrus and medial frontal lobe were activated during a social perception task based on dynamic stimuli (Larence et al., 2006). The use of neural imaging has been around for decades, but the investigation for the relationship between empathy and literature is still a work in progress. These two, previously mentioned, studies show the possibility to measure neural activity with empathetic and literary simulation. Research shows that an fMRI can result in reliable results demonstrating blood

flow in various regions central to emotion processing during specific task conditions (Gee, McEwen, Forsyth, Haut, Bearden, Addington, and Cannon, 2015). A study performed by Gee et al. (2015) has confirmed the reliability in imaging signals of activation of emotion processing regions in the brain when stimulated by relevant tasks. Study/test results are only viable when proven in research using reliable sources and equipment.

Oxytocin is a neuropeptide hormone that provokes emotional and social behavior (Lane, Luminet, Rime, Gross, de Timary, and Moira, 2013). Oxytocin is commonly used to cause or strengthen labor contractions, but this neurotransmitter is also known to release to empathy-stimulating responses. The hormone is released by the posterior pituitary and is synthesized in the hypothalamus (Crespi, 2016). Lane et al. (2013) have participated in a study to support oxytocin's ability to facilitate pair-bonding and social interactions. Their test included sixty test subjects (30 men and 30 women), and each subject was given either oxytocin or a placebo. Participants watched a film on friendship and camaraderie and were asked to write a narrative. Two judges reviewed the participants' written experience and found some papers where a subject's emotions were so intense, they could not express themselves accordingly with words. Results were significant because men, who are usually less inclined to reveal emotions, shared as much as women. These findings imply that releasing this hormone can be used to improve social interaction and patient to therapist communication (Lane et al., 2013). Lane et al. (2013) provided us with the first evidence to prove that oxytocin can maximize social interactions, such as empathy. It is also believed that 3,4-Methylenedioxymethamphetamine (MDMA) and norepinephrine produces and/or enhances empathy and sociability (Hysek et al., 2014). Hysek et al. (2014) created a study that included thirty-two participants, using an equal number of both sexes, who were in a placebo-controlled, double-blind, random-order experiment. MDMA increased plasma levels of cortisol, prolactin, and oxytocin, which have been associated with prosocial behavior (Hysek et al., 2014). "The MDMA-induced release of oxytocin and overall very similar emotional-cognitive effects of oxytocin and MDMA might implicate oxytocin as a crucial mediator of the effects of MDMA on empathy and social behavior" (Hysek et al., 2014). As per Hysek et al. (2014), because of blood–brain barrier, directly testing these effects will be difficult. Oxytocin, according to multiple researchers, is a key component for the engendering of empathic and prosocial behavior. Studies have consisted with theories that pharmacological manipulations of the previously mentioned neurotransmitters have been effective. Administering oxytocin intra-nasally has become a common way for conducting studies for its effects. As previously mentioned, oxytocin has demonstrated the enhancement of emotion, but it can also effect perception of others' emotional facial expressions. Oxytocin can sharpen the impressions such that happy faces appear happier and less angry, whereas angry expressions appear angrier and less happy (Leknes, Wessberg, Ellingsen, Chelnokova, Olausson, and Laeng, 2013). Leknes, Wessberg, Ellingsen, Chelnokova, Olausson, and Laeng (2013) also correlated oxytocin to pupil dilation; when oxytocin stimulus increased, so did pupil dilation. According to their analysis, large pupil sizes are associated with increased attractiveness and social behavior in humans. Because of the many uses of oxytocin and its positive impact on social interaction (including empathy), professionals have been searching for evidence for the physiological mechanism whereby this hormone interacts. The understanding of the hormonal mediation can lead to a better comprehension of human psychiatric disorders and enhancement of empathy.

According to Seibert, Stridh-Igo and Zimmerman (2002), "a person's culture and ethnicity determine how he/she perceives the world and its contents." Healthcare professionals are required to be aware of differences between groups of people and their attitudes/meanings attached to events such as depression, pains and disability (Seibert, Stridh-Igo and Zimmerman, 2002). Healthcare professionals are not expected to know every aspect of every culture, but acquire sensitivity in understanding cultural differences. Reading fiction literature can help envision healthcare through a patient's perspective by the use of descriptive imagery. Incorporating literary texts in the medical education can develop contextual awareness, empathy, and ethical reasoning (Flood and Farkas, 2011). Reading can also educate individuals on different cultural backgrounds and illustrate how they perceive the world or certain situations in the medical field. Literature appears to involve cognitive participation and because of the use of language, readers have more control over emotional distance (Mar, Oatley, Djikic and Mullin, 2011). A person's inclination (culture) and literary impact (through

imagination) can either affect or engender empathy (Tamir, Bricker, Dodell-Feder, and Mitchell, 2016).

Iannone (2005) claims that the fear of being unique in young Americans is not allowing them to love their own traditions, language or literature. Iannone (2005) believes that reading is a solitary activity, but those who read literature also participate in social and cultural activities. The rapid increase in the Hispanic population has brought down the literary readers, since Hispanics have the lowest share of literary readers (Iannone, 2005). Because people usually acquire what they see or are taught at home, this issue is still rising in numbers. Empathy is also influenced by culture, and culture influences literature. Culture influences the type of reading, if reading is implemented at all, and the interpretation of the literary piece. Nursing book clubs are often held to continue education, reflect and discuss challenging healthcare issues, such as empathy and cultural diversity in the field (Greenwald and Adams, 2008). This kind of social interaction helps nurses (of different cultural backgrounds) share their own perspectives on issues or topics of discussion.

Developing empathy through children's literature has been encouraged to develop social adjustment from a young age (Cress and Holm, 2000). Promoting critical thinking and social skills from literary analysis can sway a child's perspective and engender empathy. Research implies that children's literature should expose messages about the relationship among bilingual language, culture, and identity (Chappell and Faltis, 2007). Chappell and Faltis (2007) claim that educators using this literary tool can introduce an empathetic message about values, attitudes, and beliefs individuals endure on a daily basis. Culture and empathy possess the ability to alter the way one may perceive a piece of literature and the meaning of it, from learned perspective or personal experiences. Engendering prosocial skills through literature at an early stage in life increases literary skills and critical thinking. Empathy can be engendered through literature, but also influenced by culture. Considering the mixture of cultures and its differences coexisting in a certain area/region, people tend to show reduced empathy for those with dissimilar cultures (Heinke and Louis, 2009).

As the United States becomes more diverse, effectiveness of interaction and empathy begins to decrease due to the population. Interdependent cultures and independent cultures have different attitudes towards cultural diversity; independent cultures fear that it might disrupt their harmony (De Greck et al., 2012). De Greck et al. (2012) used an fMRI to examine Chinese (interdependent culture) and German (independent culture) healthy subjects during an empathy task, a control task and baseline condition. Both groups reacted differently, but results imply that enhanced emotion regulation during empathy with anger in the interdependent culture is mediated. Study results suggest that empathy is provoked in many ways, but varies depending on culture and environment (De Greck et al., 2012). Socializing is effortless with others who share the same culture, patients feel a stronger, personal connection to the healthcare provider when this similarity is present. As minority groups grow and cultural diversity in the health system is more common, challenges to clinicians or other medical professionals arise (Zayas and Torres, 2009). Acknowledging a patient/client's cultural background is important but should not negatively affect empathy from the medical professional.

Materials and Methods

The concept of empathy in nursing students was based on a measurement of self-evaluation surveys. Because there seems to be a decline of empathy in nursing students, nursing students were questioned about empathy at the beginning of nursing school and one more time as they were about to graduate. For the purpose of this research I decided to use 40, healthy first semester nursing students at West Coast University to evaluate, 20 males and 20 females. To avoid bias results, no specific race/ethnicity was required to participate, all individuals in this study were selected at random within the gender needed from an enrollment list provided by the Admissions Department. I decided not to filter participants by age or socioeconomic status to allow my results to reflect a variety of circumstances – as no nursing student is the same.

After gaining permission from West Coast University, I was able to contact students via e-mail to inform them of my research and extending to them an opportunity to be a part of a valuable study. A study that will impact the healthcare field entirely, improve patient care, and refine the nursing school process. After one week, I received forty acceptances from the nursing students. I divided the

participants into two groups of ten males and ten females, named Group A and Group B. Both groups were to be surveyed in the first semester of nursing school using the Empathy Scoring Survey (ESS) provided. At the top of the survey, students are required to write if they are a first-semester or a graduating nursing student. The ESS consists of ten statements, participants will have to reply with a number one through five; one being strongly disagree, two being disagree, three being neutral, four being agree, and five being strongly agree. Out of the possible fifty points, points were doubled for percentage purposes and answers were evaluated. Before receiving any knowledge of the importance of empathy or the general concept of patient care, I wanted to measure their understanding of it at that point in time. Participants were surveyed individually in an isolated room to avoid any distractions or answer influence from other students. From this point, the groups were divided and Group A was required to be involved in fiction literature courses throughout nursing school while Group B was not.

Group A underwent fiction literature courses that will strengthen their comprehension of empathy and its crucial effects on patient care. This course involved literary pieces written from a patient's perspective on their healthcare experiences, videos and audios on empathy involving patient care, and other helpful literary tools. Offering a variety of fiction literature exercises will target each and every learning style and guarantee the comprehension of the assignment. This course included a short quiz after each literary piece has been reviewed to measure progress, but did not count towards the student's GPA. The goal of this study was not to add more stress to nursing students, but to improve their apprehension of empathy so that they become successful, well-rounded nurses. Group B completed nursing school, like Group A, but excluding the fiction literature courses. As both groups approached graduation from nursing school, we have the participants complete the ESS one more time using the same strategy, individually tested in an isolated area/room, so there are no discrepancies. I gathered both scores collected from each group and analyzed the data. Due to the total of 100 points on the ESS, I decided to calculate averages in percentages and used an average total for the whole group to compare to the other.

Results

Survey results were collected from the individuals participating in the nursing program at West Coast University. The control group was determined to be Group B, who were not involved in a fiction literature course in nursing school. The experimental group was Group A, who were enrolled in fiction literature courses throughout nursing school. The independent variable was the fiction literature course that would be implemented in the nursing school, and the dependent variables would be the amount of fiction literature read and the level of empathy measured in first semester and graduating nursing students.

In Figure I, the graph presents the first-semester nursing students and their average empathy score alongside those who are graduating. Group A scored a total of 69%, and Group B a total of 73%; both groups had somewhat of an understanding of empathy, but they do not fully comprehend the importance of it in the healthcare field. Both averages are fairly close to one another, so the benchmark is set to compare to their scores when the ESS is repeated. The same graph compares the vast increase of empathy comprehension in Group A. In Group B, there is an increase in empathy, but not as drastic as Group A's improvement. Group A had an average score of 97%, while Group B had an average of 81%.

In Figure II, Baptist Hospital conducted a Patient Empathy Survey Questionnaire on all of their patients to evaluate West Coast University's Nursing students and their impact on the hospital's patients. After the graduating nursing students' last clinical rotation on their site, Baptist conducted the survey, and sent West Coast University the results. As you can see, patients that were treated by Group A, scored a higher empathy percentage than those who were treated by Group B. Group A earned a score of 89% from their patients, and Group B had a score of 62%.

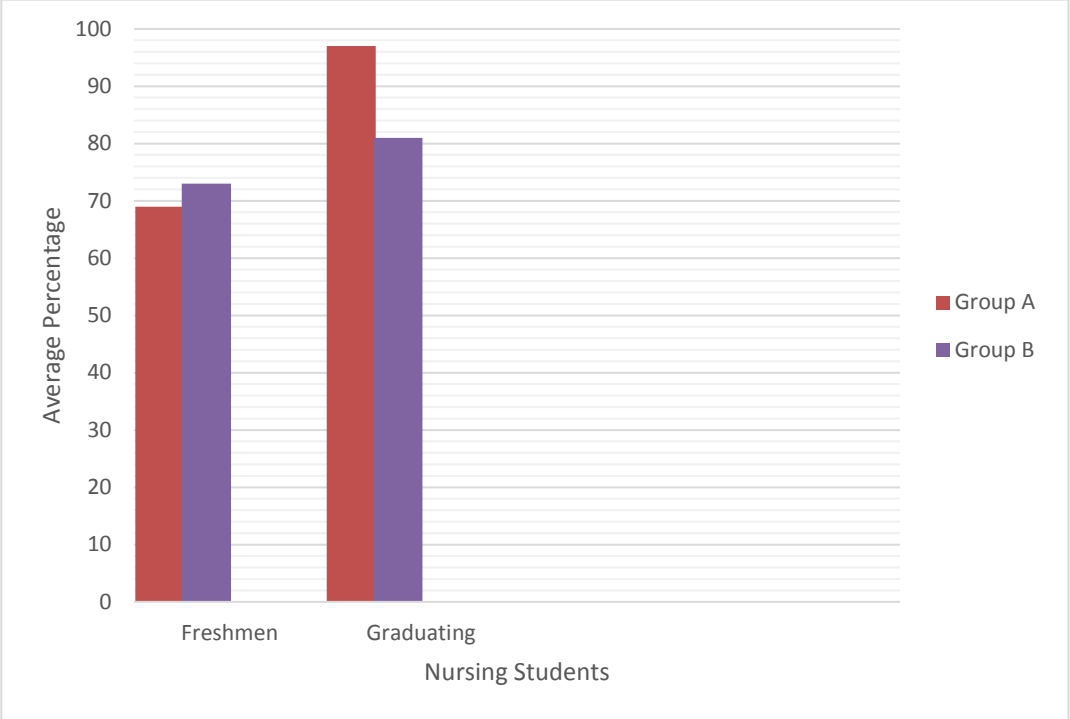


Figure I, Group A First Semester Nursing Students showed an average of 69% and Group B First Semester Nursing Students scored a 73% on the ESS. Group A Graduating Nursing Students showed an increased average of 97% and Group B Graduating Nursing Students scored an 81% on the ESS.

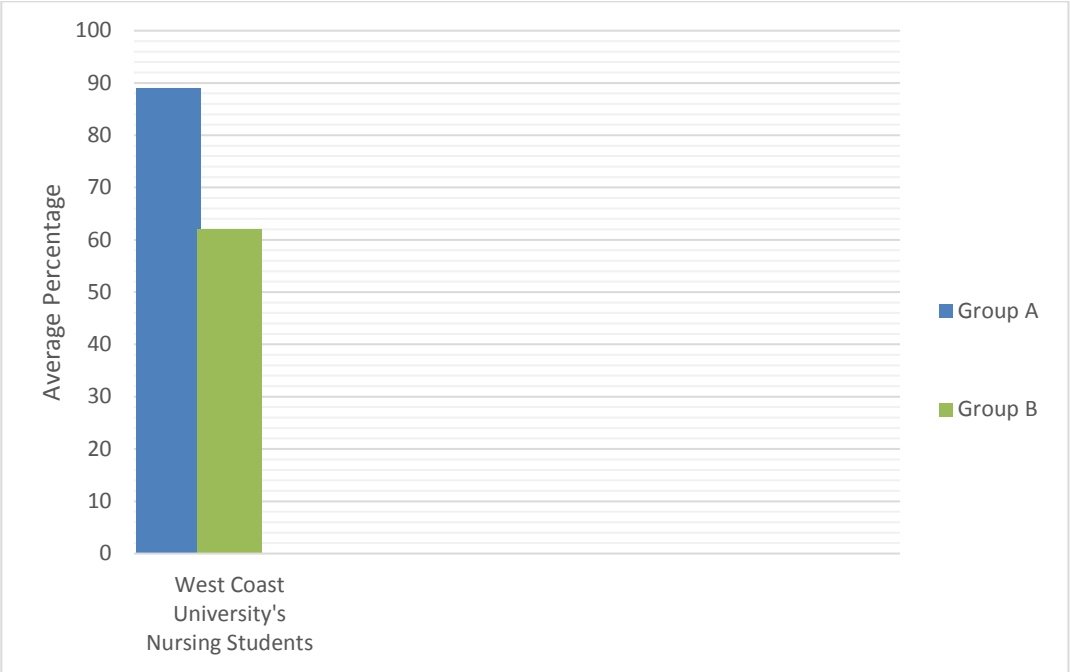


Figure II, the graph presents a Patient Empathy Survey Questionnaire that patients from the graduating nursing class' last clinical rotation at Baptist Hospital. Nursing students in Group A were graded an 89% on empathy from their patients, and Group B scored a 62%.

Empathy Scoring Survey

Nursing School Semester: _____ (First Semester, Graduating)

On a scale of 1-5, answer the questions below; **1-Strongly Disagree, 2-Disagree, 3-Neutral, 4-Agree, 5-Strongly Agree**

1. Health care providers' understanding of their patients' feelings and the feelings of their patients' families influences treatment outcomes.	

2. Patients feel better when their health care providers understand their feelings.	
3. It is easy for a healthcare provider to view things from patients' perspectives.	
4. Health care providers should allow themselves to be influenced by strong personal bonds between their patients and their family members.	
5. Health care providers' understanding of the emotional status of their patients, as well as that of their families is one important component of the health care provider - patient relationship.	
6. Even though people are different, it is easy to see things from patient's perspectives.	
7. Asking patients about what is happening in their lives is help in understanding their physical complaints.	
8. Attentiveness to patients' personal experiences influences treatment outcomes.	
9. Health care providers should try to stand in their patients' shoes when providing care to them.	
10. Patients value a health care provider's understanding of their feelings which is therapeutic in its own right.	

Conclusions

As nursing students get closer to graduating, the main focus shifts entirely towards the NCLEX boards exam. Empathy is pushed aside, to make room for other information necessary to pass this life-changing test. Results have proven the decline in empathy as nursing students reach graduation, if not reinforced. With fiction literature courses throughout nursing school, students are able to comprehend and apply empathy to their patient's care. Empathy plays a crucial part in effective treatment and recuperation, and nursing students must learn to exercise this key element. By using fiction literary pieces, students are able to relate to and understand their patients better and vice versa. Research presents that those who take these courses have higher empathy scores than those who do not, and also rank higher percentages on Patient Empathy Survey Questionnaires. Taking this data into consideration, fiction literature should be a requirement throughout nursing school. Educators can use these literary tools to assist students in understanding a patient's signs and symptoms associated with their disease. Engendering empathy through literature can also be used outside of nursing school, as it is always kind to take other peoples' perspective into consideration.

Testing correlations between empathy and fiction literature leads to advancement in the scientific community by opening new ideas for more research. Now that there is a tested theory of the interconnection of these two actions, scientists are able to point out any hormonal or neural activity when both are stimulated. Measuring oxytocin levels in nursing students taking a fiction literature course revolved around empathy and using fMRIs to test neural activity. Once the science community is able to identify the specific hormone, the public can count on revolutionary enhancements to be produced. These research results affect medical science as well by improving quality of care and treatment plan. Using empathy to connect to patients establishes trust and increases patient understanding and treatment compliance.

Due to insufficient funds, research has been limited to using surveys. If a scientific lab were available, one could test for any specific hormone release during empathy-stimulating confrontations. The use of fMRI studies during fiction literature courses can also dictate specific neural activity signaling any connections with empathy. Utilizing these two approaches, studies can offer more concrete correlations between fiction literature and empathy. Further studies can also embrace the idea of nursing students losing/gaining empathy through fiction literature. To reveal valid conclusions, future research must include progress/regress of all participants every semester of nursing school. If

self-evaluation surveys were to be performed as my research has done, I would suggest implementing lie-detecting technology to estimate the legitimacy of the participants' answers.

Nursing is an art, and students should not forget that or what it implies. As before-mentioned, nursing students are increasingly focused on the scientific portion of nursing (as they should be) but they cannot forget the other empathetic portion that allows effective nurse-patient relationships. There has been recent concerns with the decline of empathy in nursing students, educators not enforcing fiction literature, and patients not feeling the connection with their healthcare provider (nurses, in this case). Issues like these may cause serious deterioration of the concept of nursing and healthcare. Without empathy, there is no connection between the nurse and patient causing ineffective treatment. If students are not taught to comprehend and exercise empathy through fiction literature, they will be unable to fully become the nurses they sought to be. To be a nurse, one must engender empathy and have the burning passion to help/understand those in need of medical attention. If educators fail to strengthen such quality, the medical field will suffer along with its medically needy.

Introducing the idea of fiction literature courses that induce empathy in nursing schools to the Board of Nursing would highly impact the medical field for the best. Raising the awareness of the empathy declination and its importance by providing research results and statistics to the Education Board can result in a change of requirements for nursing students. Implementing the fiction literature courses in nursing school will, indeed, increase empathetic skills in graduating nurses and patient satisfaction/wellness. The general public can provide feedback on experiences with nurses who have and those who have not shown empathy during their care and how it impacted them. Obtaining perspectives from patients, families, friends, and healthcare facilities will strengthen the seriousness of empathy. The government should enable the fiction literature courses in all nursing schools as a requirement, not only in nursing schools but in all schools in general. The development of empathy through fiction literature is important in social and cognitive situations.

Although there are no legislative actions regarding empathy or fiction literature requirements for nurses or nursing students, there should be some type of requirement for implementation of them. With the studies previously conducted and new findings, I propose the Board of Nursing to implement new requirements for certification. Nursing students should complete and excel in fiction literature courses that revolve around empathy in order to graduate nursing school and take the NCLEX exam. In addition to that, registered nurses should complete an Empathy Continuing Education Course at the time of their certification renewal. By making fiction literature a requirement, nursing students and renewing Registered Nurses are given the same opportunity to acquire empathy. Making empathy a requirement for nurses emphasizes its importance and its equality to the scientific aspect that must be learned and applied as well.

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Genetics of Hypertension

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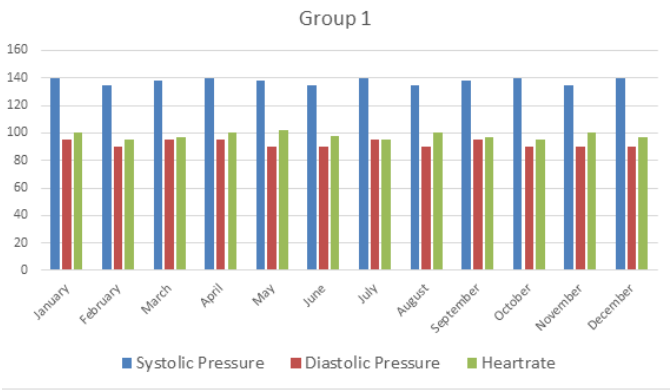
Graphical Abstract



Abstract

Review of the literature helped to establish hypertension as a multifactorial disease, meaning that malfunctions in certain genes predispose individuals to developing the condition but the genes are not particularly dominant and the expression of the final phenotype is heavily influenced by the patient's lifestyle and environment. There is also a disparity concerning the prevalence of chronic illness between individuals of a lower and higher socioeconomic status. It has been shown that people coming from low socioeconomic backgrounds are disadvantaged in terms of disease management. In this study, 30 Zucker rats were used in the first trials of a medication for patients genetically predisposed to hypertension who struggle to manage their condition. The test subjects were separated into 3 groups: a control group who merely got fed twice a day, group 2 who got fed twice a day and received the medication with their first meal, and group 3 who were fed twice a day and received the medication with both meals. The results showed that the test subjects that received the lower dosage did have a decrease in blood pressure although it was slower and less stable compared to the rats that received the higher dosage. Studies must still be conducted although the medication has been deemed safe enough to continue on to the next phase: non-human primates. The hope is that within 5 years, through government assistance such as grants, the medication will be distributed throughout community health centers for the at-risk patient base.

Note: This paper was an assignment for a nursing school General Education Capstone course. The student writer did not conduct a real

	study; she rather simulated a study to demonstrate writing/research skills, creativity, scientific knowledge, and an understanding of how to generate and analyze data. The corresponding author is the student's instructor, who guided the student on each section of the scientific paper, providing feedback on how to "conduct" the study and on how to revise the writing.

Introduction

Literature Review: Scientific

It is essential to understand the basis of inheriting genetic disorders in order to understand the genetic foundation of an illness such as hypertension. According to Coy (2005), there are four types of genetic disorders: single-gene, chromosomal, mitochondrial, and multifactorial. Single-gene disorders occur due to mutations in the DNA sequence on one particular gene versus chromosomal diseases which originate from a mutation in the structure of a chromosome such as deletion or copy of a particular gene (Coy, 2005). A defect or mutation in mitochondrial DNA, inherited solely from the patient's maternal DNA, can result in a mitochondrial disease (Coy, 2005). Hypertension is a multifactorial disease because although individuals may present with malfunctions in several genes, neither gene malfunction is particularly dominant and the patient's lifestyle and environment play an important role in producing the final phenotype (Coy, 2005). This being said, there have been rare presentations of Mendelian forms of hypertension; where a single gene mutation results in deviations of blood pressure (Singh et al., 2016).

Blood pressure is defined as the force of blood as it pushes against the walls of the arteries when the ventricles of the heart contract (National Heart, Lung, and Blood Institute, 2015). There are two readings seen when noting a patient's blood pressure, the systolic and diastolic pressure. Systolic pressure is the pressure of blood against arterial walls when the heart is contracting while diastolic is the pressure when the heart is at rest in between beats (National Heart, Lung, and Blood Institute, 2015). The National Heart, Lung, and Blood Institute (2015) denotes that high blood pressure, also known as hypertension, is a blood pressure reading of 140/90mmHg and above. This same article mentions that there are two main types or stages of high blood pressure: primary, or essential, which is the type that generally develops over time as a person ages. A study done by Marrie et al. (2012) reinforced that the risk associated with developing hypertension increased with age. There is also secondary high blood pressure which is caused by a separate medical condition or a particular medication; this type can be easier to treat as it will generally be resolved once its causative agent has been treated or removed (National Heart, Lung, and Blood Institute, 2015).

There are several genes that are considered in the synergistic association between genetics of hypertension and external environmental factors (Sousa et al., 2017). The angiotensinogen gene is responsible for coding for the substrate renin, indirectly responsible in lowering blood pressure (Singh et al., 2016). There are two genes that are considered as relatively similar and mutations in them could have the same effect: the angiotensin-converting enzyme gene and the renin gene (Singh et al., 2016). A silent mutation can exist on the angiotensin II receptor type I gene which promotes vasoconstriction and growth that provides for an especially aggressive form of essential hypertension (Singh et al., 2016). A polymorphism of the gene responsible for producing G-proteins was linked to lower levels of renin and prorenin, dually responsible for lowering blood pressure in normal healthy individuals (Singh et al., 2016) A population study revealed that polymorphisms of cystathionine β-synthase resulted in a higher risk factor for developing essential hypertension (Ying et al., 2014). Mutations in the adducin gene can provide another risk factor for persons who contain the mutation in their DNA as alterations in the gene have been shown to affect tubular sodium reabsorption (Singh et al., 2016). In

total there have been alterations in 27 genes identified as providing a predisposition for developing primary/essential hypertension in adults, further investigation and genetic testing could reveal even more (Coy, 2005). Varga et al. (2016) discussed the case of a white male aged 62 who presented with persisting dyspnea and signs of right heart failure (2016). In the case of this patient, genetic testing was able to identify his genetic predisposition for hypertension and gave his son the initiative to undergo the same genetic testing and take precautionary measure so as to hopefully not develop the condition later on in life.

Due to the status of hypertension as a multifactorial disease, environmental factors are crucial in the final phenotype of the condition (Coy, 2005). According to Chalmers, MacMahon, & Mancia, several of these risk factors include age, sex, family history of hypertension, demographic factors, overweight, diabetes mellitus, physical inactivity, smoking, excess consumption of sodium, coffee, and alcohol (as cited in Singh et al., 2016). Although age is thought of as a risk factor, this only becomes the case after adulthood, age is not a risk factor in childhood and adolescence (Beeman, 2013). A case report presented a 62 year old white male, his case involved a history of smoking combined with a genetic predisposition resulting in a particularly severe form of pulmonary hypertension (Varga et al., 2016). Common features seen in a hypertensive patient are obesity, sedentary, history of smoking, and/or diagnosis of diabetes mellitus (Coy, 2005). The sad reality is that most of these factors are modifiable, meaning that although they are predictive of the development of hypertension, they can be fixed with lifestyle changes (Beeman, 2013).

According to the American Heart Association, it is imperative to understand that hypertension is one of the leading contributors for developing coronary heart disease or having a stroke, worldwide (as cited in Ho & Rumsfeld, 2006). There are individuals who can be unaware of their hypertensive status or who have difficulty in receiving a proper diagnosis because they have a type called masked hypertension. A recent study defines masked hypertension as “a blood pressure in the hypertensive range outside the office setting” (Unsal et al., 2016, p. 1). This same study found that this type of hypertension is associated with a greater prevalence of target-organ damage. This being said, it is high blood pressure/hypertension in general that can damage a patient’s body for years before symptoms develop or if left untreated (Mayo Clinic, 2016). Damage that can be done to the patient’s body as a result of untreated hypertension includes damaged and narrowed arteries or an aneurysm as a result of the constant high pressure within arterial walls, heart failure or an enlarged left heart due to the thickening of the heart wall from the extra work put forth by the left ventricle of the heart, damage to the brain like a stroke or dementia, and even kidney failure through damage of the renal arteries leading to the tiny blood vessels that filter waste from the body (Mayo Clinic, 2016).

There are multiple methods considered to assist in regulating blood pressure, one identified by Behuliak et al. (2017) is the activation of myosin light chain phosphatase in vascular smooth muscle, this results in the dephosphorylation of myosin light chain and promotes vasorelaxation. Drug therapy has been the standard method of treatment for hypertension for over half a century (Rodman, 1969). For the most effective treatment, the right drug or even combination of drugs and supplements must be identified for the individual case of each patient (Rodman, 1969). An example of a particular case study involves a patient with a particularly severe case of hypertension who received oral anticoagulation, loop diuretics, aldosterone antagonists, and oxygen supplementation (Varga, 2016). This patient’s anticoagulation regimen had to be constantly adjusted to maintain ideal therapeutic levels. Other common pharmaceutical treatments for hypertension include thiazide diuretics, loop diuretics, angiotensin II receptor blockers, calcium channel blockers, beta blockers, and even more (Beeman, 2013).

Literature Review: Cultural

It is known that the homeless population in the United States is rising although their mean age of survival is not; this could be because of their lack of proper healthcare for chronic illnesses (Bernstein et al. 2015). A study done that examined a low-income neighborhood found three major themes arose: social connectedness, stress factors, and availability of food options (Al-Bayan et al., 2016). This same study noted that these factors along with the disorder of the neighborhood and lack of proper healthy food choices led to an increase in hypertension within its population. The study

found that out of the participant sample, 24% were pre-hypertensive and 35% were hypertensive. This is a stark contrast from the U.S. national average of 29.1% (Nwanko et al. 2013). It has also been found that patients of community health centers were 4% more likely to be on two or more blood pressure medications than those patients from a private physician's office (Fontil et al. 2017). The community health center patients Fontil et al. (2017) analyzed were also approximately 9% more likely to have stage 2 or severe hypertension than those who attended a private care physician's office.

A study conducted by Fontil et al. (2017) found that patients from community health centers were approximately 5% more likely than those who attended a private physician's office to have uncontrolled hypertension. This same study also found that patients at community health centers were less likely than those of private offices to be on fixed-dose combination drugs, this likely contributes to the higher rate of uncontrolled hypertension in these lower income communities. Rosemberg & Hsin-Chun Tsai, (2014) found that hypertension management was increasingly difficult in patients of a lower socioeconomic status. The participants underlined a lack of ability to both pay for the medication that could manage their hypertension and their bills. What many payers and insurance companies fail to realize is that between the comorbidities and complications that can arise from uncontrolled hypertension, effective treatment from the start can be drastically more effective in the long run (Cohen et al., 2001).

An essential portion of effective hypertension management is proper nutrition, Swinburne, Garfield, & Wasserman (2017) say that hospitals need to take it upon themselves to teach effective nutrition to patients. These authors say that it is not only the moral responsibility of the care provider but also their legal responsibility as a stipulation of the hospital readmission reduction program of the Affordable Care Act which enforces penalties on hospitals with excessive patient readmissions. Many communities where the residents are of a lower socioeconomic status are statistically inclined to developing hypertension as residents of neighborhoods with better safety, social connectedness, accessibility and availability of healthy were less likely to be hypertensive (Mujahid et al., 2008). It has been shown that a more cohesive social environment creates an environment less conducive to food insecurity (King, 2017). It has also been shown that there are other factors leading to an individual being disadvantaged from a perspective of disease management: being a woman, black, lower class, and an immigrant all seem to impact the patient's ability to effectively manage their hypertension (Rosemberg, & Hsin-Chun Tsai, 2014). Many of these risk factors are non-modifiable such as being a woman or black (Beeman, 2013).

The rate of patients with uncontrolled hypertension is excessively high, especially considering its increased damage on the body (Cohen et al., 2001). Oftentimes, patients are simply not aware of their hypertensive status or they receive a high blood pressure reading that is simply not reciprocated in the presence of a healthcare professional (Unsal et al., 2016). This phenomenon is known as masked hypertension and is important clinically because of the increased risks of cardiovascular disease (Unsal et al., 2016). In the study analyzed, it appears that the participants found to have masked hypertension had a mean BMI of 30.6 versus the other groups assessed, true normotension and true pre-hypertension, with a mean BMI of 25.9 and 28.5, respectively. The mean waist circumference was also 6 points higher than in the other groups, possibly alluding to a correlation between higher weight and masked hypertension (Unsal et al., 2016). A study conducted by Wang et al. (2017) tells us that the prevalence of masked hypertension is quite astounding, approximately 1 in 8 adults. This statistic is surely a contributing factor in terms of receiving effective treatment for patients with this condition as they are oftentimes misdiagnosed as non-hypertensive (Wang et al., 2017).

Stress has a profound impact on increasing an individual's blood pressure. It is recommended that if you are not able to simply eliminate your stressors, you can still make an effort to reduce your stress levels by changing your expectations, making time to relax, and do other activities you enjoy (Mayo Clinic, 2015). Things have changed quite drastically in terms of the patient's ability to be able to monitor his or her blood pressure at home and knowing when it is imperative to seek support, whether it is in their family or friends to help them manage their illness or their healthcare provider for immediate care (Mayo Clinic, 2015).

Patients must consider at the end of the day that while many factors leading to increased risk for the development of hypertension are considered as non-modifiable, most of them are not (Beeman, 2013). There are approximately 27 genes that lead to a genetic predisposition for hypertension,

however there is often a synergistic involvement between genetic factors and external environmental influences such as weight, diet, salt intake, alcohol consumption, smoking, and various other lifestyle choices (Coy, 2005).

Fortunately, according to the Mayo Clinic (2015), hypertension can be controlled by non-pharmaceutical means for many individuals. Weight does have an impact on an individual developing high blood pressure and hypertension as they are more likely to have a buildup of plaque within their arteries (Unsal et al., 2016). The staff at the Mayo Clinic (2015) tells of how losing just 10 pounds can reduce your blood pressure. The Mayo Clinic also recommends exercising regularly and eating a healthy diet with plentiful whole grains, fruits, and vegetables. Reducing your salt intake can also have a profound impact in terms of lowering your blood pressure (Behuliak et al., 2017).

Materials and Methods

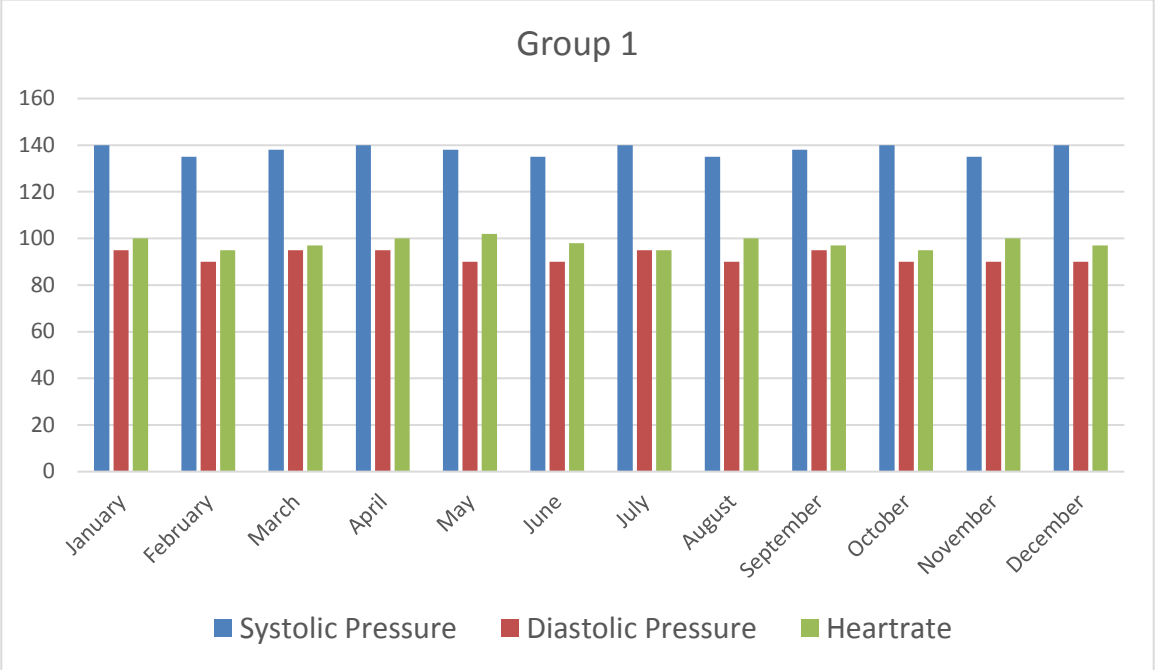
The development of this drug was conducted over a period of 1 year at a research lab in Miami, Florida. The researchers involved with this project consistently complied with all the guidelines set for ethical conduct in the care and use of nonhuman animals in research by the committee on animal research and ethics (CARE) affiliated with the American Psychological Association (2012). This experiment began by obtaining 30 young female Zucker rats and running various tests in the laboratory setting to insure that they did not have any medical conditions that would interfere with the procedures of this study such as Diabetes. The rats in this experiment were fed their normal caloric intake through two meals each day. They were kept in air controlled units so as to keep them comfortable and not raise their stress levels and cause erroneous blood pressure or heart rate results. The rats were kept in cages of an appropriate size, 40 square feet, and each cage kept a consistent supply of water to the rats along with appropriate ventilation (American Psychological Association, n.d.).

The 30 rats were divided into three groups; a first group which received their two meals a day with nothing added, a second that received one crushed pill of the experimental drug in the morning meal only, and a third group which received one pill crushed in each meal daily. Each pill contains 50 mg of the medication, group 2 would only receive this singular dosage while group 3 would receive a total of 100 mg of the medication. The control group in this experiment was group 1 while the experimental groups were groups 2 and 3. The effects of two doses were measured in this case in the effort to figure out what the appropriate amount of medication would be to stabilize, at a consistent rate, the blood pressure and heart rate of the ‘patients’. The independent variables in this experiment would be the amount of medication provided to the rats. The dependent variables in this experiment would be the resulting heart rate and blood pressure measurements.

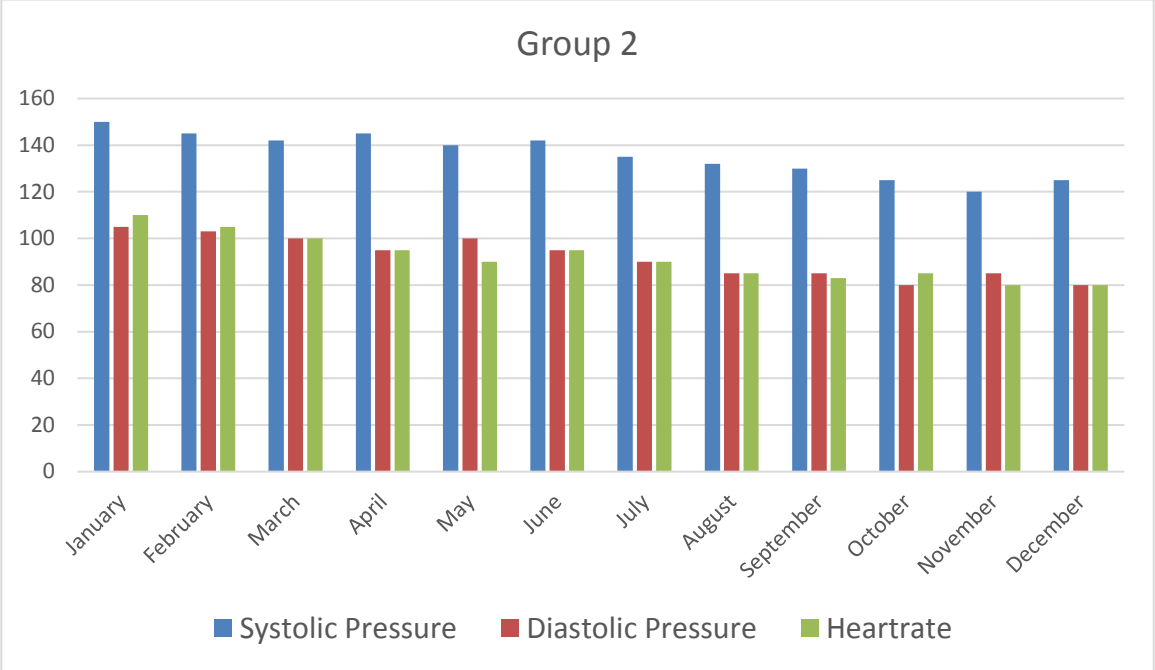
Every day, after their morning feeding and after their evening feeding, the blood pressure, heart rate, and temperature of each rat was taken with the use of a Passive Infrared Transponder (PIT) and a CODA surgical monitor. The PIT was chosen as it includes a sensor for temperature and could be injected subcutaneously while still obtaining an accurate reading, it is able to withstand internal body temperature. The CODA surgical monitor is a tail cuff system that allows for an accurate reading of systolic, diastolic, mean BP, heart rate, tail blood volume, and blood flow in rodents as small as 8 grams to rats as large as 950 grams. This CODA surgical monitor is also applied through a noninvasive procedure, helping maintain the stress levels of the rats used in the experiment low.

Once a month, the rats underwent a complete blood count (CBC) to check their levels of blood glucose, potassium, sodium, and cholesterol. These levels were obtained to ensure that the rodents would be able to withstand the pharmaceutical treatment throughout the duration of the experiment. To ensure that the hearts of the lab rats would be able to withstand the experiment, an EKG was also conducted each month with the Animal Bio Amp including PowerLab with LabChart software. The measurements obtained each day were kept diligently in a log; this included systolic blood pressure, diastolic blood pressure, mean blood pressure, heart rate, tail blood volume, and tail blood flow. Along with these measurements were the cholesterol levels for each rat. Meal times and measurement times were strict so as to prevent faults throughout the experiment and eliminate much of the chance of obtaining erroneous results.

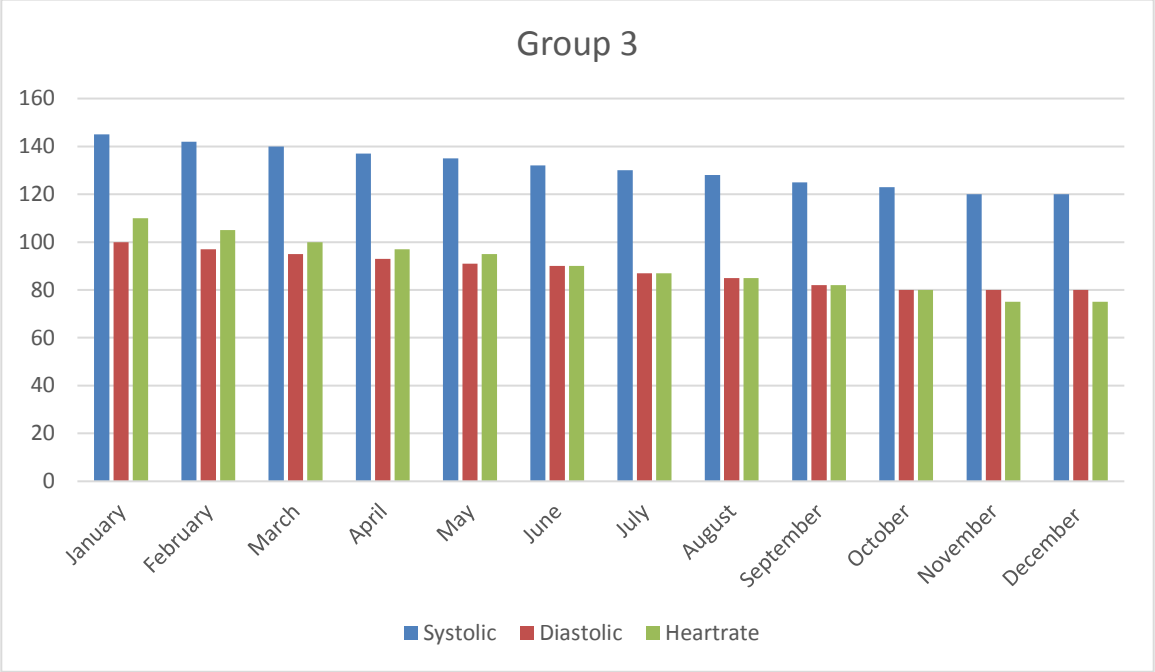
Results



This graph represents the average blood pressure, systolic and diastolic, along with heart rates for the group 1 control Zucker rat group, each month for the year tested. In the month of January, the average systolic pressure was 140mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 100 bpm. In the month of February, the average systolic pressure was 135mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 95 bpm. In the month of March, the average systolic pressure was 138mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 97 bpm. In the month of April, the average systolic pressure was 140mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 100 bpm. In the month of May, the average systolic pressure as 138mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 100 bpm. In the month of June, the average systolic pressure was 135mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 98 bpm. In the month of July, the average systolic pressure was 140mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 95 bpm. In the month of August, the average systolic pressure was 135mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 100 bpm. In the month of September, the average systolic pressure was 138mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 97 bpm. In the month of October, the average systolic pressure was 140mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 95 bpm. In the month of November, the average systolic pressure was 135mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 100 bpm. In the month of December, the average systolic pressure was 140mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 97 bpm.



This graph represents the average blood pressure, systolic and diastolic, along with heart rates for the group 2 Zucker rat group receiving one pill, each month for the year tested. In the month of January, the average systolic pressure was 150mmHg, the average diastolic pressure was 105mmHg, and the average heart rate was 110 bpm. In the month of February, the average systolic pressure was 145mmHg, the average diastolic pressure was 103mmHg, and the average heart rate was 105 bpm. In the month of March, the average systolic pressure was 142mmHg, the average diastolic pressure was 100mmHg, and the average heart rate was 100 bpm. In the month of April, the average systolic pressure was 145mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 95 bpm. In the month of May, the average systolic pressure was 140mmHg, the average diastolic pressure was 100mmHg, and the average heart rate was 90 bpm. In the month of June, the average systolic pressure was 142mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 95 bpm. In the month of July, the average systolic pressure 135mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 90 bpm. In the month of August, the average systolic pressure was 132mmHg, the average diastolic pressure was 85mmHg, and the average heart rate was 85 bpm. In the month of September, the average systolic pressure was 130mmHg, the average diastolic pressure was 85mmHg, and the average heart rate was 83 bpm. In the month of October, the average systolic pressure was 125mmHg, the average diastolic pressure was 80mmHg, and the average heart rate was 85 bpm. In the month of November, the average systolic pressure was 120mmHg, the average diastolic pressure was 85mmHg, and the average heart rate was 80 bpm. In the month of December, the average systolic pressure was 125mmHg, the average diastolic pressure was 80mmHg, and the average heart rate was 80 bpm.



This graph represents the average blood pressure, systolic and diastolic, along with heart rates for the group 3 Zucker rat group receiving two pills, each month for the year tested. In the month of January, the average systolic pressure was 145mmHg, the average diastolic pressure was 100mmHg, and the average heart rate was 110 bpm. In the month of February, the average systolic pressure was 142mmHg, the average diastolic pressure was 97mmHg, and the average heart rate was 105 bpm. In the month of March, the average systolic pressure was 140mmHg, the average diastolic pressure was 95mmHg, and the average heart rate was 100 bpm. In the month of April, the average systolic pressure was 137mmHg, the average diastolic pressure was 93, and the average heart rate was 97 bpm. In the month of May, the average systolic pressure was 135mmHg, the average diastolic pressure was 91mmHg, and the average heart rate was 95 bpm. In the month of June, the average systolic pressure was 132mmHg, the average diastolic pressure was 90mmHg, and the average heart rate was 90 bpm. In the month of July, the average systolic pressure was 130mmHg, the average diastolic pressure was 87mmHg, and the average heart rate was 87 bpm. In the month of August, the average systolic pressure was 128mmHg, the average diastolic pressure was 85mmHg, and the average heart rate was 85 bpm. In the month of September, the average systolic pressure was 125mmHg, the average diastolic pressure was 82mmHg, and the average heart rate was 82 bpm. In the month of October, the average systolic pressure was 123mmHg, the average diastolic pressure was 80mmHg, and the average heart rate was 80 bpm. In the month of November, the average systolic pressure was 120mmHg, the average diastolic pressure was 80mmHg, and the average heart rate was 75 bpm. In the month of December, the average systolic pressure was 120mmHg, the average diastolic pressure was 80mmHg, and the average heart rate was 75 bpm.

Conclusions

The lower dosage of the results shows the effectiveness of the drug. However, in contrast to the higher dosage which resulted in a more stable decline in blood pressure, the lower dosage was less stable in lowering and regulating the test subjects' blood pressure. The higher dosage of the experimental medication led to a steady decline from hypertensive status to one of normotension and effectively maintained it at that level. The lower dosage of medication could work well for patients who have just reached hypertensive status as they have not already sustained extensive damage to their cardiovascular system and body. Having the higher dosage of medication which lead to a steadier decline in blood pressure would be better for a patient whose condition necessitates a much faster solution.

The hope of these results is that the scientific community will only continue to make these advances in terms of treating hypertension. The more widespread these types of medications become

the more people will be able to access them in order to regulate their high blood pressure. With a chronic illness such as hypertension affecting 29.1% of the adult population in the United States (Nwankwo et al., 2013) and a lower likelihood that patients with Medicaid will be on fixed dose-combination drugs and have their hypertension controlled (Fontil et al., 2017), it is imperative that the scientific community, insurance companies, physicians or other medical professionals, and even legislators all come together to ensure that people of any socioeconomic status are able to access effective medication.

The scientific community has an ethical responsibility to make an effort to inform the public about the dangers of hypertension, especially if the condition goes uncontrolled. Public health officials can initiate a community outreach program that involves going out to schools to educate students at an early age and also making visits to community health centers or areas that are underprivileged and thus will tend to have a higher rate of uncontrolled hypertension (Conen et al., 2009). Government medical assistance for managing chronic illness for individuals of a lower socioeconomic status would be one good solution for effectively managing hypertension in this group. Another could be allotting more money for the supplemental nutrition assistance program (SNAP) while simultaneously placing stricter guidelines on what can be purchased with the benefits. This would save money in the long run as a preventative measure, it would cost more to then pay for these individual's medical treatments for end-stage hypertension and the possible complications.

Making healthy food choices and maintaining a steady balanced diet is one of the most important factors in managing hypertension in the vast majority of patients. However, availability of food options in lower socioeconomic neighborhoods contributes to a higher rate of hypertension occurring in these households. One of the pieces of legislation affecting the cost of healthier food options is the farm bill. The farm bill is currently comprised of regulations surrounding farm commodity price and income supports, agricultural conservation, farm credit, trade, research, rural development, bioenergy, foreign food aid, and domestic nutrition assistance (Johnson & Monke, 2017). One of the problems with this bill is that it is written by Congress members and not by experts or those who it would affect. The purpose of the farm bill is to create an alliance with farms and promote healthy living but trends indicate otherwise (Roberts et al., 2012). The legislation has allowed for unhealthy food choices to become less expensive and healthier food options have grown into the more expensive option. This only increases the health disparity in the United States.

The research conducted is certainly limited in terms of its patient base which happens to be Zucker rats. Studies and trials still need to be conducted to determine not only the effectiveness of the drug in human patients but also any possible complications. The study was also limited by time, it was conducted over the course of only one year while an ideal trial of a medication would be conducted over a course of time closer to at least three years. It would be recommended to conduct more trials of the proposed medication on patients whose hypertension is otherwise uncontrolled by other combined-dose drug treatments and who provide informed consent for the experimental medication. Before this point however, another trial can be conducted with non-human primates to ensure the medication is safe for the next phase with humans. Not only would they indicate further safety but also effectiveness of the medication as the genome of the primates is even closer to humans than the Zucker rats.

Particular organizations such as People for the Ethical Treatment of Animals (PETA) and the Humane Society would have serious objections over the utilization of animals as test subjects. They argue that animals used in a laboratory setting are treated inhumanely, that they needlessly suffer and then die in vain. There is legislation such as the Animal Welfare Act (AWA) and organizations such as the Committee on Animal Research and Ethics (CARE), affiliated with the American Psychological Association, who exist for the purpose of ensuring the fair and humane treatment of animals in experimentation. Another issue surrounding the proposed pharmaceutical is cost. Being a generic versus a name brand medication the hope is that the medication tested in this study will be more accessible to the general public. We would also like to propose a government grant for further accessibility to the general public. A grant covering at the bare minimum the manufacture of the medication would allow the drug to be offered at low-cost or even free at community health centers for at-risk populations.

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In Silico Repurposing, Design, Synthesis and Biological Evaluation of Bisacodyl Analogues

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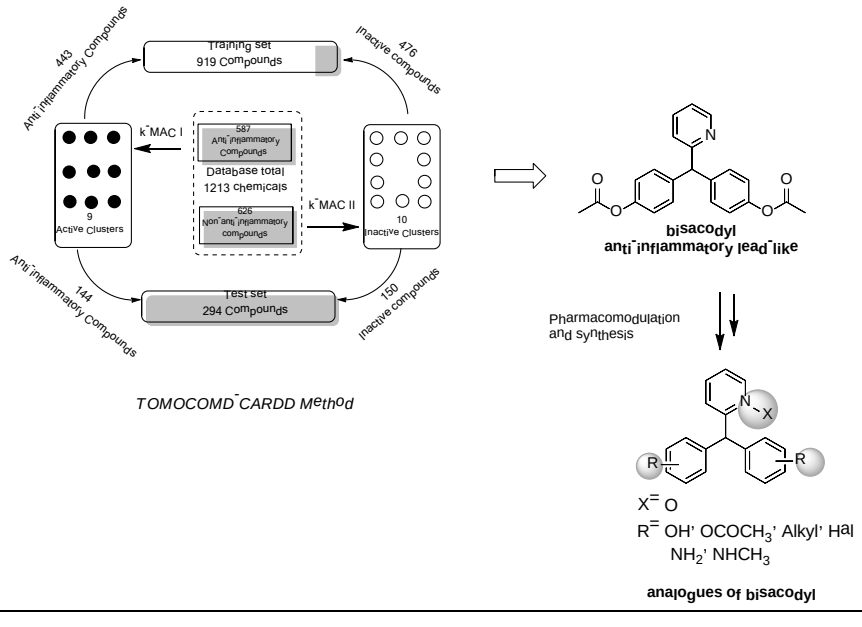
Graphical Abstract



Abstract.

In a recent article was described in silico repositioning, design, synthesis, biological evaluation and structure-activity relationship (SAR) of an original class of anti-inflammatory agents based on a polyaromatic pharmacophore structurally related to bisacodyl (BSL) drug used in therapeutic as laxative.

Keywords *TOMOCOMD-CARDD* Software, Atom-based bilinear indices, Anti-inflammatory database, Bisacodyl, Repurposing, Diarylmethylpyridines, Anti-inflammatory assay

Introduction

Drug repositioning allows the development of new indications for existing drugs with identified pharmacokinetic profiles, known safety profile and already resolved manufacturing issues [1]. The aim of this research was to identify new anti-inflammatory drug-like agents using in silico repurposing from a diverse series of known drugs, then design, synthesis and biological evaluation of analogs [2].

Materials and Methods

The potential of TOMOCOMD-CARDD (topological molecular computational design-computer aided rational drug design) methods to find out new anti-inflammatory drug-like agents from a diverse series of compounds using the total and local atom based bilinear indices as molecular descriptors was used [3]. Several biological in vitro (Nitrite and PGE2 production in LPS-stimulated cells, inhibitory effect on TNF-α and IL-6 release in cells) and in vivo (LPS-enhanced leukocyte migration to the injury zone in Zebrafish, TPA-induced mouse ear oedema, carrageenan-induced paw oedema test in rats) assays were performed in order to understand the mechanism of action of the identified known drug. A set of analogues of this drug was prepared using low-cost synthetic procedures and further biologically investigated in zebrafish models using LPS-enhanced leukocyte migration assay.

Results and Discussion

The models obtained with the TOMOCOMD-CARDD suites were validated by biological studies. BSL was identified as the first anti-inflammatory lead-like using in silico repurposing from commercially available drugs. At 30 μM, BSL showed the best result with an anti-inflammatory activity superior to the value obtained by positive control indomethacin (85%). BSL reduced oedema and inhibited leukocyte infiltration comparable to indomethacin at 0.5 mg/ear. At dose of 20 mg/kg, BSL showed equipotent anti-inflammatory activity in protecting rats from carrageenan-induced inflammation when compared to indomethacin, while the effect was higher at 40 mg/kg. Considering the biological results, it was suggested that anti-inflammatory activity of BSL observed in vivo assays may be related to the release of cytokines, in particular with IL-6.

Diarylmethylpyridines and their corresponding *N*-oxides were synthesized by Friedel-Crafts hydroxylalkylation reaction with no more than two steps from commercially available inexpensive reagents. Among others, eighteen new compounds were synthesized in this work. Best anti-inflammatory activities reached 10 μM in the pyridyl series and *N*-oxide respectively. The *N*-oxide functionality generally improved the anti-inflammatory activity and decrease toxicity in most series of BSL analogues.

Conclusions

Two compounds exhibited higher anti-inflammatory activities than BSL and represent new promising anti-inflammatory agents for further preclinical development.

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Bioenergy: A Sustainable Energy option

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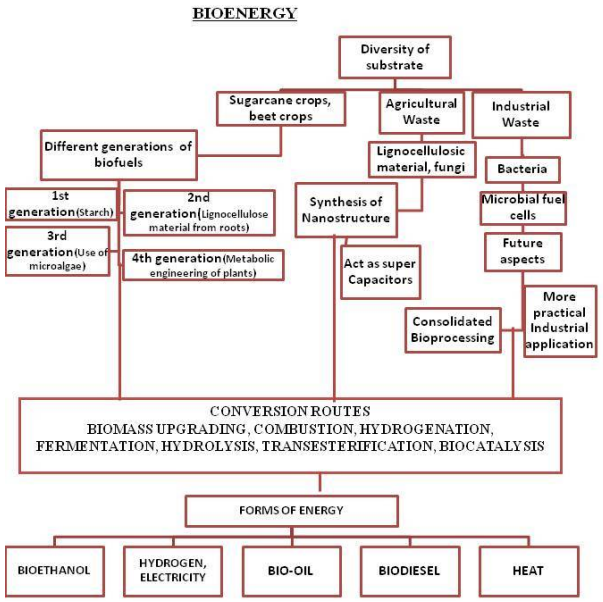
Graphical Abstract



Abstract.

Bioenergy is the renewable and sustainable source of energy produced from organic matter. The challenge of depleting non-renewable resources can be addressed by exploiting the capability of biotic systems to produce bioenergy.The study talks about switching from first generation biofuels produced from sugars and seed oils to fourth generation biofuel that involves metabolically engineered plants. Recent developments in molecular biology techniques have provided valuable tools that could effectively optimize and control the processes involved in bioenergy production in the near future. Production of biofuels employing fungi that have high potential for bioconversion of lignocellulosic materials abundant in nature can also be an effective means. Synthesis of nanostructures using fungi that can serve as super capacitors would be a solution to the problem of storage of bioenergy. The paper also discusses the role of bacteria in Microbial Fuel Cell (MFC). General biochemistry involved in MFC is also presented. Possible limitations or shortcomings are also identified and importance of identifying newer approaches is stressed upon in order to match the future demands.

Keywords

Bioenergy, Biofuels, Microbial Fuel Cell

Introduction

Bioenergy is the energy produced by means of living systems, involving whole cells, enzymes produced by specific microbes or through metabolic activities of living organisms [1]. The challenge of depleting non-renewable resources can be addressed by exploiting the capability of biotic systems to produce bioenergy. Under

favorable conditions substantial growth for bioenergy production is possible over the next 20 years. Bioenergy potential from biomass residues and energy crops is estimated to range between 4.4 - 24 EJ by 2030 in EU [2]. Over the coming decades, supply of sustainable energy in adequate amount would be one of the main challenges that mankind will face, particularly because of the need to address climate change. Environmental concerns and the depletion of oil reserves have also resulted in governmental actions and incentives to establish greater energy independence and promotion of research on environmentally friendly & sustainable biofuels such as bioethanol and biodiesel.

Agriculture and industry are the driving forces of the Indian economy. However, both agriculture and Industry produce large amounts of waste that causes significant pollution in the environment. Microbes, specifically fungi and bacteria, can serve a dual purpose in treating these organic wastes while providing us bioenergy [3]. Production of biofuel through fungal action upon lignocellulosic materials holds high biotechnological value. The low-cost remediation by fungi captivates high application rate. Industrial wastes that mainly contain effluent with lots of carbohydrates can well serve as a substrate for microbial growth and hence can be the principle component of Microbial Fuel Cells (MFC), another effective way of bioenergy generation. Moreover, these MFC's helps in reducing COD (chemical oxygen demand) by 80% and thus can also aid in reducing pollution due to putrification of biomass. Table1 presents different stages through which biomass associated bioenergy production has evolved. Crop biotechnology and plant genetic engineering has the potential to optimize biomass productivity in favor of energy crops. This aspect has been implemented in the fourth generation energy crops. These modified crops have resulted in enhanced biomass conversion into biofuels [4]. Biologists are using genetic engineering to overcome two major difficulties that hinders the conversion of lignocellulose into fuels: higher requirement of cellulases which adds to the processing cost and the limited ability of the microbes to ferment the breakdown products which affects the process and product quality.

Table1. Different stages of evolution in biomass associated bioenergy production

Generation	**Feedstock and technology**	**Advantages**	**Disadvantages**
1stgeneration biofuel	Starch, sugar and seed oil	Use of renewable sources	Food ethics issues, blended with conventional fuel
2ndgeneration biofuel	Lignocellulosic material from grasses and trees	Not competing with food, environment friendly	High energy input, high cost bio fuel
3rdgeneration biofuel	Use of microalgae because of high rapid growth	Higher energy yield, lower requirement for fertilizer and land	Capital and operating costs
4thgeneration biofuel	Metabolically engineered plants and algae	Carbon negative fuel due to carbon capture	High research and investment at primary stage

Fungi as source of Bioenergy

Accumulation of lignocellulosic residues from woods, grass, agricultural, forestry waste and municipal solid wastes in large quantities results not only in deterioration of environment but also in loss of possible utilization, especially in bio-energy generation [5]. Bioconversion of lignocellulosic residues to useful, higher price products commonly needs multi-step processes that include: (1) Biological pretreatment (2) hydrolysis of polymers to supply readily metabolizable molecules (hexose, simple sugars); (3) Use of these molecules to support microbial growth or to supply chemical products; and (4) Separation and purification. Numerous life forms degrade and utilize cellulose and hemicellulose as carbon and energy source. The structural complexity of lignin, its high relative molecular mass, and its insolubility make its degradation very difficult. However, filamentous fungi belonging primarily to the basidiomycetous group have an ability to degrade or modify lignin, the most obstinate part of the plant cell wall. There are several advantages utilizing fungi including higher capacity to degrade lignocellulosic material due to their proficient enzymatic framework and their applicability as low cost bioremediation ventures [5]. Fungi have two types of extracellular enzymatic systems: the hydrolytic system responsible for degrading polysaccharides and the oxidative ligninolytic system, which degrades or modifies lignin. The most efficient and widely studied white-rot organism capable of degrading polysaccharides and lignin simultaneously is *P. chrysosporium*. Efficient hydrolysis of polysaccharides requires the action of three enzymes: 1. endo-glucanases to cleave random inter monomer bonds; 2. exoglucanases to remove mono and dimers at the end of the glucose chain; and 3. β-glucosidase, hydrolyzing the glucose dimer. The lignolytic system includes phenol oxidases (lignin peroxidase (LiP), manganese peroxidase (MnP)) and laccasees. While LiP and MnP oxidize the substrate by two consecutive one-electron oxidation steps with intermediate cation radical formation, the laccasees have broad substrate specificity and oxidise phenols and lignin substructures with the formation of oxygen radicals [6]. Biodegradation of lignocellulosic wastes has several uses including its use as raw material for ethanol production, paper manufacturing, compost making for cultivation of edible mushroom, and even as direct animal feed [6]. Ethanol as biofuel would cut back gas emissions and improves air quality while providing strategic economic benefits. Ethanol is currently used as blended fuel in petrol engines.

According to recent research, fungi can be used as templates for the synthesis of nanostructures with potential applications in biosensors, batteries and super capacitors. Supercapacitors are currently considered promising energy storage systems. Supercapacitors store energy in the electric field generated at the interface between a metal electrode and an electrolyte. Fungal cell wall is considered as two-phase system consisting of a chitin skeleton framework embedded in an amorphous polysaccharide matrix [7]. Fungal cell walls can act as cation exchangers due to the different functional groups (e.g., carboxylic, phosphate, amine or sulfhydryl) present. Fungal cells have walls that mainly contain chitin which becomes a rich source for metal binding ligands. NiO microtubes were synthesized using the fungus *C.cladosporioides* as a biotemplate, exhibiting pseudo-capacitive properties with high capacitance, long cycle life and good coulombic efficiency [8]. Such technologies can further empower wider storage and utilization of bioenergy.

Bacteria as source of Bioenergy

Microbial fuel cells (MFC) are a sustainable source of energy. They employ micro-organisms to generate electricity from the energy produced

during metabolism of organic substrates. MFCs facilitate direct conversion of chemical energy of substrate into electrical energy. Bacteria are the preferred source of microorganisms in MFC. Research suggests that waste water sources (municipal, domestic, industrial) rich in organic substances can be used as a substrate for bacteria in MFC, thus serving the dual purpose of waste water utilization and generation of bioenergy.

A typical MFC has two compartments: an anodic and one cathodic compartment. In the anodic compartment, the microorganisms are provided with the substrate rich in organic compounds (*viz*., organic waste). The cathodic compartment is provided with a continuous supply of oxygen or a potential electron acceptor. The two compartments are separated by Proton Exchange Membrane. The anode and cathode are connected by an external circuit with a resistor at which power is obtained. MFCs work when bacteria switch from a natural electron acceptor such as Oxygen to an insoluble one like MFC anode. Bacteria oxidize the substrate (electron donor), resulting electron is then passed onto anode and goes through the external circuit through resistor and reach cathode, whereas the proton generated passes through the proton exchange membrane and reach cathode to complete the circuit. The oxygen in the cathodic compartment gets reduced to form water. The transfer of electrons from the bacterial surface to anode is a critical step and there are several ways which can be employed for the same. Mediators such as phenazines, phenothiazines and Quinone's are well known for electron shuttling property [9]. Also, bacteria transfer electrons through nanowires. The electron transfer from the microbial cell to the fuel cell anode, as a process that links microbiology and electrochemistry, represents a key factor that defines, the theoretical limits of the energy conversion. The more positive the redox potential of a terminal electron acceptor (with a given substrate—the electron donor), the higher is the energy gain for an organism [10].

Future of energy Systems: Microbial fuel cell (MFC) has failed at Industrial Scale. Some strategies to overcome the limitations can be: a. Over expression of genes that code for nanowires and pili that could enhance the electrogenic capacity of microbes and increasing the production of mediators that shuttle the electrons like flavins and phenazines; b. Preventing bacteria from dispersing from anode could be targeted; c. Sometimes, there is a nutrient limitation for biofilm bacteria by the matrix surrounding it. So, a manipulation that can cause the dispersion of non-biofilm bacteria can be targeted.

Conclusions

Traditionally India's energy system is dominated by ancient feedstock, conventional energy systems and petroleum products, but these methods failed to meet the growing energy requirements of the population. According to recent studies, it has been proved often that bioenergy technologies have the potential to provide ample energy production to fulfill the power desires, and contribute to bridge the demand–supply gap. Accumulation of lignocellulose residues presents a disposal problem along with deterioration of environment. The use of fungi in low cost bioremediation projects might be attractive given their highly efficient lignocellulose hydrolysis enzyme machinery. Microorganisms that can couple the oxidation of organic compounds to electron transfer to electrodes offer the promise of self-sustaining systems that can effectively convert waste organic matter and renewable biomass into electricity. Significant optimization of microbial fuel cells will be required for most applications. Further investigations into the physiology and ecology of microbes that transfer electrons to electrodes are essential to carry out these optimizations in a rational manner.

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Ecotoxicological assessment of pharmaceuticals using computational toxicology approaches: QSTR and interspecies QTTR modeling

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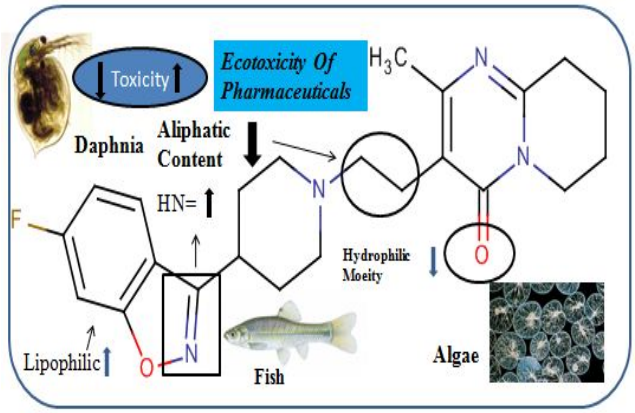
Graphical Abstract



Abstract

Although pharmaceuticals have been exposed to the environment with no or very little care, their environmental toxicity has been studied experimentally only to a limited extent till date. There are reports of measurable quantities of drug molecules and other bioactive metabolites in rivers and other surface water bodies. It is next to impossible to carry out experimental evaluation of the impact of pharmaceuticals on all relevant and exposed organisms – this is also both unethical, costly and slow. However, computational tools such as Quantitative Structure-Activity Relationship (QSAR) can be used to fill the data gaps where limited number of experimental data is available. In the current study, we have developed Quantitative Structure-Toxicity Relationship (QSTR) models for toxicity of pharmaceuticals on three different organisms namely algae, daphnia and fish. In order to study relationship between structural feature and toxicity response; the models were developed by partial least squares regression approach using descriptors selected through a genetic algorithm approach and the developed

models were subsequently extensively validated following OECD guidelines. An additional interspecies quantitative structure-toxicity-toxicity relationship (QSTTR) modelling has been performed to check for the interrelationship of various pattern of response as we move across the hierarchy of genetics in different taxonomical class. Various descriptor calculating software such as PaDEL-Descriptor, DRAGON and SiRMS were used to compute a wide array of 2D descriptors for capturing chemical information required to correlate the biological properties (toxicities) inherited in the chemical structure of the molecules. All the obtained models showed that with an increase in hydrophobic characteristics (in terms of Log P) toxicity also increases linearly while with an increase in hydrogen bond donating groups, toxicity decreases. An applicability domain study was carried out in order to define the scope of developed model and to highlight compounds falling outside the domain of the respective models. The obtained QSTTR models were finally utilized to fill the data gaps of 275 pharmaceuticals, by using as a template to predict toxicity of pharmaceuticals where experimental data were missing for at least one of the endpoints. Finally, the developed QSTR models were used to predict a large dataset of approximately 7000 drug like molecules in order to prioritize the existing drug like substances in accordance to their acute predicted aquatic toxicities.

Keywords: QSAR, QSTR, QSTTR, Ecotoxicity, Pharmaceuticals

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