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MOL2NET International Conference on Multidisciplinary Sciences

(2nd edition)

MOL2NET: FROM MOLECULES TO NETWORKS

(PROCEEDINGS BOOK) Vol. 2.

YEAR-ROUND CONFERENCE

15 January–15 December 2016

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Welcome Message

We are glad to invite all colleagues worldwide to participate on a new International Conference Series. The official title of this conference series is MOL2NET International Conference Series on Multidisciplinary Sciences. 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 between experimentalists and theoretical scientists.

This is an International Conference Series to Foster Interdisciplinary Collaborations in Sciences with emphasis on Experimental Chemistry (all branches), Materials Science, Nanotechnology, Life Sciences, Medicine, and Healthcare, along with Data Analysis, Computer Sciences, Bioinformatics, Systems Biology, and Complex Networks Sciences.

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).

In addition, professors / researchers from 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, and many other institutions are also founders and supporters of this conference, please see full committees lists.

The publication of communications will be online via the platform SciForum of the Editorial Molecular Diversity Preservation International (MDPI), with HQ in Basel, Switzerland, and Beijing -Wuhan, China. This year the second edition of MOL2NET is planed to be held from **2016**-Dec-05 to **2017**-Jan-25 (including interactive discussions, posts, comments, questions, and answers about papers in the online platform Sciforum). However, the online submission platform is open and the publication of communications will be asap upon acceptance, all the year. For more details, see Schedule & Program page and to submit a communication use the Submission link. Remember, these are the dates for the online conference and not for the face-to-face workshops associated to the conference.

MOL2NET Past Edition

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.

Face-to-Face Associated Workshops (In person participation)

MOL2NET International Conferences Series on Multidisciplinary Sciences is a scientific conference that runs totally online at the SciForum platform promoted by the editorial of the Molecular Diversity Preservation Institute (MDPI), Basel, Switzerland. http://sciforum.net/conference/mol2net-02. Consequently, no physical presence is needed saving traveling costs. However multiple workshops associated to the conference run in person (face-to-face) at their organizing universities. This year our conference is the online host of more than 10 of these workshops (see sections 7 to last section). The participants in these workshops will be allowed also to participate and comment online in our platform including cross-section comments. Some of the workshops organized are:

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, Bio-molecular Sciences, etc. **Chairpersons:** Prof. David Quesada and Prof. Humberto Gonzalez-Diaz (Online Publication). (Closed, received **38 communications**)

IWMEDIC-04, IV International Workshop on Medical Imaging, Medical Coding, and Clinical Data Analysis of University of Coruña (UDC). The IWMEDIC-04 workshop will be 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. **Chairpersons:** Prof. Alejandro Pazos and Prof. Humberto Gonzalez-Diaz (Online Publication). (Closed, received **30 communications**)

UFIQOSYC-01 , 1st Young Scientist 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. (Closed, received **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). (Closed, received **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), and Prof. Humberto Gonzalez-Diaz (Publication). (Closed, received **12 communications**)

MODEC01, Workshop on Natural Products and Agro-Industrial Procesess in Amazon, Ecuador. The workshop was held at Amazon State University, 2016. **Topics**: Computational chemistry, Cheminformatics, Bioinformatics, Organic and Functional Foods, BioTrade: Natural Products of the Amazon, Environmental sciences, Agro-industrial development processes. **Chairperson**: Prof. Amaury Pérez-Martinez. (Closed, received **22 communications**)

MDPI JCR Journals Special Issues

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/). See, as example, the special issue on the International Journal of Molecular Sciences (IJMS), IF = 3.257), with 18 papers in total including papers from the conference, link: Special Issue on Data Analysis in Molecular Sciences. In order to send a proposal of associated workshop and/or special issue contact the chairperson of the conference and UPV/EHU Ikerbasque Professor Prof. H. González-Díaz .

MOL2NET 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: GOOGLE+ account with +50000 viewers; 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].

NOTES for participants

The MOL2NET conference is Totally Online; no physical presence is needed 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). Please, see the following instructions: (1) Read call for papers [Link], (2) Read [Instructions to Authors] and download template .doc file MOL2NET 2016 Microsoft Word template file, (3) Submit short communications (2-3 pages), reviews, papers, or videos: [Link]. For details about in person (face-to-face) participation on associated workshops contact the respective members of the local committees.

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Editorial Paper

González-Díaz, H. Editorial: MOL2NET 2016, International Conference Series on Multidisciplinary Sciences.. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 00001; doi:10.3390/mol2net-02-00001, http://sciforum.net/conference/mol2net-02/paper/3777

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List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (1)

00001 Editorial: MOL2NET 2016, International Conference Series on Multidisciplinary Sciences. show abstract
by Humberto González-Díaz *

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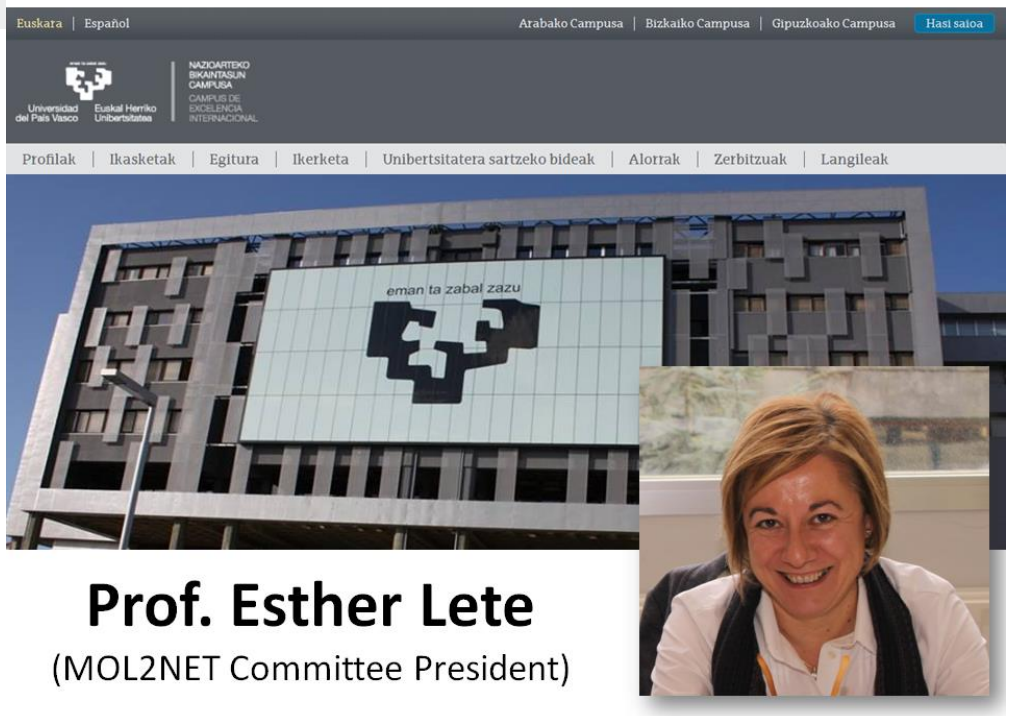

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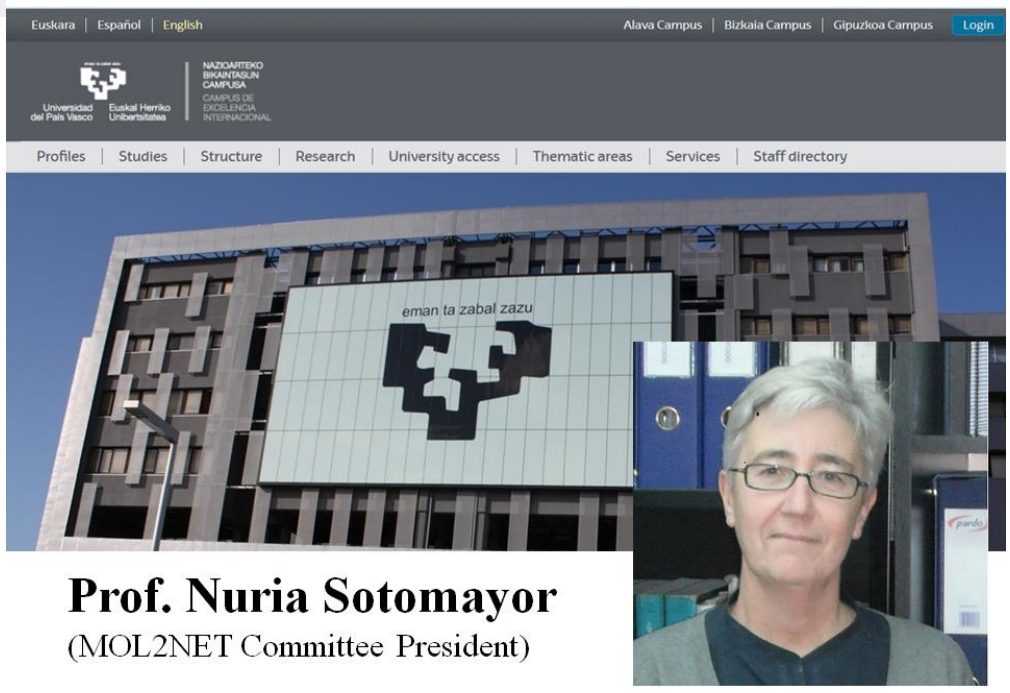

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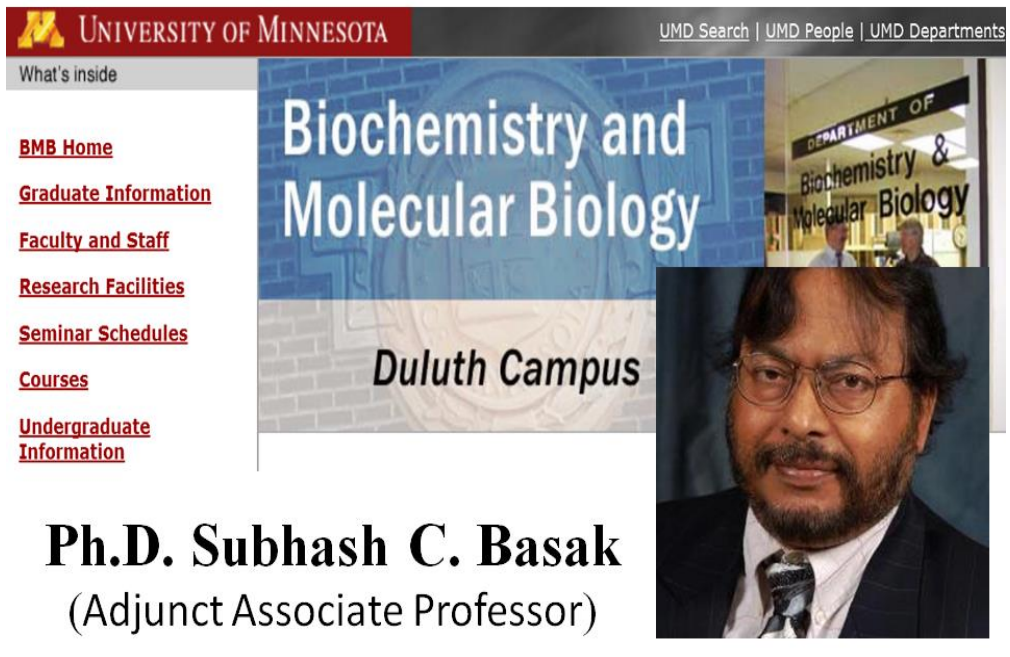

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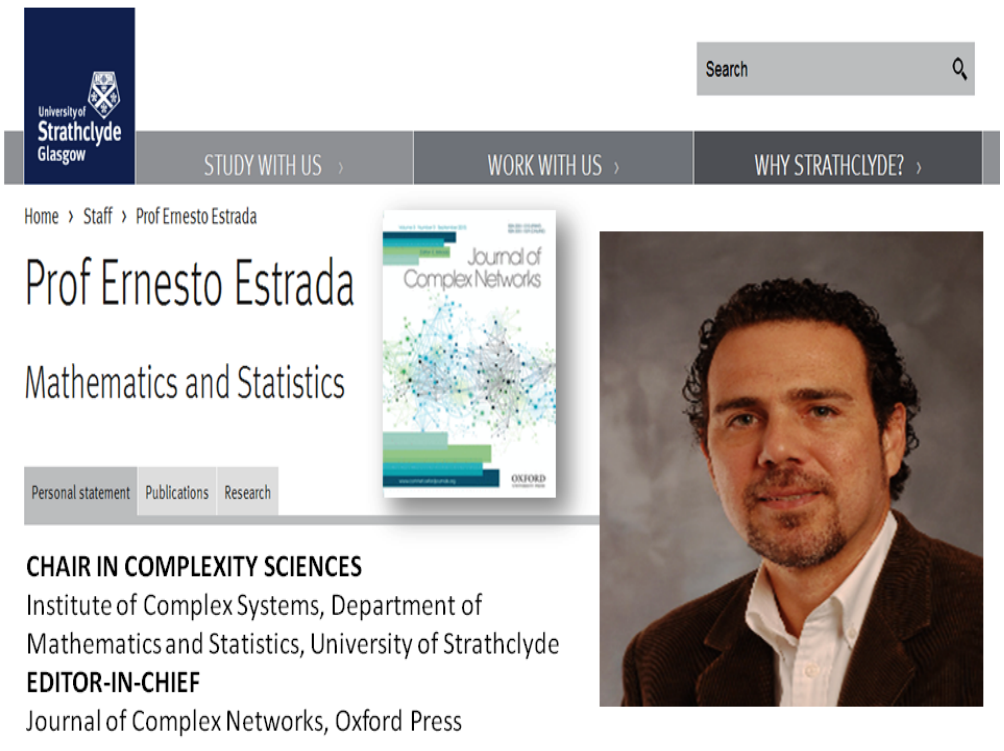

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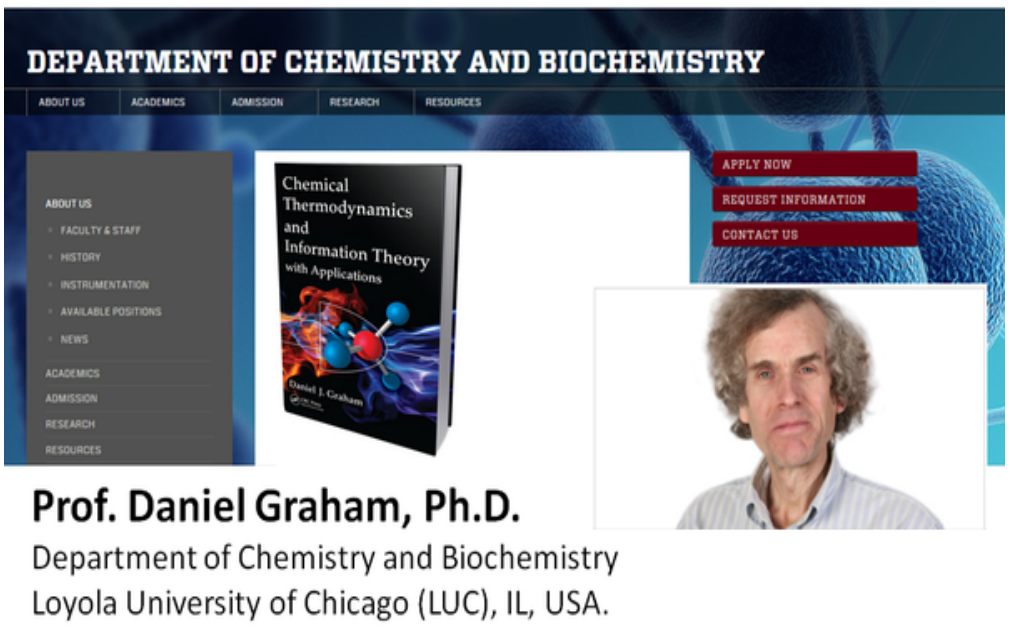

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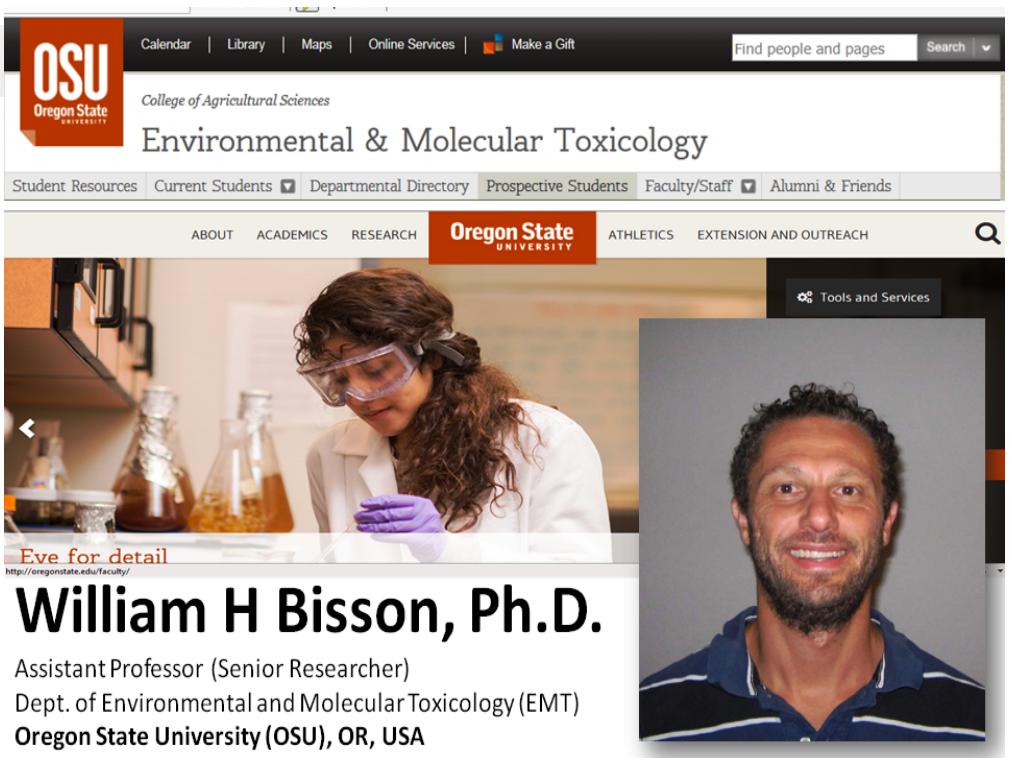

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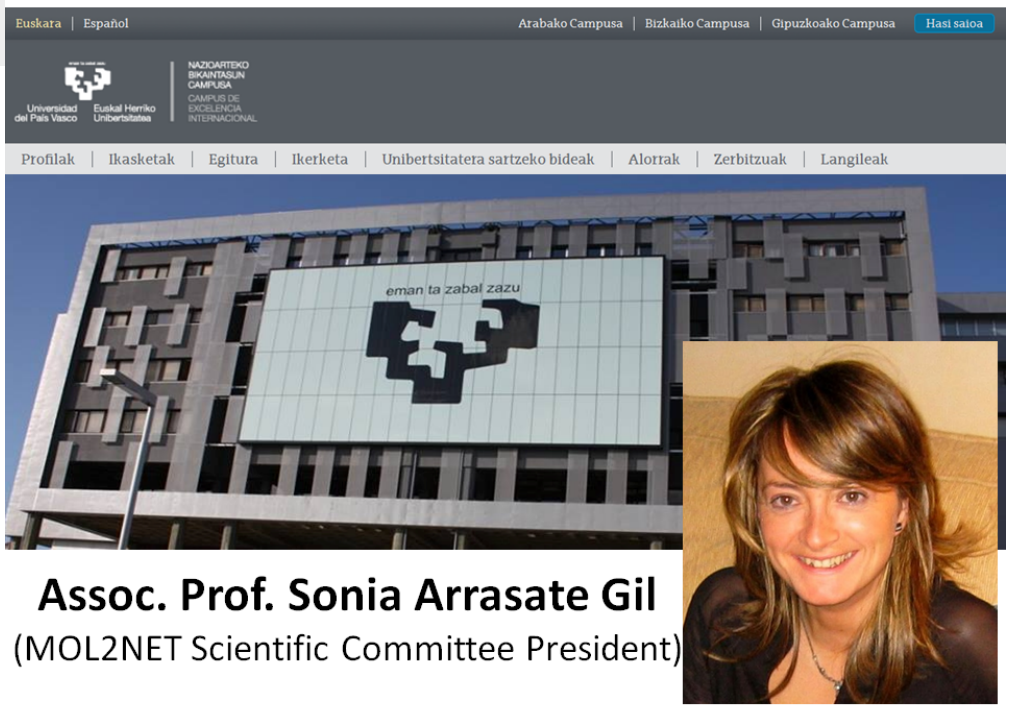

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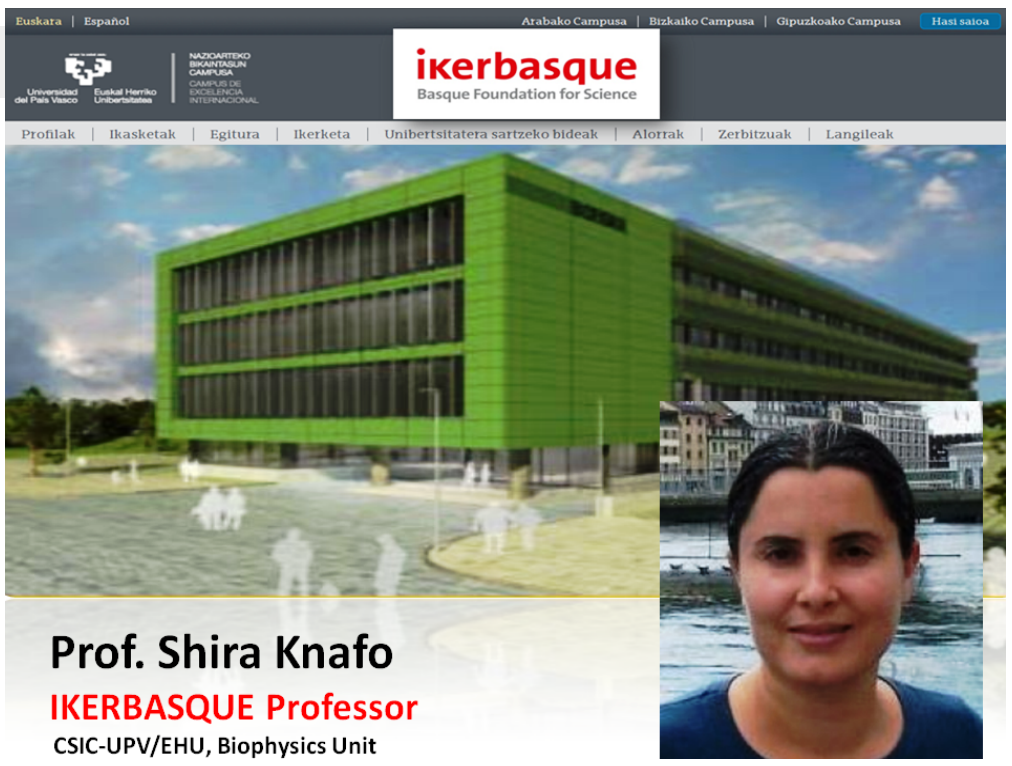

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Assist. Prof. Efraim Reyes Martín, Department of Organic Chemistry II, UPV/EHU, **Campus Biscay**

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Assoc. Prof. Maite Insausti, Department of Inorganic Chemistry, UPV/EHU, **Campus Biscay**

Assoc. Prof. Jose Luis Vilas, Department of Physical Chemistry, UPV/EHU, **Campus Biscay**

Assoc. Prof. Izaskun Gil de Muro, Dept. of Inorganic Chemistry, UPV/EHU, **Campus Biscay**

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Ph.D. Ailette Prieto Sobrino, Dept. of Analytical Chemistry, UPV/EHU, **Campus Biscay**

Ph.D. Jose Manuel Laza Terroba, Dept. of Physical Chemistry, UPV/EHU, **Campus Biscay**

Prof. M.D. Marta Arrasate Gil, Adjunct Prof., Faculty of Medicine, UPV/EHU, **Campus Biscay**

Assoc. Prof. Jose Luis Ayastuy Arizti, Dept. of Chemical Engineering, UPV/EHU, **Campus Biscay**

Adjunct Prof. Uxue Uria Pujana, Department of Organic Chemistry II, UPV/EHU, **Campus Biscay**

PhD. Eider Aranzamendi, Department of Organic Chemistry II, UPV/EHU, **Campus Biscay**

Adjunct Prof. Irantzu Barrio, Ph.D. Department of Applied Mathematics and Statistics, UPV/EHU, **Campus Biscay**

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

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, 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. Chemometrics, Experimental Design, and Data Analysis in Analytical chemistry.

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (16)

Computer aidded design of new inhibitors of acetylcholinesterase. show abstract
by Ana L. Pascoini , Leonardo B. Federico , Ana L. F. Arêa , Barbara A. Verde , Ihosvany Camps *

Computational Study of Natural Phenolic Acid Solubility and Their Interactions with Chitosan show abstract
by Pablo Henrique Delmondes , Ricardo Stefani *

Chemometric highlighting of inter- and intra-molecular diversification factors by a new training simplex-based approach: application to *Astragalus* saponins show abstract
by Abir Sarraj-Laabidi , Habib Messai , Asma Hammami-Semmar , Nabil Semmar *

TARGET METABOLOMIC APPROACH LOOKING FOR BETTER STRAWBERRY GROWING CONDITIONS TO ENHANCE BIOACTIVE CONTENT. show abstract
by Ikram Akhatou *, Angeles Fernández-Recamales , Ana Sayago , Raúl González-Domínguez , Rafael Beltrán

NMR Fingerprint to Classify Spanish Olive Varieties show abstract
by Ana Sayago *, Ángeles Fernández-Recamales , Raúl González-Domínguez , Juan Urbano , Rafael Beltrán

DEVELOPEMENT AND OPTIMIZATION OF ACALORIC SWEETENERS EXTRACTION PROCEDURE FROM S*tevia rebaudiana* Bertoni show abstract
by Verónica López Carbón *, Ángeles Fernández-Recamales , Ana Sayago , Raúl González-Domínguez , Rafael Beltrán

Development and Characterization of New Fermented Beverages of Low Alcoholic Graduation from Strawberry. show abstract
by Angeles Fernández Recamales *, Ana Sayago , Raúl González-Domínguez , Veronica López-Carbón , Ikram Akhatou , Rafael Beltrán

List of Accepted Abstracts (3)

C−H Activation of Diamondoids by $Pd(OAc)_2$ show abstract
by Marta Larrosa Ferreiro *

Electrochemical Capacitive Detection of DNA Modification and Hybridization Processes using Interdigitated Gold Microelectrodes Arrays show abstract
by Nadja E Solis-Marcano *, Marjorie Lopez-Nieves , Carlos R. Cabrera

Ureolysis system for water recovery from urine using a microbial carbon based electrode show abstract
by Myreisa Morales *, Roberto Alexis Martinez , Carlos Raul Cabrera

02: OMICs, Biotechnology, Bioinformatics, and Biomedical Engineering

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 also includes: 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.

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (12)

Multiple Linear Regression Model of Thermolysin Inhibitors show abstract
by Juan Alberto Castillo-Garit *, Yudith Cañizares-Carmenate , Karel Mena-Ulecia , Yunier Perera-Sardiña , Francisco Torrens

An approach toward the identification of new antileishmaniasic compounds. show abstract
by Juan Alberto Castillo-Garit *, Naiví Flores-Balmaseda , Orlando Álvarez

QSAR Models and Virtual Screening for Discovery of New Analgesic Leads show abstract
by Juan Alberto Castillo-Garit *, Arelys López , Yovani Marrero-Ponce , Gerardo M. Casañola-Martín , Vicente J. Arán

Nanoparticles mutagenicity: search for matches and potential limitations of Comet assay and Ames test show abstract
by Natalia Sizochenko *, Bakhtiyor Rasulev *, Jerzy Leszczynski

List of Accepted Abstracts (2)

Analysis and refining the homology modeled to the Protein Kinases J and L from *mycobacterium* tuberculosis: topological and 3D analysis. show abstract
by Alejandro Morales-Bayuelo *

Nanoparticles mutagenicity: search for matches and potential limitations of Comet assay and Ames test show abstract
by Natalia Sizochenko *, Bakhtiyor Rasulev , Jerzy Leszczynski

03: Computational Sciences (Applied to Social, Legal, and Life Sciences)

Computational Sciences

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!

Note: Former Section 03, Soft Matter Physics, Materials, Nanosciences, etc., was decommissioned and merged into **Section 01**, Chemistry (All Areas), Physical Chemistry, etc., please follow the link http://sciforum.net/conference/mol2net-02/section-01

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (7)

05001 **Linear Indices Bob-Jenkins operators for development of multi-output models using multi-target inhibitors of ubiquitin-proteasome system** show abstract
by Gerardo Casañola-Martin *, Facundo Perez-Gimenez , Matilde Merino-Sanjuan

05002 **Artificial Neural Network Schedulers for Food Webs** show abstract
by Humberto González-Díaz *, Alejandro Pazos , Cristian Robert Munteanu , Enrique Barreiro

Artificial Neural Networks and Multilinear Least Squares to Model Physicochemical Properties of Organic Solvents show abstract
by Jesus Vicente de Julián-Ortiz *, Lionello Pogliani , Emili Besalú

Co-evolution importance on binding Hot-Spot prediction methods show abstract
by José G. Almeida , António J. Preto , Rita Melo , Zeynep H. Gümüş , Joaquim Costa , Alexandre M.J.J. Bonvin , Irina Moreira *

Fuzzy Membership Roster Method based Selection Rule for Parameter Reductio show abstract
by Sreyasi Ghosh *, Sarbari Ghosh , Pradip Sen

Efficient RL Algorithm by Combing AC with Dual Piecewise Model Learning show abstract
by Shan Zhong *, Quan Liu *, Qiming Fu

Complex Network Analysis of General Tax Law show abstract
by Aliuska Duardo-Sanchez *

List of Accepted Abstracts (5)

A Study on Automatic Algorithm Configuration Based on ParamILS show abstract
by Yonggang Zhang *, Sainan Qin , Yuxiao Wang , Mingyuan Yang , Yue Gu , Chunlei Li

A study of the immune epitope database for fungi species using network Topological Indices show abstract
by Severo Vázquez-Prieto *, Esperanza Paniagua , Hugo Solana , Florencio M. Ubeira

Experimental studies and perturbation models of microbiome cell membrane permeability and cell surface hydrophobicity show abstract
by Yong Liu *, Shaoxun Tang

Fuzzy Membership Roster Method based Selection Rule for Parameter Reduction show abstract
by SREYASI GHOSH *, SARBARI GHOSH , pradip kumar sen

Speech recognition methods in task of automated detection of pronunciation defects show abstract
by Viktor Malyuchenko *

04: Welcome Videos (English, Chinese, Hindi, Spanish, Portuguese, French, and Euskera)

Welcome Video Presentations

1. MOL2NET Introductory talk by Ph.D. Yong Liu, Audio: Chinese Mandarin (官话). *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–10 January 2017; Sciforum Electronic Conference Series, Vol. 2, **2016**; doi:10.3390/mol2net-02-06001, Sciforum communication: http://sciforum.net/conference/mol2net-02/paper/3447
2. MOL2NET Introductory talk by M.Sc. Mitali Hardikar, Jr. Scientist, Audio: Hindi (हिन्दी). Hardikar, M.; Nayarisseri, A. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–10 January 2017; Sciforum Electronic Conference Series, Vol. 2, **2016**; doi:10.3390/mol2net-02-06002, Sciforum communication: http://sciforum.net/conference/mol2net-02/paper/3448

3. MOL2NET Welcome Message by Prof. Marcus Tullius Scotti, Audio: Portuguese. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–10 January 2017; Sciforum Electronic Conference Series, Vol. 2, **2016**; doi:10.3390/mol2net-02-06003, http://sciforum.net/conference/mol2net-02/paper/3450
4. MOL2NET Welcome Message by PhD. Veronica Ortiz de Elguea, Audio: Spanish (Castellano). *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–10 January 2017; Sciforum Electronic Conference Series, Vol. 2, **2016**; doi:10.3390/mol2net-02-06004, http://sciforum.net/conference/mol2net-02/paper/3451
5. MOL2NET Welcome Message by Prof. Yagamare Fall Diop, Audio: French (Français) *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–10 January 2017; Sciforum Electronic Conference Series, Vol. 2, **2016**; doi:10.3390/mol2net-02-06005.
6. MOL2NET Welcome Message by Prof. Sonia Arrasate and Prof. Inmaculada Arostegui, Audio: Basque (Euskera). *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–10 January 2017; Sciforum Electronic Conference Series, Vol. 2, **2016**; doi:10.3390/mol2net-02-06005.

7. MOL2NET Welcome Message by Adj. Prof. Ph.D. Eider Aransamendi, Audio: Basque (Euskera). *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–10 January 2017; Sciforum Electronic Conference Series, Vol. 2, **2016**; doi:10.3390/mol2net-02-06005.

Note: The original Section 04, Healthcare & Clinical Medical Sciences, Biomedical Engineering, and Medical Informatics, was merged into Section 02, http://sciforum.net/conference/mol2net-02/section-02

05: News and Channels

MOL2NET Social Media

We are also releasing the videos as well as other conference materials such as links to papers to other media like MOL2NET Google+, MOL2NET Twitter, etc. In fact, we have opened channels, webs, or social media pages on different media to publish updates about the conference and promote synergies of experimental molecular and biomedical science groups with networks and data analytics experts. MOL2NET list of social media includes, but is not limited to, the following accounts in well known social networks: FACEBOOK group (with >10 000 members), RESEARCH GATE Project (with details, updates, and links to scientific profiles of committee members), GOOGLE+ page (>50 000 viewers, flyers of the conference, videos, etc.), andTWITTER account (twits, comments, flyers, updates, etc.)

MOL2NET Video Channel

This international science conference focus on multidisciplinary sciences. The official working language is English, the main form of publication is palin text or presentations (.doc, .pdf, .ppt), and the official page is on Sciforum: http://sciforum.net/conference/mol2net-02

However we have an initiative to promote the use of other languages, forms of presentations, and different social network platforms in order to reach people from non-English speaking country, that prefer not read text at first glance, and/or are present on social media but do not know our official platform yet. This initiative is also aimed to show the cultural heritage of different people in the world by using other languages.
One action in this sense includes the compilation of a playlist with welcome videos and/or scientific talks of different scientist talking about both the conference and/or their own work in Hindi (हिन्दी), Chinese Mandarin (官话), Spanish, French, Portuguese, and very specially in Euskera a very ancient Europe language from Basque Country (Euskadi), used today.
Do not hesitate to contact Prof. Gonzalez-Diaz H. (conference ch.) humberto.gonzalezdiaz@ehu.eus, if you want to publish a similar video introducing the conference and/or your own work in English or other language. All these videos will be uploaded to the main page of the conference on Sciforum paltform with the form of video conferences but we are also releasing them to authors video channels. Please, watch also the current playlist of the conference chairman in Youtube [MOL2NET Youtube Channel].
Please, be aware that to submit a talk or video communication, you need to submit both the video and a short video communication description in a .doc or .pdf file in the official template of the conference (1 page only) with title, author, affiliation, short asbtract, and link to your private channel if any, you need to send us also the video file, please [Submit video communication].
Please, read also the following instructions: (1) Read call for papers [Link], (2) Read [Instructions to Authors] and download template .doc file MOL2NET 2016 Microsoft Word template file, (3) Submit short description of video communications description (**1 page only**) and video: [Link].
See one example of video communication description [Video comm. description example].

Note: The original Section 5 (Statistics, Computing, etc.) have been re-numerated as Section 03. Computational Sciences.

http://sciforum.net/conference/mol2net-02/section-03

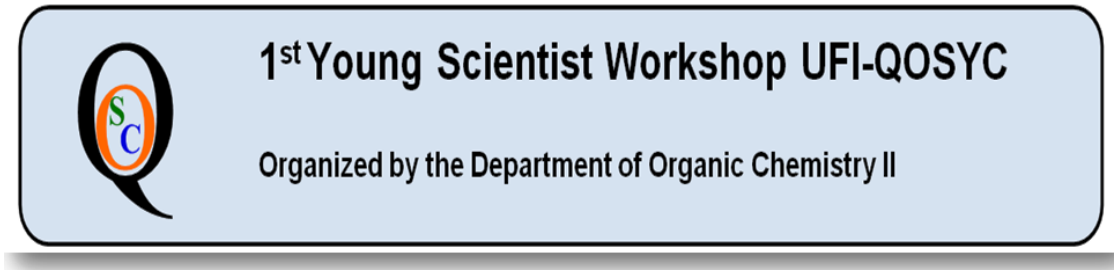
06: Associated Workshops

MOL2NET ASSOCIATED WORKSHOPS (in person)

MOL2NET International Conferences Series on Multidisciplinary Sciences is a scientific conference that runs totally online at the SciForum platform promoted by the editorial of the Molecular Diversity Preservation Institute (MDPI), Basel, Switzerland. http://sciforum.net/conference/mol2net-02. Consequently, no physical presence is needed saving traveling costs. However multiple workshops associated to the conference run in person (face-to-face) at their organizing universities. This year our conference is the online host of many of these 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, Chairperson Prof. David Quesada and Advisory Chairperson Prof. Humberto Gonzalez-Diaz (Online Publication).

IWMEDIC-04, IV International Workshop on Medical Imaging, Medical Coding, and Clinical Data Analysis of University of Coruña (UDC). The IWMEDIC-04 workshop will be 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). The chairman of this workshop is the Chair Professor and Director of Department of Computer Sciences, UDC, Coruña, Spain Prof. Alejandro Pazos and Advisory Chairperson Prof. Humberto Gonzalez-Diaz(Online Publication).



UFIQOSYC-01 , 1st Young Scientist Workshop hold at the Department of Organic Chemistry II, University of Basque Country UPV/EHU. This workshop have brought together early career researchers (postdocs and graduate students) from the area of organic chemistry and catalysis across the UFI QOSYC to exchange information and practice presenting their research work in a supportive scientific environment. Chairpersons Prof. Esther Lete, Prof. Esther Domínguez Pérez, and Prof. Jose Luis Vicario

SUIWCS-01, Soochow University International Workshop Series on Computer Sciences. The SUIWCS-01 workshop will be held presentially at the the School of Computer Science and Technology of Soochow University, PCR, China (Summer, 2016). The chairman of this workshop is the Chief of Department of Software Engineering and Professor of Computer Sciences, School of Computer Sciences and Technology, Soochow University (SUDA), Suzhou, China, Prof. Quan Liu .

BMEICB-02 Second Bioinformatics Meeting of The School of Bioinformatics Engineering, University of Talca, Talca, Chile, (Oct, 13-14, 2016). Advisory chair and connection with MOL2NET conference Prof. Julio Caballero

CIESABIO-01 , 2016, the Workshop Series on Biotechnology and Zoonotic Diseases of the CIESA, Center for Invetigations and Advanced Studies on Animal Health of the FMVZ Faculty of Medical Veterinary and Zootechnique, of the UAEMEX Autonomous University of the State of Mexico. The Chairperson of this workshop is the Prof. Esvieta Tenorio.

See other workshops following the respective links of the the different workshop sections in the homepage. http://sciforum.net/conference/mol2net-02

07: SRI08: The 8th Annual Symposium of Summer Research Insitute, STU, Miami, USA, 2016

SRI-08: The 8th Annual Symposium of Summer Research Institute, Saint Thomas University, September 16th, 2016

Submit New Communications

Dear colleagues we welcome you to the symposium of the Summer Research Institute (SRI) with Head Quarters (HQ) at Saint Thomas University (STU), and supported by Miami Dade College (MDC), Miami, Downtown, FL, USA . This is face-to-face (in person) workshop associated to and hosted online by MOL2NET-2, International Conference of Multidisciplinary Sciences, 2016, MDPI, Sciforum, Basel, Switzerland, with HQs, University of The Basque Country (UPV/EHU), and supported by IKERBASQUE, Basque Foundation for Science, Basque Country, Bilbao, Spain.

Welcome Message from Chairman

Eight years have passed since the First Annual Undergraduate Summer Research Symposium. Over this period of time, the School of Science, Technology, and Engineering Management of Saint Thomas University (STU) in partnership with Miami Dade College (MDC) and with the continuous support of the faculty and staff has provided an excellent internship program to our students and those coming from our partner's institutions. From the start the Carnival Cruise Lines Science and Technology Building focused on the hands-on research experience. This places our students in an excellent position to gain entrance into graduate, or professional Schools, or to directly enter the workforce in South Florida. Most students in other institutions do not have this research opportunity until they reach graduate school.

The faculty and staff of the School of Science are committed to providing a quality education in the sciences and offer the unique opportunity to talented undergraduate students, to experience hands-on research in ten research laboratories alongside their professors. In addition, the Summer Research Institute (SRI) has enhanced the instrumentation capacity of our institution. Such improvements have allowed deepening our research projects as well as to establish new alliances in research and development. Results from our projects already circulate in local, national and international conferences, augmenting this way the visibility of the institution and the pride that students might have for their faculty and work accomplished. The eight edition of the SRI offered a continuous lecture series "Moving into the Future" on a weekly basis. Ten speakers from University of Miami, Florida International University, and local Technological companies came to St. Thomas to share their wisdom as an in kind contribution.

This year, the memories of the Annual Symposium will be published online in the open source forum MOL2NET of 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, inter-disciplinarity, teamwork and networking. I hope you will enjoy the program and the presentations.

Special thanks to our sponsors, Miami Dade College, STEM-TRAC grant, St. Thomas School of Science, Technology, and Engineering Management, Yager Foundation, Monet Coiffure, In Fashion Forever, and Physics & Mathematics Solutions, who provided funding for major and minor activities associated with the 2016 edition. Follow the link to download and/or read the full program [SRI08 Program]

Program Overview

8:00 – 9:00 Registration and Poster Setup. Judges meet in room CCL 111
9:00 – 9:05 Invocation by Rev. Alfred Cioffi
9:05 – 9:20 Opening Comments by Dr. Adrienne Vynne, Dean of School of STEM
9:20 – 9:35 Opening Comments by Dr. Irma Becerra, Provost of St. Thomas University
9:35 – 9:45 Session Introduction by Dr. David Quesada, Coordinator SRI 2016
9:45 – 10:00 Oral Presentation 1
10:00 – 10:15 Oral Presentation 2
10:15 – 10:30 Oral Presentation 3
10:30 – 10:55 Break
11:00 – 11:15 Oral Presentation 4
11:15 – 11:30 Oral Presentation 5
11:30 – 11:45 Oral Presentation 6
12:00 – 13:00 Lunch Break courtesy of the School of STEM
13:00 – 15:00 Poster Session
15:00 – 15:30 Judges meet in CCL 111 to choose the awards
15:30 – 15:45 Announcement of Awards

Submission Notes

This workshop is planned to be held on Sept, 2016. However, 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. After abstract approval you need to send your communication. Firstly, you need to submit only the title, authors, and short abstract. Secondly, you could submit your full work in .doc and .pdf format after receiving the approval email from the chairperson.

Please, download and use the following .doc file as [SRI08 2016 Official Template] to submit your work. For more details contact the Chairman of the workshop.

Be aware:

on step 1, you should select MOL2NET 2016, International Conference on Multidisciplinary Sciences, 2nd edition (Conference),

on step 2, you should select SRI08: Saint Thomas University Research Experience for Undergraduates, Miami, 201**6.**

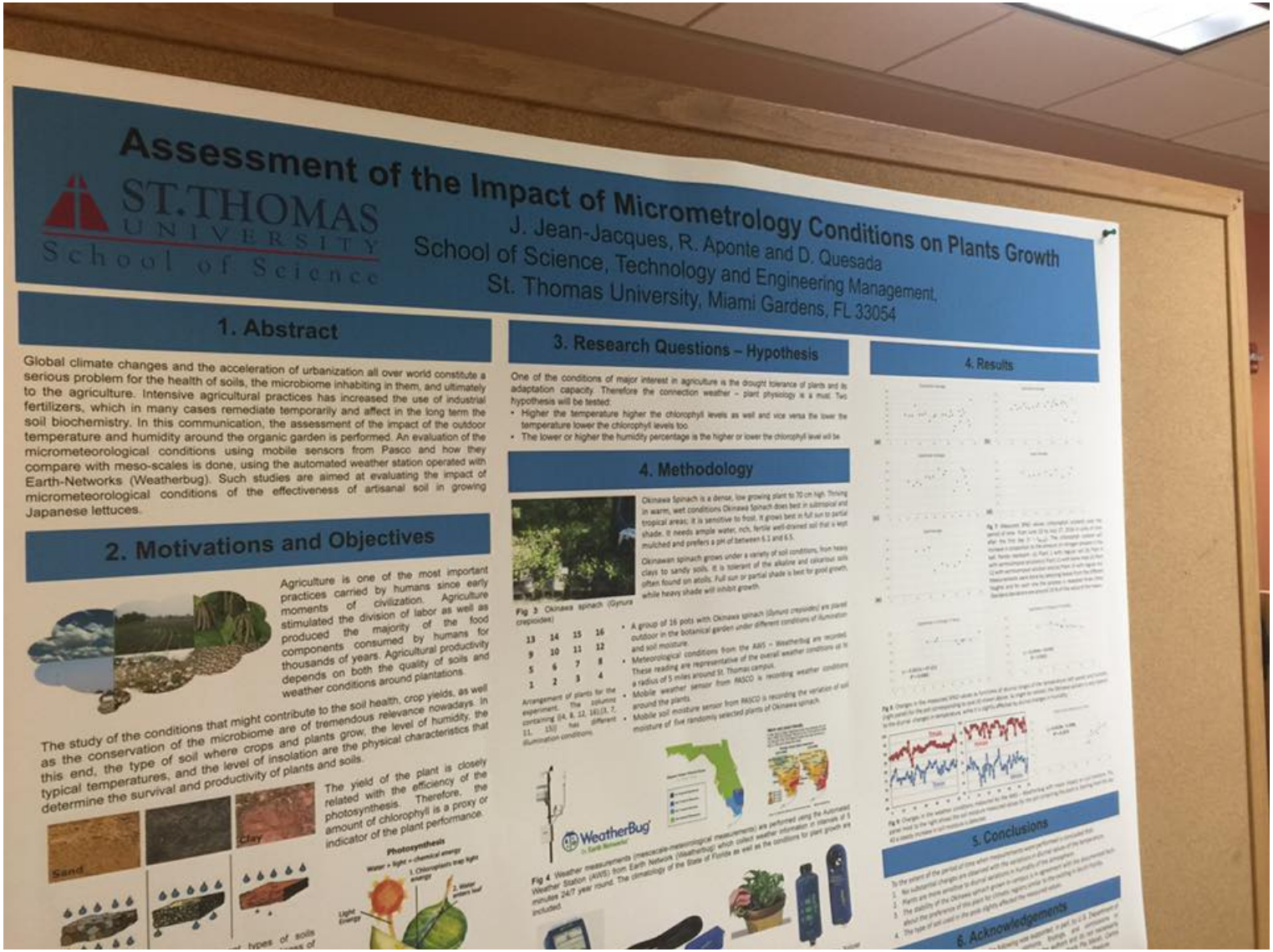
Symposium Committee

SRI Chairperson: Prof. David Quezada, Associate Professor of Physics, Email: dquesada@stu.edu
Saint Thomas University (STU), Carnival Building, Room 115, Miami, FL, USA. (MOL2NET Honor Committee)

Publication Advisory Chair: Prof. Humberto González-Díaz, IKERBASQUE Prof., Ph.D., Email: humberto.gonzalezdiaz@ehu.eus
(1) Department of Organic Chemistry II, University of Basque Country (UPV/EHU), Campus Bizkaia, Basque Country, Spain.
(2) IKERBASQUE, Basque Foundation for Science, Bilbao, Bizkaia, Basque Country, Spain.
(MOL2NET Conference Chairman).

Workshop Photo gallery

See a photo gallery with pictures of the participants posted by the chairperson of the workshop on a Facebook [Album].



List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (21)

07001 SRI-08: The 8th Annual Undergraduate Summer Research Symposium of Saint Thomas University show abstract

by Humberto González-Díaz *, David Quesada *

07002 **Initial Description of the Local Ionospheric Response to Geomagnetic Storms** show abstract

by Santiago Mejia , Eduardo A Araujo-Pradere *, Ali Khan

07003 **The seasonality of upper respiratory tract infections and their relationship to asthma** show abstract

by David Quesada *, Aidin Alejo

07004 **Effect of Brain network topologies on the synchronization of neuronal oscillations – Is this the gateway to the understanding of Central Nervous disorders?** show abstract

by David Quesada *, Natasha M. Astudillo , Manuel A Garcia-Russo

07005 **The effect of Hedgehog signaling on *in vivo* neuronal morphogenesis.** show abstract

by Alexis Tapanes-Castillo *, Leana M. Ramos

07006 **Contrasting Effects of Aqueous Vermicompost Extract Mixtures on Growth of *Brassica oleracea* var. *sabellica*** show abstract

by Carlos E Vazquez , Dora P Maul *, Luis A Cendan

07007 **Analysis of Oyster Plant (Tradescantia Spathacea) Extracts via Maceration, Soxhlet Extraction and Thin Layer Chromatograpy** show abstract

by Maria D Pina *, Daniel A Russo , Cristina Balistreri , Kelly R O'Reilly , Luis A Cendan , Carlos E Vazquez , Dora P Maul

07008 **Culturing Human Neural Stem Cells and Quantifying Lentiviruses to Study Autism.** show abstract

by Alexis Tapanes-Castillo *, Derek Dykxhoorn , Leana M. Ramos , Milagros D Mulero , Deliabell Hernandez , Vadym Trokhymchuk

Mathematical Modeling of the Optical response of photovoltaic cells show abstract

by David Quesada *, Henry M Morales

Modeling the oscillating Belousov – Zhabotinsky chemical reaction show abstract

by David Quesada *, Steven Centeno

Braess Paradox in Electrical Networks – When more might mean less show abstract

by David Quesada *, Javier F Rojas , Alexander R Alonso

Assessment of the impact of micrometeorological conditions on plants growth. show abstract

by David Quesada *, Jonathan Jean-Jacques , Richard M Aponte

Belousov-Zhabotinsky Reaction: Effects of Magnetic Field Variations show abstract

by Luis C Fernandez-Torres *, Amanda L Penton , Brandon F Gamboa , David Quesada *

Solvent Variations of the Briggs-Rauscher Reaction show abstract

by Luis C Fernandez-Torres *, Chelsea E Trost , Ana C Figuereo , Marie V Roche , Leonardo Albertini

Mighty Fruits: Antioxidant Performance of Various Fruits show abstract

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

Structure – Activity Relationships (SARs) of Antioxidant Molecule show abstract

by Luis C Fernandez-Torres *, Luis E Castellar , Langeda Bontemps , Leslie R Robinson

Antioxidant Capacity of Selected Teas and Cocoa show abstract

by Luis C Fernandez-Torres *, James D Hankemeyer , Kasey L Rivera , Kelnisha N Lightbourne , Sara N Salamah

Powerful Plants: Antioxidant Capacity of Selected Plants show abstract

by Ana C Figuereo , Chelsea E Trost , Christine Curiac , Jason Alvarodiaz , Jennifer J Cerda , Langeda Bontemps , Luis E Castellar , Luis C Fernandez-Torres *

Antioxidant Capacity of Common Dietary Supplements show abstract

by Ana C Figuereo , Chelsea E Trost , Leonardo Albertini , Luis C Fernandez-Torres *, Marie V Roche

Determination of the Antioxidant Capacity of Coffee show abstract

by James D Hankemeyer , Kasey L Rivera , Kelnisha N Lightbourne , Luis C Fernandez-Torres *, Sara N Salamah

CRISPR-Cas Gene Editing: Regulatory Issues and Applications show abstract

by Aliuska Duardo-Sanchez *

List of Accepted Abstracts (18)

A Comparison of Liquid Organic Fertilizers in the growth of Okinawan Spinach (*Gynura bicolor*), an Asian Medicinal Plant show abstract

by Carlos E Vazquez , Dora P Maul *, Luis A Cendan , Tiffany A Brown

Bacterial Communities Associated with Rhizospheres of Four Organically-Grown Plant Species. show abstract

by Dora P Maul *, Andrea Gonzalez

Bacterial Communities Associated with Rhizospheres of Four Organically-Grown Plant Species. show abstract

by David Quesada *

Can DLGAP1 antisense RNA 2 “cure” autism *in vitro*? show abstract

by Alexis Tapanes-Castillo *, Leana M. Ramos , Derek Dykxhoorn

Characterizing Potential Anticancer Properties of the "Medicinal" Oyster Plant *Tradescantia Spathacea.* show abstract

by Alexis Tapanes-Castillo *, Dora P Maul , Leana M. Ramos , Luis A Cendan , Maria D Pina , Milagros D Mulero , Vadym Trokhymchuk , Marrisa L Lee , Tashani T Brown , Carlos C Planchart , Mang S Cing , Carlos E Canales Silva

Comparing Primer Sets in Polymerase Chain Reaction Analysis of *Candidatus* Liberibacter asiaticus show abstract

by Dora P Maul *, Oriana C Chacin

Comparing the Differential Expression of Selected Genes in Native Peruvian Potatoes in Response to Early and Late Drought Conditions. show abstract

by Carlos E Vazquez , Dora P Maul *, Laynet Cornelio , Indira Perez , Diana Martinez , Olga P Ponce , Emi Murata , Yerisf Torres , Gisella Ojeda ,Carlos Merino , Luz N Zuniga

Confirming Huanglongbing in Miami Dade County show abstract

by Dora P Maul *, Daniel G Diaz

Cube Quest Challenge show abstract

by Javier F Rojas *, Monsi Roman

Development of histological techniques and initial analysis of putative stem cell populations following spinal cord injury in adult zebrafish. show abstract

by Jeffery A Plunkett *, Abdiel J Badillo , Martin Oudega , Kevin J Perez , Melanie M. Gonzalez

Evaluation of an Autonomous Vehicle Utilizing Self-Adaptive Controller for Obstacle Avoidance show abstract

by Steve O Hernandez *, Carlos J Caro , Yasshin A Lozano , Vanessa Jean-Francois , Ksenia M. Slavina , Eric J Covach , J. Castillo , J. Armas

Microbiome analysis of arugula rhizospheres in plants fertilized with vermicompost-based solutions show abstract

by Jose Calera , Dora Pilar Maul *, Luis Cendan , Carlos Vazquez , Rachael Karns , Cole Easson , Jose Lopez

Microbiome analysis of arugula rhizospheres in plants fertilized with vermicompost-based solutions show abstract

by Carlos E Vazquez , Dora P Maul *, Luis A Cendan , Jose M. Calera , Rachael Karns , Cole G Easson , Jose Lopez

Molecular Cloning of DLGAP1 Short Hairpin RNA Vectors to Study Autism Biology. show abstract

by Alexis Tapanes-Castillo *, Derek Dykxhoorn , Leana M. Ramos , Milagros D Mulero , Arielis Ortiz , Claudia Martinez Crespo

Nitrogen Determination Using Kjeldahl Method in Soil and Fertilizer to Grow Okinawa Spinach Plants show abstract

by Carlos E Vazquez , Dora P Maul , Kelly R O'Reilly , Luis A Cendan , Maria D Pina *, Anne D Noel , Karla F Rodriguez , Myly Fabre

Spectrophotometric Iron Determination in Organic-Fertilizer Treated Soil Analyzed Using 1,10-Phenantroline Method show abstract

by Carlos E Vazquez , Cristina Balistreri , Daniel A Russo , Dora P Maul , Kelly R O'Reilly , Luis A Cendan , Maria D Pina *

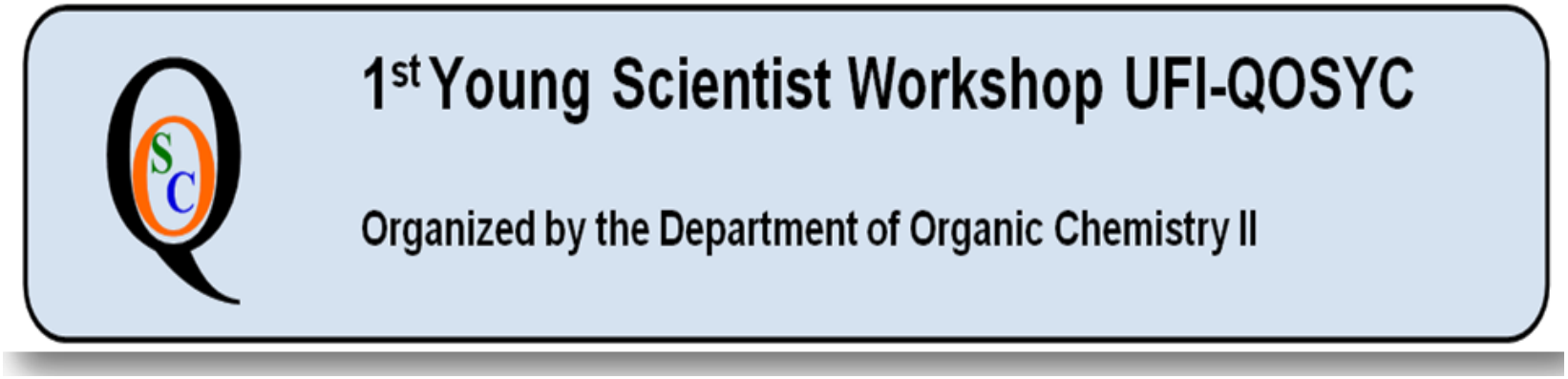
The role of CSPGs in neuronal differentiation of stem cells from the adult zebrafish brainstem. show abstract

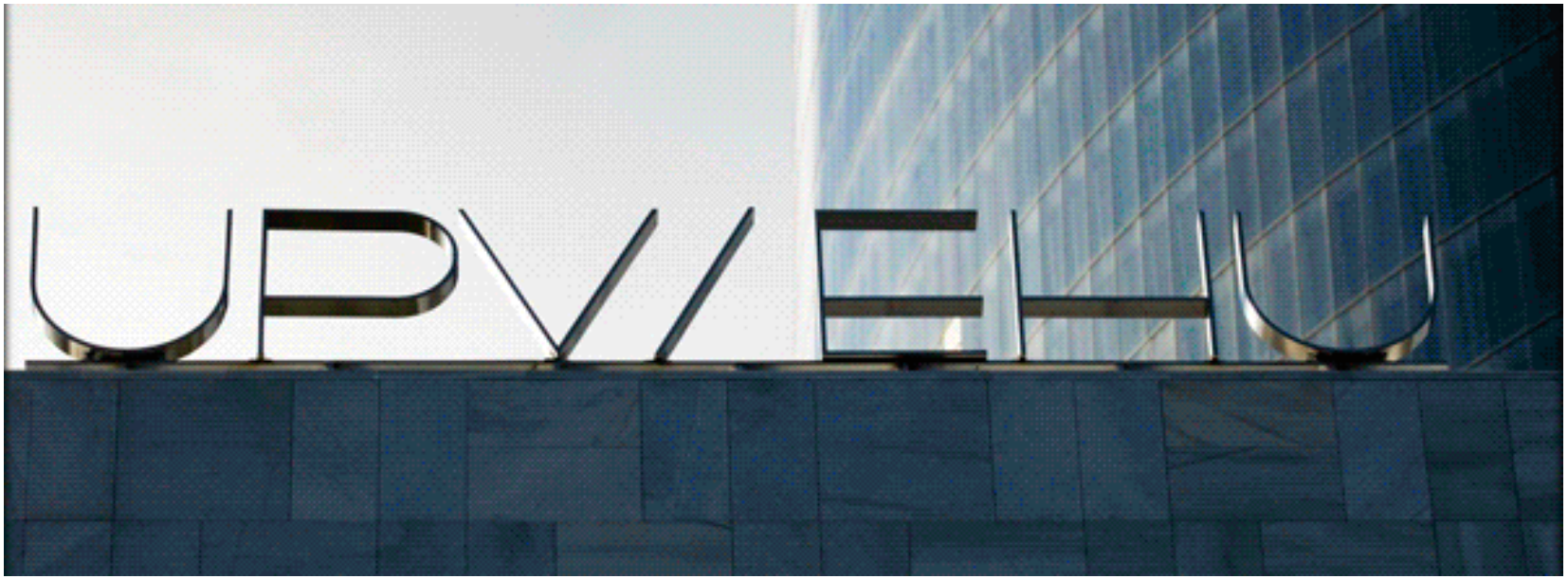
by Abdiel J Badillo , Jeffery A Plunkett *, Martin Oudega , Andrea G Solano , Rayshell L Sands , Stephanie Mangels

The role of putative stem and neural progenitor cell populations following traumatic brain injury in adult zebrafish. show abstract

by David Quesada *, Jeffery A Plunkett *, Michael B Fernando , Raul Banos , Abdiel J Badillo , Martin Oudega

08: UFI-QOSYC01: 1st Young Scientist Workshop on Organic Chemistry, Bilbao, Spain, 2014





1st Young Scientist Workshop UFI-QOSYC

MOL2NET Conference Series on Multidisciplinary Sciences is the host conference of a workshop of UFI QOSYC for young researchers. The UFIs are Training and Research Units (TRUs), or in Spanish UFIs - Unidades de Formación e Investigación, formed by different Departments of the University of Basque Country UPV/EHU.

The UFI QOSYC is the Organic Chemistry, Synthesis and Catalysis unit devoted to developing new procedures for chemical synthesis and catalysis and their application to the preparation of molecules of biological and industrial use.

The "1st Workshop for Young Scientists – UFI QOSYC" will bring together early career researchers (postdocs and graduate students) from the area of organic chemistry and catalysis across the UFI QOSYC to exchange information and practice presenting their research work in a supportive scientific environment. This event will showcase the high value that young researchers bring to UFI QOSYC through their research accomplishments and provide them an opportunity to explore potential research collaborations in the University of the Basque Country UPV/EHU. Besides, it offers an excellent platform to demonstrate UFI QOSYC research activities to external audiences.

Chairpersons of 1st Young Scientist Workshop UFI-QOSYC

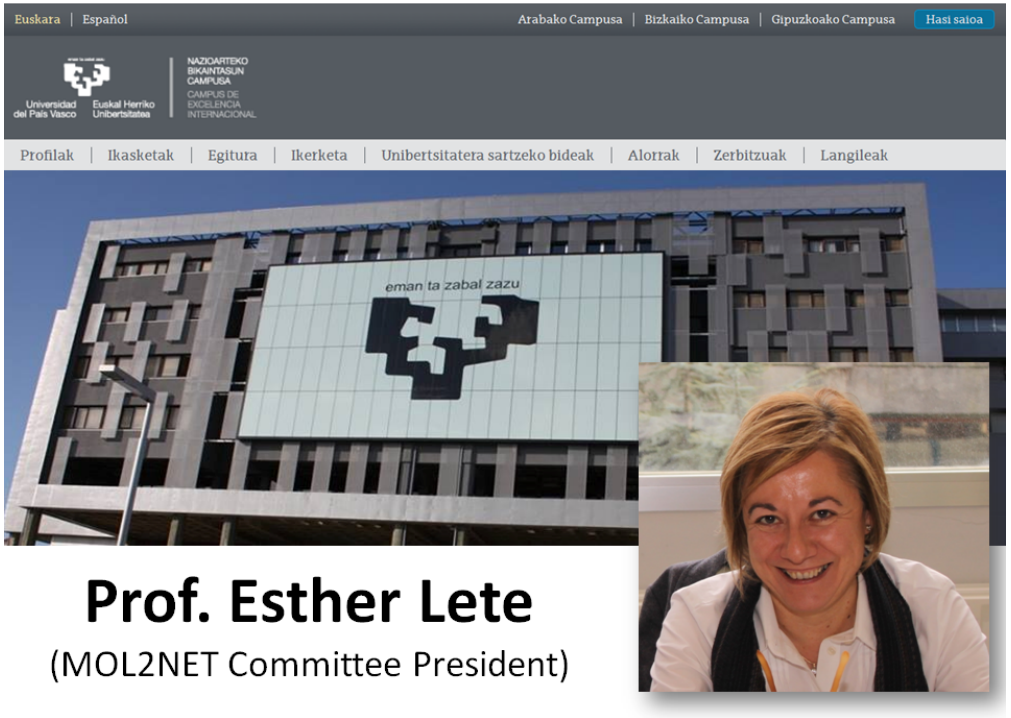
Prof. Esther Lete

Department of Organic Chemistry II,

University of Basque Country
(UPV/EHU), Leioa,

Sarriena w/n,
Bizkaia. esther.lete@ehu.eus

(Chairperson Session I)



Prof. Esther Domínguez Pérez

Dean of Faculty of Science and Technology,

Department of Organic Chemistry II,

University of Basque Country (UPV/EHU),

Leioa, Sarriena w/n, Bizkaia.

(Chairperson Session II)

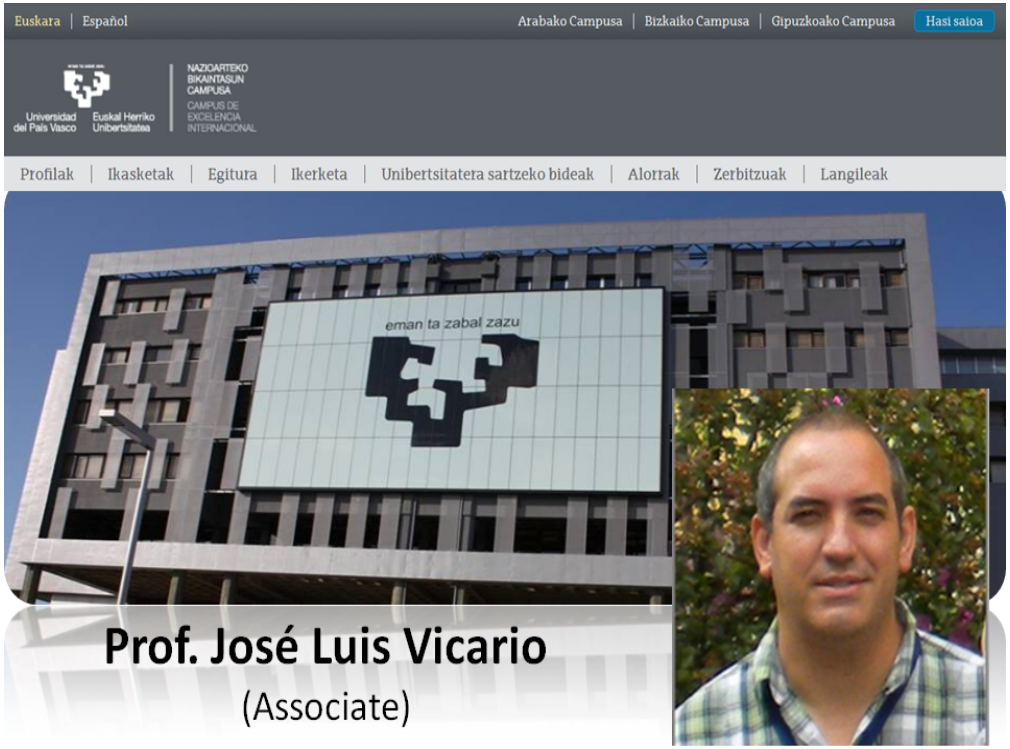
Prof. Jose Luis Vicario

Department of Organic Chemistry II,

University of Basque Country (UPV/EHU),

Leioa, Sarriena w/n, Bizkaia.

(Chairperson Session III)



UFI-QOSYC Council*

Prof. Claudio Palomo Nicolau

Director Department of Organic Chemistry I,

University of Basque Country (UPV/EHU),

Donostia - San Sebastián Campus, Gipuzkoa.

(UFI-QOSYC Council Coordinator)

Prof. Fernando P. Cossío

President of IKERBASQUE,

Basque Foundation for Science,

Prof. Department of Organic Chemistry I,

University of Basque Country (UPV/EHU),

Donostia - San Sebastián Campus, Gipuzkoa.

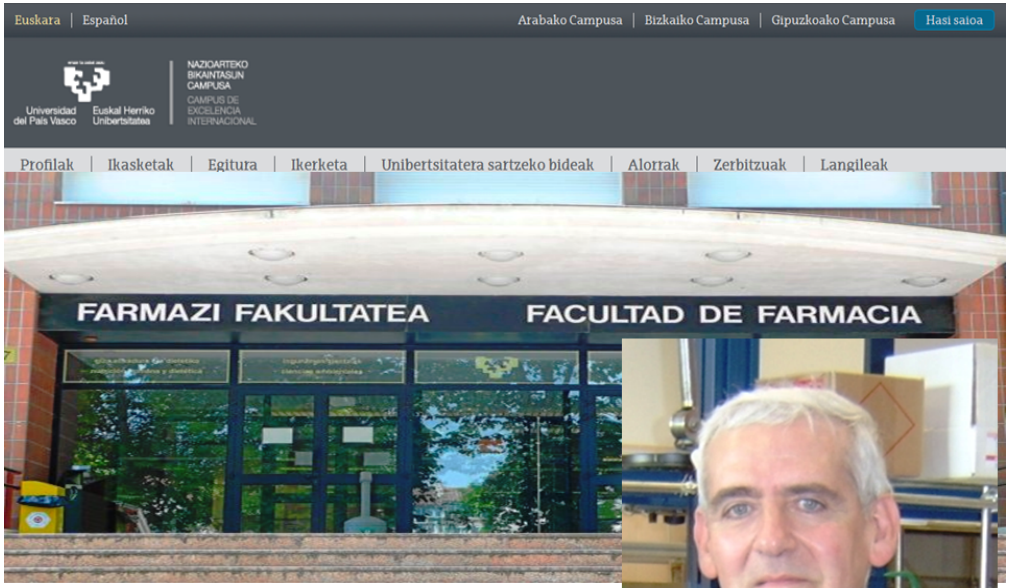
Prof. Francisco Palacios Gambra

Prof. Department of Organic Chemistry I,

Faculty of Pharmacy / Farmazi Fakultatea

University of Basque Country (UPV/EHU),

Álava Campus, Vitoria-Gasteiz.



Prof. Francisco Palacios Gambra
(UFI-QOSYC Council)

*** Professors Esther Domínguez, Esther Lete, and Jose Juis Vicario are also members of the council.**

UFI-QOSYC Research and Training groups

Asymmetric Catalysis and Chemical Synthesis Group

Lead researcher: Palomo Nicolau, Claudio
Centre: Faculty of Chemistry
Department: Organic Chemistry I

Asymmetric Synthesis Group

Lead researcher: Vicario Hernando, José Luis
Centre: Faculty of Science and Technology

Department: Organic Chemistry I

Bioorganic and Computational Chemistry Group

Lead researcher: Cossío Mora, Fernando
Centre: Faculty of Chemistry
Department: Organic Chemistry

New Synthetic Methodology Development Group

Lead researcher: Domínguez Pérez, Esther
Centre: Faculty of Science and Technology
Department: Organic Chemistry II

Organometallics in Synthesis Group

Lead researcher: Lete Expósito, Esther
Centre: Faculty of Science and Technology
Department: Organic Chemistry II

Phospho-Fluorochemistry Group

Lead researcher: Palacios Gambra, Francisco
Centre: Faculty of Pharmacy
Department: Organic Chemistry I

List of Oral communications (abstracts), Short communications (pdf)s (17)

H001 α'-Oxy Enones for Construction of All-Carbon Quaternary Stereogenic Centers: Azlactones as Pronucleophiles show abstract
by Eider Badiola , Julen Etxabe , Aitor Landa , Antonia Mielgo , Iurre Olaizola , Iñaki Urruzuno , Jesus Garcia , Jose M Odriozola , Jesus A Razquin Lizarraga , Mikel Oiarbide Garmendia , Claudio Palomo Nicolau *

H002 A more efficient entry to phenanthridinone show abstract

by Nerea Conde , Fátima Churruca , Raul SanMartin *, María Teresa Herrero , Esther Domínguez

H003 Brønsted acid catalyzed enantioselective inter and intramolecular α-amidoalkylation reactions in the synthesis of isoquinoline derivatives show abstract

by Eider Aranzamendi Uruburu *, Nuria Sotomayor , Esther Lete

H004 Enantioselective Synthesis of Chiral Proline Derivatives show abstract

by Iker Riaño , Estibaliz Diaz , Luisa Carrillo Fernández *, Jose Luis Vicario *, Efraim Reyes Martín , Uxue Uria

H005 Catalytic Enantioselective Quick Entry to Aldol-Tethered 1,6- and 1,7-Enynes and Their Synthetic Application show abstract

by Mikel Oiarbide Garmendia , Jesus Garcia , Jose M Odriozola , Jesus A Razquin Lizarraga , Irati Lapuerta , Iñaki Urruzuno *, Silvia Vera , Claudio Palomo Nicolau

H006 New Methods For Stereocontrolled Cycloaddition/Dearomatization Reactions Under Catalytic Conditions show abstract

by Aitor Lacambra , Ivan Rivilla , Fernando P. Cossío *, Stéphane Quideau

H007 **Searching for new applications of the hypervalent iodine reagents in the construction of nitrogen containing compounds** show abstract

by Izaskun Dávila Rodríguez *

H008 Intramolecular Palladium-catalyzed C-H activation reactions: Synthesis of substituted quinolones show abstract

by Verónica Ortiz de Elguea *, Nuria Sotomayor , Esther Lete

H009 Favouring Trienamine Activation through Unconjugated Dienals show abstract

by Liher Prieto , Garazi Talavera , Uxue Uria , Efraim Reyes Martín *, Jose Luis Vicario *, Luisa Carrillo Fernández

H010 **New catalytic systems for oxygen-mediated oxidative processes** show abstract

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

08011 Dimers Derived From Densely Substituted Unnatural Prolines As Precursors Of γ-Peptides And Their Use In Organocatalysis show abstract

by Maddalen Agirre , Maria de Gracia Retamosa Hernández , Andrea Ruiz-Olalla , Fernando P. Cossío *

08012 Depsipeptides and peptide-mimetics, cyclics and acyclics, integrin $\alpha_V\beta_3$ inhibitors. show abstract

by Nerea Zabala Uncilla , Claudio Palomo Nicolau *, Jose Ignacio Gamboa Landa , Jose Luis Castrillo , Silvia Avila

08013 **Intramolecular enantioselective Mizoroki-Heck reactions of 2-alkenyl (o-halobenzyl)pyrroles** show abstract

by Estibaliz Coya , Nuria Sotomayor , Esther Lete *

08014 **The Role of Pyranones in Asymmetric Organocatalytic Cascade Reactions** show abstract

by Uxue Uria , Jose Luis Vicario *, Efraim Reyes Martín *, Luisa Carrillo Fernández , Ane Orue

08015 **Propargylic-Amines and Alcohols Through *anti*-Selective Mannich and Aldol Reactions** show abstract

by Enrique Gomez-Bengoa , Jesus Garcia , Jacqueline Jiménez , Irati Lapuerta , Antonia Mielgo , Miguel Maestro , Jose M Odriozola , Mikel Oiarbide Garmendia , Itziar Otazo , Jesus A Razquin Lizarraga , Iñaki Urruzuno , Silvia Vera , Claudio Palomo Nicolau *

08016 **Synthesis and reactivity in the Michael reaction of unnatural densely functionalized Proline-derived organocatalysts** show abstract

by Andrea Ruiz-Olalla , Maria de Gracia Retamosa Hernández , Fernando P. Cossío *

08017 **Catalytic Enantioselective Synthesis of Tertiary Thiols from 5*H*-Thiazol-4-ones: Bifunctional Ureidopeptide-Based Brønsted Base Catalyst** show abstract

by Saioa Diosdado , Julen Etxabe , Joseba Izquierdo , Aitor Landa , Antonia Mielgo , Iurre Olaizola , Rosa López Alvares , Claudio Palomo Nicolau *

09: IWMEDIC04: International Workshop in Medical Informatics and Integration of Clinical Data, Coruña, Spain, 2016

IV International Workshop in Medical Imaging and Integration of Clinical Data

[Editorial Paper] [Workshop Flyer]

Workshop Description

This year the MOL2NET is the online host conference for IWMEDIC-04 (see details on Section I). IWMEDIC-04 is the IV International Workshop on Medical Imaging, Medical Coding, and Clinical Data Analysis of University of Coruña (UDC). The IWMEDIC-04 workshop will be 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). The chairman of this workshop is Prof. Alejandro Pazos; Ph.D., M.D., Chair and Director of Department of Computer Sciences, UDC, Coruña, Spain. The topics include, but are not limited to, Medical Imaging Processing, Medical Informatics, Medical Coding, Bioinformatics, Computer Aided Drug Desing, Data Analysis, *etc*. English will be the official language for online publication and presentations, as per MDPI rules, presential lectures may be in English, Spanish, or Galician.

Workshop Schedule

9:00 AM Reception, Welcome Speak, and Opening by *José Manuel Vázquez Rodríguez.*

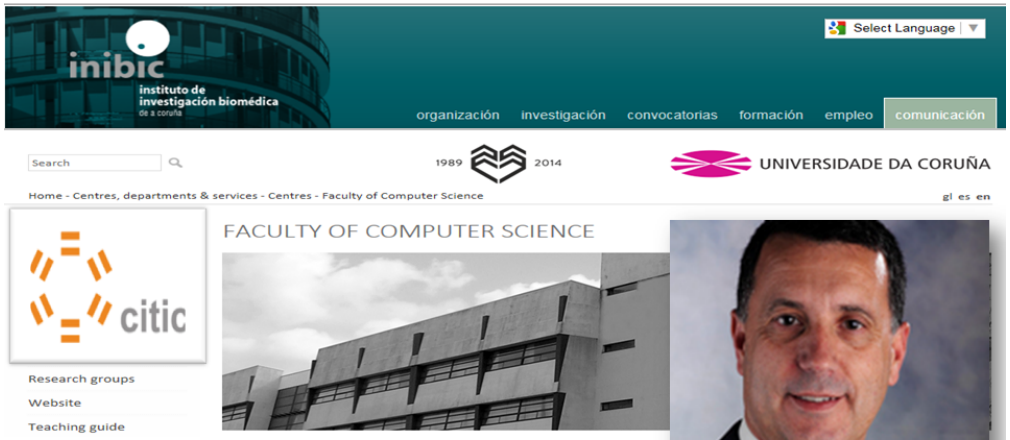
Coordinador Docencia e innovación , EOXI A Coruña

Click here to download conference schedule [pdf]

Workshop Chairman

Prof. Alejandro Pazos

Ph.D., M.D., Chair Professor of Department of Information and Communication Technologies, Faculty of Computer Sciences, University of Coruña (UDC), Coruña, Spain



Prof. Alejandro Pazos Sierra, MD. PhD.
(MOL2NET Committee President)

Department of Information and Communication Technologies UDC,
Research Center on Information and Communication Technologies (CITIC),
Medical Informatics & Radiological Diagnosis Center (IMEDIR),
Institute of Biomedical Research (INIBIC)

Scientific Committee

Dr. Francisco Blanco García

Prof. Víctor Maojo García

Dr. Antolín Rodriguez Martínez

Dr. José M. Vázquez Rodríguez

Dr. Guillermo Vázquez González

Local Committee

Prof. Julián Dorado de La Calle

Ph.D. Carlos Fernandez-Lozano

Prof. Cristian Robert Munteanu

Prof. Nieves Pedreira

Prof. Javier Pereira

Prof. Ana B Porto-Pazos

Prof. Juan R. Rabuñal

Organizing Committee

Prof. Marcos Gestal Pose

Assist. Prof. drián Carballal Mato

Ph.D. Enrique Fernández Blanco

M.Sc. Candela Rodríguez Suárez

(RNASA-IMEDIR Project Manager)

Advisory Committee

 Humberto González-DíazProf. Humberto González-Díaz

(Online Publication)

Conference Proceedings

Video Conferences Playlists

Please, visit the channel of the RANASA-IMEDIR group and/or follow the present link to visualize all the video conferences presented o this workshop [IWMEDIC playlist]. You can see these conference also on the IWMEDIC - MOL2NET shared playlist as alternative landing page [IWMEDIC mirror]

Selected Video Conferences

01. Fernández., M. Analysing the value of online health communities. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09002; doi:10.3390/mol2net-02-09002

02. Seoane, J. Models of Molecular Data Integration on Cancer. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 ; doi:10.3390/mol2net-02-03827

03. Martínez Romero, M. Using biomedical ontologies to improve metadata management in CEDAR project. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09013; doi:10.3390/mol2net-02-09013, http://sciforum.net/conference/mol2net-02/paper/3722

04. Esquerra, N. Posibilidades de Financiación en el NIH en el ámbito de la Imagen Médica e integración de datos clínicos. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09008; doi:10.3390/mol2net-02-09008, http://sciforum.net/conference/mol2net-02/paper/3658

05. Pazos Sierra, J. Integration of medical data - expert systems and virtual reality on chirurgic procedures. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09009; doi:10.3390/mol2net-02-09009, http://sciforum.net/conference/mol2net-02/paper/3660

06. Lizcano Casas, D. Preprocessing and data integration through automated reasoning techniques with descriptive logic. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09010; doi:10.3390/mol2net-02-0901

07. Acevedo Costa, C. Collaborative Environments in Medical Imaging. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09011; doi:10.3390/mol2net-02-09011

08. Rodriguez, A. RIS3 and Health. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09012; doi:10.3390/mol2net-02-09012, http://sciforum.net/conference/mol2net-02/paper/3717

09. Ruíz Romero, C. Aplications of mass spectrometry to medical imagin . *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09006; doi: 10.3390/mol2net-02-09006

10. Martinez-Sapiña Llanas, M. Virtual Colonoscopy: State of art. *In Proceedings of the MOL2NET, International Conference on Multidisciplinary Sciences*, 5 December 2016–25 January 2017; Sciforum Electronic Conference Series, Vol. 2, 2016 , 09007; doi:10.3390/mol2net-02-09007, http://sciforum.net/conference/mol2net-02/paper/3656

SPONSORS

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (14)

09001 IWMEDIC04: International Workshop in Medical Informatics and Integration of Clinical Data, Coruña, 2016 show abstract

by Julia Dorado de La Calle *, Cristian Robert Munteanu *, Humberto González-Díaz *, Alejandro Pazos *

09002 Analysing the value of online health communities show abstract

by Miriam Fernández. *

09005 BioMedical Informatics: its role in merging participatory health and personalised medicine show abstract

by Guillermo López Campos *

09006 **Aplications of mass spectrometry to medical imagin** show abstract

by Cristina Ruíz Romero *

09007 Virtual Colonoscopy: State of art show abstract

by María José Martinez-Sapiña Llanas *

09008 Posibilidades de Financiación en el NIH en el ámbito de la Imagen Médica e integración de datos clínicos show abstract

by Norberto Esquerra *

09009 Integration of medical data - expert systems and virtual reality on chirurgic procedures show abstract
by Juan Pazos Sierra *

09010 **Preprocessing and data integration through automated reasoning techniques with descriptive logic** show abstract
by David Lizcano Casas *

09011 **Collaborative Environments in Medical Imaging** show abstract
by Carlos Manuel Acevedo Costa *

09012 RIS3 and Health show abstract
by Antonio Rodriguez *

09013 Using biomedical ontologies to improve metadata management in CEDAR project show abstract
by Marcos Martínez Romero *

09014 **Identification of high affinity small molecules targeting ESR1 inhibitors for the treatment of Breast Cancer** show abstract
by Saphy Sharda , Anuraj Nayarisseri *

Models of Molecular Data Integration on Cancer show abstract
by Jose Antonio Seoane *

Advanced Techniques of Quantitative Magnetic Resonance show abstract
by Juan Antonio Hernández *

11: SUIWML01: International Workshop on Machine Learning in Biomedicine, Soochow, 2016

SUIWML2016: Soochow University International Workshop Series on Machine Learning and its Applications in Biomedicine

Submit New Communications

Dear all we are glad to welcome you to the SUIWML2016: Soochow University International Workshop Series on Machine Learning and its Applications in Biomedicine. This is one scientific conference series of the School of Computer Science and Technology of Soochow University, PCR,

China. This is workshop is also a section of MOL2NET-2.

This workshop is focus on Machine Learning. Machine learning is the most growing branch of computer science, driven by the ongoing explosion in the availability of data. Machine learning evolved from artificial intelligence and deals with many different problems and aspects to solve various tasks, including knowledge discovery, data mining, decision support and etc. A grand challenge is to discover relevant structural patterns and/or temporal patterns in complex data, which are often hidden and not accessible to the human expert.

Biomedicine is a branch of medical science that applies biological and other natural-science principles to clinical practice. The branch especially applies to biology and physiology, which has been the dominant health system for more than a century. Nowadays, the dramatic growth of medical and biological data has created an unprecedented opportunity for machine learning in the pattern recognition and machine learning community. Many medical and biological problems involve challenging approaches to pattern discovery and learning.

This workshop aims at highlighting the on-going research both the advancement of machine learning technologies and the improvements of biomedicine, and trying to bringing together researchers from the related fields to foster discussion and elicit open problems on machine learning and its applications in biomedicine. The workshop will consist of invited talks, contributed presentations, and posters. We plan to include an opening tutorial and an overview of the state-of-the-art techniques. Invited talks will be given by leading experts from both machine learning and biomedicine. We hope this workshop will not only provide an opportunity for international researchers to exchange ideas and present the latest promising work, but also create a platform to discuss and identify important future topics and directions in related fields for further research and collaboration.

This workshop is planned to be held in middle of Nov, 2016. However, the submission **is open and the publication of communications will be ASAPafter acceptance, all the year**. For more details, see Schedule & Program page and 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. After abstract approval you need to send your communication. Please, download and use the following template to write your communication SUIWML template

Be aware:

on step 1, you should select **MOL2NET 2016, International Conference on Multidisciplinary Sciences, 2nd edition (Conference),**

on step 2, you should select SUIWML2016: Soochow University International Workshop Series on Machine Learning and its Applications in Biomedicine.

Topics of particular interest include (but not limited to)

Machine Learning; Supervised learning; Unsupervised learning; Reinforcement learning; Transfer learning; Manifold Learning; Multi-task learning; Active Learning; Applications in Biomedicine; Biomedical decision support systems; Medical informatics; Bioimage mining; Medical (business/financial) intelligence; Electronic medical record (EMR) data mining; Patient (customer) relationship management; Protein structure prediction and protein fold recognition; Disease/cancer classification; Microarray data analysis

Advisory Committee

Prof. Quan Liu

Chief of Department of Software Engineering
Ph.D. Professor of Computer Sciences,
School of Computer Sciences and Technology, Soochow University (SUDA), Suzhou, China

(SUIWCS2016 Chairman)

Prof. Fangzhang Li

Ph.D. Professor of Computer Sciences
Dean of School of Computer Sciences

and Technology, Soochow University (SUDA), Suzhou, China

(SUIWCS2016 Institutional Co-Chair)

Prof. Bairong Shen

Director of Center of Complex Systems,

University of Soochow (SUDA), PRC, China.

China Coordinator of SUDA-UVP/EHU

Collaboration Agreement.

Prof. Humberto González-Díaz

IKERBASQUE Prof., Ph.D., Pharm.Lic.,

(1) Department of Organic Chemistry II,

University of Basque Country (UPV/EHU),

Campus Bizkaia, Basque Country, Spain.

(2) IKERBASQUE, Basque Foundation for Science,

Bilbao, Bizkaia, Basque Country, Spain.

humberto.gonzalezdiaz@ehu.eus

(MOL2NET2016 Conference Chairman)

(SUIWCS2016 Advisory Chairman)

Assist. Prof. Xiaoke Zhou

Assistant Professor of Computer Sciences
School of Computer Sciences and Technology, Soochow University (SUDA), Suzhou, China

PhD Fellow of Department of Organic Chemistry II, University of Basque Country UPV/EHU, Faculty of Science and Technology, Campus Bizkaia, Spain.

(SUIWCS2016 President of Organizing Committee)

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List of Proceedings (1-2 pages), Videos, Slide Presentation (ppt)s (13)

11001 **SUIWML01: International Workshop on Machine Learning in Biomedicine, Soochow, China, 2016** show abstract

by Liu Quan , Fengzhang Li , Bairong Shen , Xiaoke Zhou *

11002 **SUIWML2016: Introductory talk to Soochow University International Workshop Series on Machine Learning and its Applications in Biomedicine, by Assist. Prof. Xiaoke Zhou., Audio in Chinese (官话)** show abstract

by Xiaoke Zhou *

Machine-Learning models to predict the antioxidant capacity of food show abstract

by Estela Guardado Yordi *, Raúl Koelig , Maria Joao Matos , Yailé Caballero Mota , Eugenio Uriarte , Amaury Pérez Martínez , Lourdes Santana ,Enrique Molina

Single Trajectory Learning: Exploration VS. Exploitation show abstract

by qiming fu , quan liu , heng luo , JianPing Chen *

Adaptive Exploration in Stochastic Multi-armed Bandit Problem show abstract

by Qian Zhou , Xiaofang Zhang *, Peng Zhang , Quan Liu

Study on Optimal Control Strategy of Automatic Transmission Based on Policy Search show abstract

by Hao Wang *, Quan Liu *

Building Domain-Specific Sentiment Lexicon by Sentiment Seed Expansion show abstract

by 斌 梁 *

Video Description with Spatio-temporal Feature and Knowledge Transferring show abstract

by Xin Xu , Haibin Liu , Yi Ji , Xin Lin , Chunping Liu *

Fusing Augmented Spatio-temporal Features for Action Recognition show abstract

by Rui Ge , Xiaoyi Wan , Yi Ji , Chunping Liu *, Shengrong Gong *

Attention-based CNNs for Aspect-level Sentiment Classification show abstract

by xu jin *

Person Re-identification by Null Space Marginal Fisher Analysis show abstract

by Husheng Dong , Shengrong Gong *, Chunping Liu *, Yi Ji , Mengfei Li

Trajectory-pooled Spatial-temporal Structure of Deep Convolutional Neural Networks for Video Event Recognition show abstract

by Yonggang Li , Xiaoyi Wan , Zhaohui Wang , Shengrong Gong *, Chunping Liu *

Residual Value Iteration Algorithm based on Function Approximation show abstract

by wen Hu *

List of Accepted Abstracts (5)

Building a time-proof secure wireless sensor network through interacting the environment by efficient sampling show abstract

by Hui Wang , Fei Zhu , Quan Liu *

The Adaptive ETLBO Algorithms with K-Armed Bandit Model show abstract

by Jiaxu Cui , Yonggang Zhang *, Bingyi Sun , Dantong Ouyang

Reinforcement Learning based Dynamic Adaptive Controller show abstract

by hu lingyao *

Retinal vessel segmentation method based self-adaptive classification strategy show abstract

by Ping Jiang *, Quansheng Dou *

Single Trajectory Learning: Exploration VS. Exploitation show abstract

by QiMing Fu *

12: CIESABIO01: Workshop Series on Biotechnology and Zoonotic Diseases, Toluca, Mexico, 2016

CIESA Workshop Series on Biotechnology and Zoonotic Diseases

(CIESABIO 2016)

<< Submit a New Abstract or Contribution>>

Dear colleagues worldwide we are glad to invite you to the CIESABIO 2016, the Workshop Series on Biotechnology and Zoonotic Diseases of the CIESA, Center for Invetigations and Advanced Studies on Animal Health of the FMVZ Faculty of Medical Veterinary and Zootechnique, of the UAEMEX Autonomous University of the State of Mexico.

This workshop focus on topics related to Biotechnology, Emerging, and Zoonotics Diseases and Medical Veterinary in general, as well as topics topics from Multidisciplinar Sciences like Molecular Biology, Medicinal Chemistry, Bioinformatics, Chemoinformatics, Statistics, etc. applied to Emerging, and Zoonotics Diseases.

We welcome submissions of short proceedings (1-2 pages), posters, short communications (3-5 pages), etc. from research groups around the world. We encourage more specifically students of MSc. / Ph.D. programs to send their contributions, in particular the students of CIESA along with students of other research centers in the world.

Submissions of contributions will be open from now on until 5 December 2016, the publication of results will be asap upon acceptance by one of the chairs of the conference. The workshop runs presentially at CIESA Center, FMVZ, UAEMEX, Toluca, México. However, the online platform will be also open to worlwide participants to post comments, questions, and answers online from 5–15 December 2016 together with all other contributions submited to the MOL2NET conference. Manuscripts for the proceedings issue must have the configuration specified on the official template of Mol2Net conference (please download template from conference web or contact us).
Mol2Net Microsoft Word template file

Sincerely yours,

STEERING COMMITTEE

Ph.D. Esvieta Tenorio-Borroto, CIESA, Faculty of Veterinary Medicine and Animal Science,
Autonomous University of the State of Mexico, Toluca, 50090, Mexico.
(CIESABIO 2016 Chairperson), etenoriob@uaemex.mx

Prof. Juan Carlos Vázquez Cagoyán, CIESA, Faculty of Veterinary Medicine and Animal Science,
Autonomous University of the State of Mexico, Toluca, 50090, Mexico.
(CIESABIO 2016 Chairperson)

Prof. Simón Martínez Castañeda, CIESA, Faculty of Veterinary Medicine and Animal Science,
Autonomous University of the State of Mexico, Toluca, 50090, Mexico.
(CIESABIO 2016 Chairperson)

Prof. Humberto González-Díaz, IKERBASQUE Prof., Ph.D., Pharm.Lic.,
Department of Organic Chemistry II, University of Basque Country (UPV/EHU), Campus Bizkaia, Basque Country, Spain.
IKERBASQUE, Basque Foundation for Science, Bilbao, Bizkaia, Basque Country, Spain.
(MOL2NET Chairman), humberto.gonzalezdiaz@ehu.eus

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (9)

12003 **Design and Evaluation of cruzipain gene using *Saccharomyces cerevisiae* as a vaccine vector against *Trypanosoma cruzi* experimental infection** show abstract

by Wael Hegazy *, Juan Carlos Vázquez Chagoyán *, Alberto Barbabosa Pliego , Esvieta Tenorio Borroto

12004 Immunohistochemestry vs. Immunofluorescence: Comparative analysis via software of total colorimetric reaction of GPR43 protein in adipose tissue show abstract

by Silvia Gisell Vega-Damián , Eugenio Torres-García , Rigoberto Oros-Pantoja *, Jorge Luis De-la-Rosa-Arana , Daniela Rodríguez-Muñoz , Elia Yadira Peniche-Moreno

An approach to *Trypanosoma cruzi* Vaccine through the epitope prediction from proteins surface with IEDB show abstract

by Gilberto Yong Macias , Viridiana Camacho Sierra , ESVIETA TENORIO *

Coenurosis an emerging disease in wild rabbits show abstract

by Benjamin Valladares-Carranza *

Immune protection against *Trypanosoma cruzi* induced by TcVac1 vaccine in a murine model using an intradermal/electroporation protocol show abstract

by Wael Hegazy *, Alberto Barbabosa Pliego , Esvieta Tenorio Borroto , Juan Carlos Vázquez Chagoyán *, José Guillermo Estrada-Franco , José Antonio Zepeda-Escobar , Laucel Ochoa García , José Esteban Aparicio-Burgos

Evaluation of animal welfare during transport of sheep for slaughter. show abstract

by miguel angel pulido *

***Cryptosporidium spp.* Frequency identified through Zn staining in lambs from Michoacán, México.** show abstract

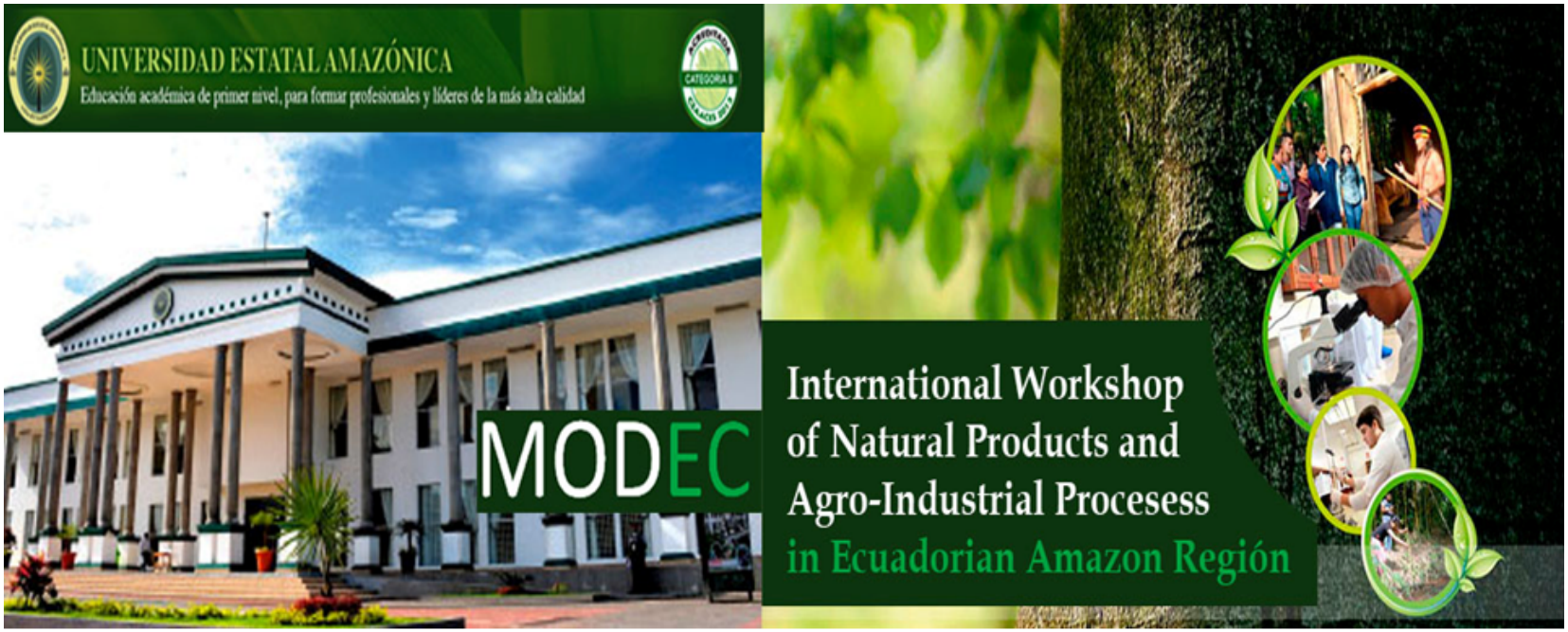
by María Uxúa Alonso Fresán *, Ana Dalia Ocaña-Soto , Soledad Díaz-Zarco , Alberto Barbabosa-Pliego , Juan Carlos Vázquez-Chagoyán ,Benjamín Valladares-Carranza , Valente Velázquez-Ordoñez

List of Accepted Abstracts (1)

Identification of *Trypanosoma cruzi* in three species of marsupials (*Philander opossum , Didelphis marsupialis, and Didelphis virginiana*) in the bio-reserve of "El Zapotal ", Tuxtla Gutiérrez, Chiapas, Mexico. show abstract

by viridiana camacho *

13: MODEC01: Workshop on Natural Products and Agro-Industrial Procesess in Amazon, Ecuador, 2016



[Workshop Editorial & Full Committee]

MODEC 2016 (Submissions Open)

SUBMIT TO MODEC HERE!!!

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 includes the application of Information and Communications Technologies (ICTs) for data analysis and computational model including the fields of Agro-industrial Engineering, Chemistry, Chemical Engineering, Biotechnology, Veterinary Medicine, and/or Environmental Sciences, *etc*.

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 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

All summaries should be sent no later than November 25, 2016. The acceptance of a publication will be given December 15, 2016 and completed papers need to be submitted by January 15, 2017. Final acceptance of the finished paper will be given on January 30, 2017 and papers will be published on February 1, 2017. 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]

Chairperson

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 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

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (22)

13001 MODEC2017, International Workshop on the Natural Products and Agro-Industrial Processes in Ecuadorian Amazon region show abstract
by Amaury Pérez Martínez *, Oscar Miguel Rivera-Borroto , Gerardo M. Casañola-Martín , Karel Dieguez Santana

13002 Food sources and emerging methods to obtain Ellagic Acid show abstract
by Estela Guardado Yordi *, Amaury Pérez Martínez , Reinaldo Demesio Alemán Pérez , Enrique Molina , Eugenio Uriarte , Lourdes Santana ,Orlando A. Abreu , Maria Joao Matos

Relationship between cattle and age nematodes in the Ecuadorian Amazon. show abstract
by Juan Carlos Moyano Tapia *, Andrea Riofrio , Juan Carlos López , Roberto Quinteros , Julio Cesar Vargas , Pablo Roberto Marini

Antimicrobial peptides of *Lactobacillus plantarum* UTNCys3.4 isolated from native fruits of Ecuadorian Amazonia inhibit the growth of foodborne pathogens show abstract
by Gabriela N Tenea *, Karina Garzón , Alejandro Barrigas , Clara Ortega

Ilex guayusa: A systematic review of its Traditional Uses, Chemical Constituents, Biological Activities and Biotrade Opportunities show abstract
by Matteo Radice *, Laura Scalvenzi , Neyfe Sablón Cossio

Environmental impact of livestock systems in the Ecuadorian Amazon show abstract

by Carlos Alfredo Bravo Medina *, Bolier Torres , Daysi Changoluisa , Haidee Marín , Reinaldo Alemán , Roldán Torres

Relationship between two species of age and cattle protozoa in the Ecuadorian Amazon show abstract

by Juan Carlos Moyano Tapia *, Juan Carlos López , Roberto Quinteros , Julio Cesar Vargas , Andrea Riofrio , Pablo Roberto Marini

Agricultural production units (APU's) at amazon region of Ecuador, Pastaza province and diversified farm as efficient production system show abstract

by Reinaldo Aleman *, Jorge Freile Almeida , Miguel Angel Iparraguirre , Roldan Torres , Carlos Bravo , Eufemia Caballero

The milk industry seen from the farms of producers in the Ecuadorian Amazon. show abstract

by Alina Ramìrez Sànchez *, María Isabel Viamonte Garcés2 , Diocle Benítez Jiménez , Verena Torres Cárdenas

Quantification and characterization of native microorganisms under contrasting rainforest environment in Ecuadorian Amazon show abstract

by Roldán Torres Gutiérrez *, Carlos Alfredo Alfredo Bravo Medina , Reinaldo Demesio Alemán Pérez , Jorge Freile , Tania Paulina Ramos Ramos ,María José José Chiliquinga Rodríguez , Daniela Elizabeth Elizabeth Marizande Lozada , Danny Javier Guevara Llerena

Low productivity and quality of the primary link of the cattle production chain as an input for the industry in the Ecuadorian Amazon Region. show abstract

by María Isabel Viamonte Garcés *, Diocles Benítez Jímenez , Alina Ramírez Sánchez , Verena Torres Cardenas

Amazonia, healthy food and rural communities, Pastaza-Ecuador. show abstract

by Manuel Lázaro Pérez Quintana *, Ruth Irene Arias Gutiérrez , Neyfe Sablón Cossio

Ecological models: A management tool of promising species with biomass potential in the Ecuadorian Amazon. show abstract

by Yudel Garcia Quitana *, Yasiel Arteaga Crespo , Reinier Abreu Naranjo , Bolier Torres Navarrete , María de Decker

Diagnosis of the behavior of the African snail (*Lissachatina fulica*) by means of its mucous membrane interspecies communication vector show abstract

by Grace Catalina Navarrete-Naranjo , Karel Diéguez-Santana *, Edgar Ruben Chicaiza Reisancho *, Pedro Damian Ríos-Guayasamin , Oscar M Rivera-Borroto , Raul Valverde , Jessy Paulina Guerrero Rubio , Bartlomiej Goldyn

Impacts of exploitation of stone in the River Tena, Napo province, Ecuador. show abstract

by Karel Diéguez-Santana *, Liliana Bárbara Sarduy-Pereira , Amaury Pérez-Martínez , Gerardo M. Casañola-Martin , Víctor Felipe Gualinga Santi ,Lilian Paola Bravo Vásquez , María Jasmina Sánchez Rivadeneria , Melania Verónica Huatatoca Chimbo , William Alcides Izurieta Maza

Nestedness between aphids and parasitoids populations in plants associated with an organic citrus grove show abstract
by CAROLINA BAÑOL *, NICOLAS PÉREZ , JOSEP PIÑOL , JOSE ANTONIO BARRIENTOS

Traditional use of plants as antihypertensive in Jipijapa, Manabí. Comparison with the literature. show abstract
by Maria del Rosario Velázquez Herrera *, Orelvis Palmero Rodríguez , Lorena Cortiñas Torregrosa , Maribel Celi Paucar Vásquez , Eduardo Faramiñán Blanco , Liliana Macías Calderón

Design of an industrial process focused on the elaboration of cosmetics using Amazonian vegetal oils: a biotrade opportunity show abstract
by Diana Paulina Romero *, Angel Freire , Fernanda Elizabeth Aillon , Matteo Radice

Study of the functional properties of the corn flour proteins (*Zea mays*), barley (*Hordeum vulgare*), quinoa (*Chenopodium quinoa*), potato (*Solanum tuberosum)*, and wheat (*Triticum aestivum*) national and imported intended for use in baking and noodles show abstract
by Liliana Cerda-Mejía *, Víctor Rodrigo Cerda Mejía , Galo Aníbal Sandoval Chasi

Influence of the uncertainty of the operational parameters in obtaining cane syrup in sensorial attributes show abstract
by Víctor Rodrigo Cerda Mejía *, Walter Francisco Quezada Moreno , Amaury Pérez-Martínez , Hilda Oquendo-Ferrer , Verena Torres , Liliana Cerda-Mejía , Erenio González Suárez

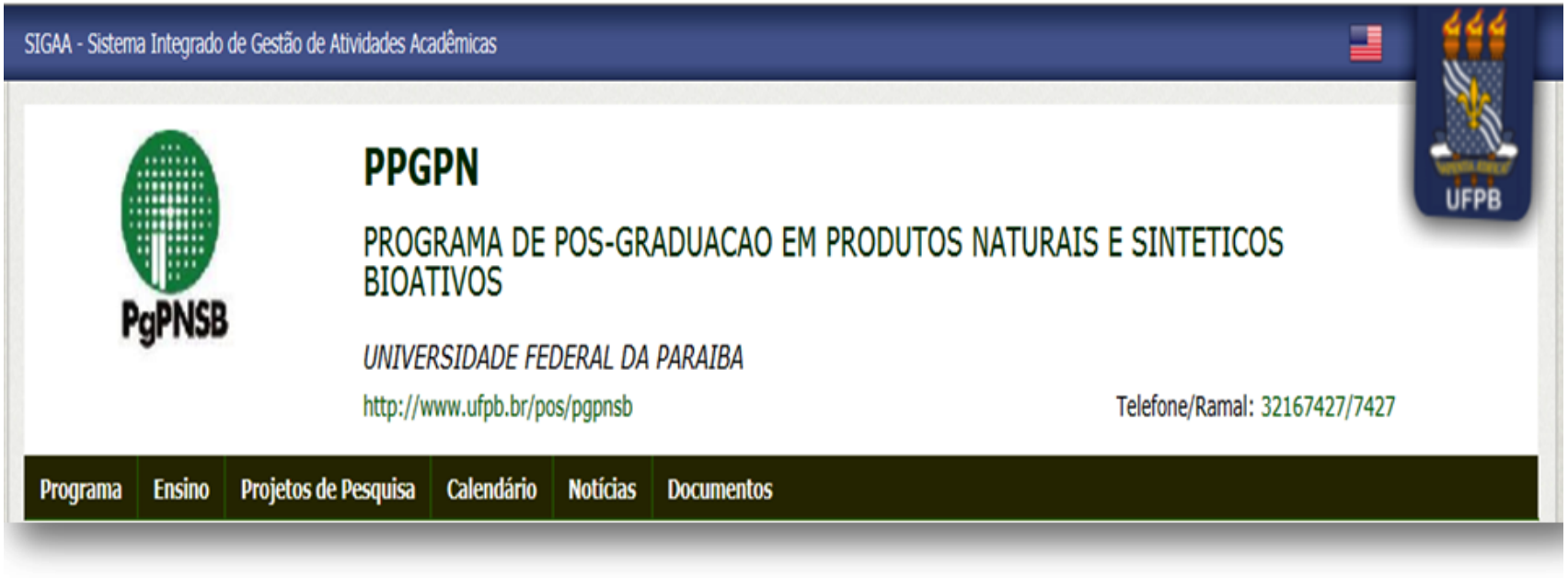
Isolation of native *Aspergillus niger* from Ecuadorian Amazon to produce citric acid from sugarcane bagasse. show abstract
by Henrry Tuquerres *, Aldo Carrera , Shirley Pomavilla , Viviana Tenemaza , Gladis Casco , Andrea Piedra , Daysi Changoluisa , Luis Bravo , Karel Diéguez-Santana , Karina Carrera , Roldan Torres

Thyme and rosemary essential oils as an alternative control of plant-parasitic nematodes. show abstract
by Diana Iler-Iler , Gabriel Moreno-Toasa , Roman Rodríguez-Maecker , Mirari Arancibia *

List of Accepted Abstracts (1)

Macroscopic model for Bacillus licheniformis growth in a sucrose-based medium. show abstract
by Mariela Rizo-Porro *, Nemecio González-Fernández , Luis B. Ramos-Sánchez

14: WRSAMC01: Workshop in Research Sciences Applied to Medicinal Chemistry, Paraiba, Brasil, 2016



WRSAMC2016: Workshop in Research Sciences Applied to Medicinal Chemistry

Submit New Communications

Dear all we are glad to welcome you to the workshop in research sciences applied in medicinal chemistry 2016, 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 is workshop also is associated to MOL2NET-2 International Conference on Multidisciplinar 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 05-09 December 2016, online via the platform SciForum.

The online submission is now **open and the publication of communications will be ASAP after acceptance, all the year**.

Paper submission: **November 27, 2016**

Paper acceptance: **November 29, 2016**

For more details, contact the chairs of the workshop and to submit a communication use the Submission link.

After you successfully register, you can submit your paper online. Communications are expected to be short papers 1-3 pages **You need to register and send your abstract first**. **After abstract approval you need to send your communication**. Please, donwload and use the following template WRSAMC template to submit your communication.

Be aware:

on step 1, you should select **MOL2NET 2016, International Conference on Multidisciplinary Sciences, 2nd edition (Conference).**

on step 2, you should select WSRAMC2016 workshop in research sciences applied in medicinal chemistry.

Workshop Chairs

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

Prof. Luciana Scotti, Hospital Universitário, Universidade Federal da Paraíba - Campus I, Cidade Universitária, CEP: 58.051-900, João Pessoa - Paraíba - Brasil. Emails: luciana.scotti@gmail.com

Local Committee

Prof. Wallace Duarte Fragoso, 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.

Prof. Francisco Jaime Bezerra Mendonça Junior, Centro de Ciências Biológicas e Sociais Aplicadas, Universidade Estadual da Paraíba, Campus V - João Pessoa. Rua Horácio Trajano de Oliveira s/n, CEP>, 58020540 - João Pessoa, Paraíba – Brasil

Prof. Fabio Correia Sampaio, Departamento de Odontologia Clínica e Social, Centro de Ciências da Saúde Universidade Federal da Paraíba, Campus I, Cidade Universitária, CEP: 58.051-900, João Pessoa - Paraíba - Brasil.

Prof. Emidio Vasconcelos Leitão da Cunha, Departamento de Farmácia, Centro de Ciências Biológicas e da Saúde, Campus I, 58429-500, Campina Grande, Paraíba – Brasil

Dr. Vicente Carlos de Oliveira Costa, Instituto de Pesquisa em Farmacos e Medicamentos, Universidade Federal da Paraíba - Campus I, Cidade Universitária, CEP: 58.051-900, João Pessoa - Paraíba – Brasil.

Prof. Mateus Feitosa Alves, Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal da Paraíba - Campus I, Cidade Universitária, CEP: 58.051-900 João Pessoa - Paraíba – Brasil.

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (6)

14004 **Alkaloids Menispermaceae family: a new source of compounds selective for beta-adrenergic receptors** show abstract

by Mateus Feitosa Alves *, Marcus Tullius Scotti *, Luciana Scotti *, Sócrates Golzio dos Santos *, Margareth de Fátima Formiga Melo Diniz *

Aactions of Angiotensin-(1-7) in cardiomyocite proliferation and cardiac regeneration show abstract

by Breno Feitosa da Silva *, ENEAS RICARDO DE MORAIS GOMES

Ligand-based virtual screening of a benzylisoquinoline alkaloids dataset with anti-inflammatory potential activity. show abstract

by Chonny Herrera Acevedo , Luciana Scotti , Mateus Feitosa Alves , Margareth F.F.M. Diniz , Marcus Tullius Scotti *

List of Accepted Abstracts (3)

Determination of phenolic constituents in *Hyptis crenata* (Pohl) ex Benth. By high performance liquid chromatography coupled to diode array detectors show abstract

by Ana Rita Rodrigues *

Identification of Phenolic constituents of *Mentha x villosa* by HPLC-DAD show abstract

by Ramon Lima *

Determination of phenolic constituents in Rhaphiodon echinus (Nees & Mart.) Schauer by CLAE-DAD. show abstract

by jociano silva lins *

16: EMBS01: Eminent Biosciences Workshop on Bioinformatics, Indore, India, 2016

EMBS2016: Eminent Biosciences Workshop on Bioinformatics

Submit New Communications

Dear all we are glad to welcome you to the workshop on Bioinformatics of the laboratory Eminent Biosciences.

This workshop deal with applications of Bioinformatics and Data Analysis algorithms to Bioimolecular sciences and Biomedicine. This workshop is planned to be held in middle of Nov, 2016. However, the submission **is open** and the **publication of communications will be ASAP after acceptance, all the year**. For more details, please contact the chairperson of this workshop and 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. After abstract approval you need to send your communication.

Be aware:

on step 1, you should select **MOL2NET 2016, International Conference on Multidisciplinary Sciences, 2nd edition (Conference),**

on step 2, you should select **EMBS2016: Eminent Biosciences Workshops on Bioinformatics**

Topics of particular interest include (but not limited to)

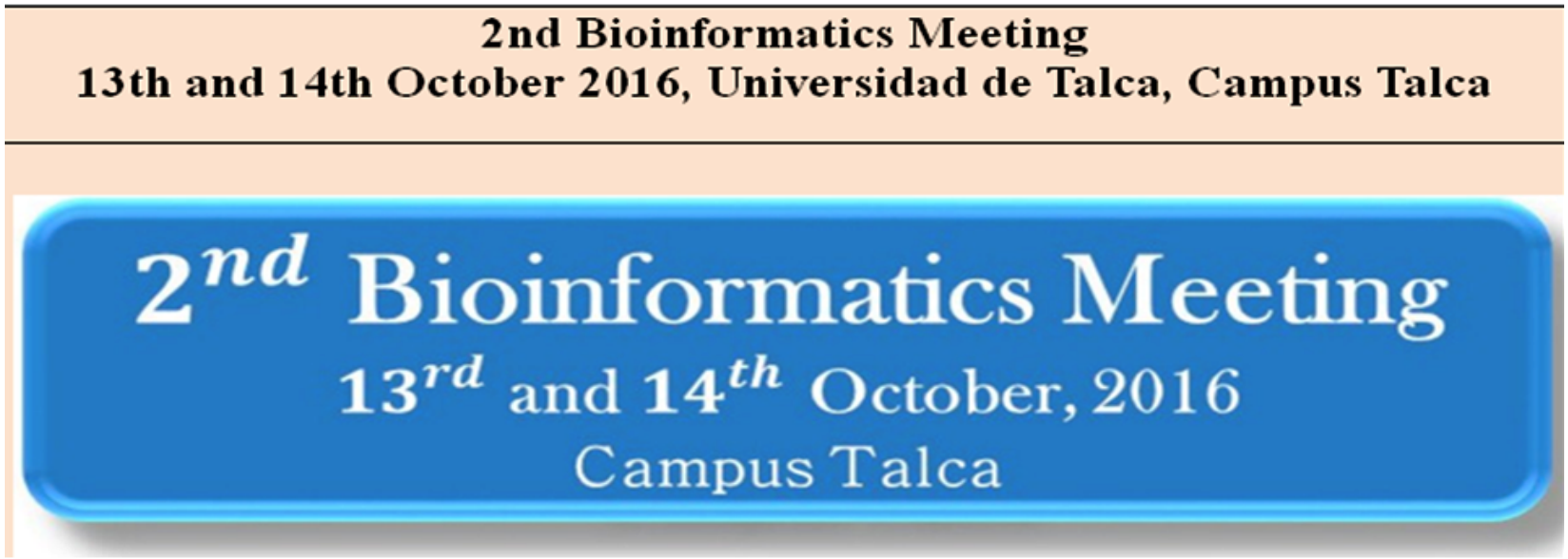
Machine Learning; Bioinformatics; Cheminformatics; Medical Informatics; Applications in Biomedicine; Biomedical decision support systems; Microarray data analysis, Next-Generation Sequencing (NGS) data analysis, etc.

Advisory Committee

Workshop Chairperson:

Dr. Anuraj Nayarisseri, Principal Scientist, Eminent Biosciences, Opp Meghdoot Garden, Shekhar Tower, First Floor, Vijaynagar, Indore - 452010, Madhya Pradesh, India. Email: anuraj@eminentbio.com

17: BMEICB-02 Second Bioinformatics Meeting of The School of Bioinformatics Engineering, Talca, Chile, 2016



Submit New Communications

On behalf of the School of Civil Engineering in Bioinformatics and the Center of Bioinformatics and Molecular Simulation (CBSM), it is our great honour to invite you to join the 2nd Meeting on Engineering in Bioinformatics, which will be held on the 13rd to 14th of October, 2016 at University of Talca in Talca, Chile.

In 2003, the University of Talca created the career “Engineering in Bioinformatics” (this year updated to “Civil Engineering in Bioinformatics”), which is a multidisciplinary career with a confluence of Computer Sciences, Biology, Chemistry, Physics, Mathematics, and Administration. It was the first career in Chile and Latin America oriented to train professionals with expertise in Bioinformatics and informatics skills applied to solve scientific problems, driven by new challenges of science and the growing need for professionals who are able to understand phenomena and biological processes, addressing their studies through the development of computational tools.

At present, more than 120 Engineers in Bioinformatics have graduated at University of Talca. They have abilities for simulating biomolecular systems and processing biological data. Many of them are working in scientific projects where they provide their knowledge about computational methods to support experimental research in Biological and Chemical research, Agriculture, Pharmaceutics, Biotechnology, etc.

This meeting is for Engineers in Bioinformatics in Talca: for graduate students, and for those who are still studying. This meeting showcases the latest research of our graduated students in all areas of bioinformatics. We also have visitor lecturers that come to share their current research topics. As the organizers, we sincerely hope that all of you will join us for what will definitely become a memorable meeting.

Be aware:

on step 1, you should select **MOL2NET 2016, International Conference on Multidisciplinary Sciences, 2nd edition (Conference),**

on step 2, you should select CBSMW01: Fisrts Worskhop of Center of Bioinformatics and Molecular Simulation**, Talca, Chile, 2016**

*Note: Doubts and queries write to ganunez@utalca.cl or jcaballero@utalca.cl

Advisory committee

Prof. Gabriel Nuñez

Prof. Julio Caballero (connection with MOL2NET conference)

Workshop Chairmans

Hector Urbina

Marcelo Rojas

Secretariat: Claudia González

TI support: Fabio Duran

UTALCA Bionformatics Engineering flyer

Video: [link], Audio: Spanish min 00:00, English min 02:45

List of Participants

* Dr. Samuel Ortega-Farías, Universidad de Talca

* Dr. Vinicius Maracaja-Coutinho, Universidad Mayor

* Dra. Gabriela Repetto, Clinica Alemána.

* Ing. Marcelo Rojas, BeagleBioinformatics

* Dr. Cesar Astudillo, Universidad de Talca

* Ing. Mg. Aucan Pedroso Rovira, Independiente

*Ing. Constanza Campano, Instituto de Salud Pública

* Ing. Dr(c) Raul Arias, Universidad Mayor

* Ing. Javier Romero, Independiente

*José Cuevas V., PhD., Viña Concha y Toro

*Héctor Urbina, Ing. En Bioinformática

*Miguel Reyes Parada,

*Aldo Acevedo, Ing. En Bioinformática

*Gonzalo Muñoz, Ing. En Bioinformática

*Camila Rojas, Estudiante de Ing. En Bioinformática

*Matías Fuentes, Estudiante de Ing. En Bioinformática

*Iñaki Hojas, Estudiante de Ing. En Bioinformática

*Camilo Fuentes, Estudiante de Ing. En Bioinformática

*Luis Letelier, Estudiante de Ing. En Bioinformática

List of Abstract, Short Proceedings Paper Papers (DOC, DPF), Presentation, Poster, (PPT, PDF)s (12)

by Carlos Catalan , Gabriel Nuñez-Vivanco , Pablo Moya *

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α'-Oxy Enones for Construction of All-Carbon Quaternary Stereogenic Centers: Azlactones as Pronucleophiles

Eider Badiola, Julen Etxabe, Aitor Landa, Antonia Mielgo, Iurre Olaizola, Iñaki Urruzuno, Jesús García, José M. Odriozola, Jesús Razkin, Mikel Oiarbide, and Claudio Palomo*

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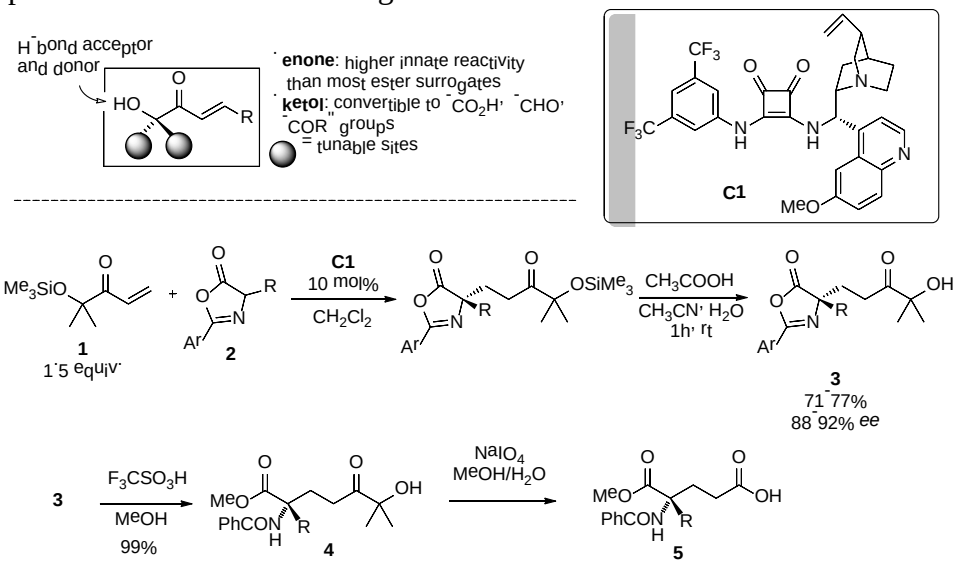
Studies in our group have shown that α'-oxy enones react in the presence of Brønsted base catalysts with 3-substituted oxindoles, cyanoesters, 5*H*-oxazol-4-ones, 1*H*-imidazol-4-(5*H*)-ones and azlactones to give the corresponding 1,5-dicarbonyl Michael adducts with a fully substituted carbon center in high enantioselectivity. For example, the reaction between azlactones **2** and enone **1** is efficiently promoted by catalyst **C1** to led, after desilylation, to the corresponding products **3** with good yields and *ee*'s. In each case, reactions proceed with high site selectivity and no products from reaction at the C2-possition of the azlactone ring are observed.[1]



Besides their utility for the installation of aldehyde and ketone functionality, α'-oxy enones, through simple oxidative cleavage of the ketol moiety in the resulting adducts, act as α-β-unsaturated carboxylic acid surrogates for which successful methodologies are notably deficient.

[1] J. S. Fisk, R. A. Mosey, J. J. Tepe, *Chem. Soc. Rev.* **2007**, *36*, 1432-1440.

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A more efficient entry to phenanthridinones

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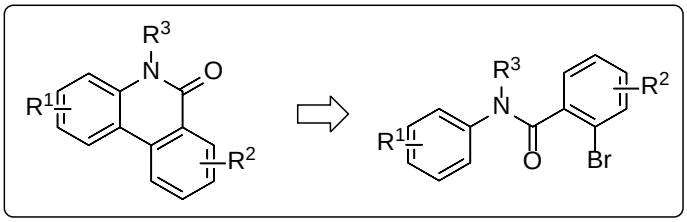
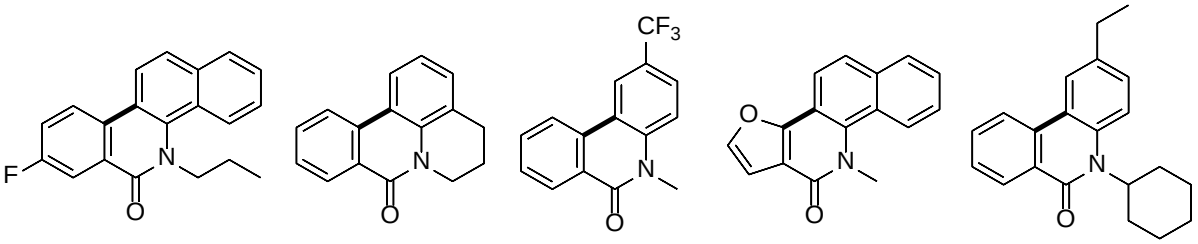
The presence of the phenanthridinone core in natural products and biologically active compounds has encouraged research on more efficient approaches to such valuable tricyclic framework.[1] Due to the lack of transmetallating agents and the atom-economy implied, palladium-catalyzed direct arylation is an appealing alternative for the ring closure step. However, the relative high amount of the catalyst employed may become a serious drawback from a practical view.[2] In this context, we wish to present the application of a palladacyclic complex to this reaction and the significant reduction of the catalytic amount achieved (0.05 mol%) in the successful preparation of a series of phenanthridinone derivatives.





78% 80% 73% 98% 86%

[1] Bhakuni, B. S.; Kumar, A.; Balkrishna, S. J.; Sheikh, J. A.; Konar, S.; Kumar, S. *Org. Lett.* **2012**, *14*, 2838-2841.
[2] Rousseaux, S.; Gorelsky, S. I.; Chung, B. K. W.; Fagnou, K. *J. Am. Chem. Soc.* **2010**, **132**, 10692-10705.

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Brønsted acid catalyzed enantioselective inter and intramolecular α-amidoalkylation reactions in the synthesis of isoquinoline derivatives

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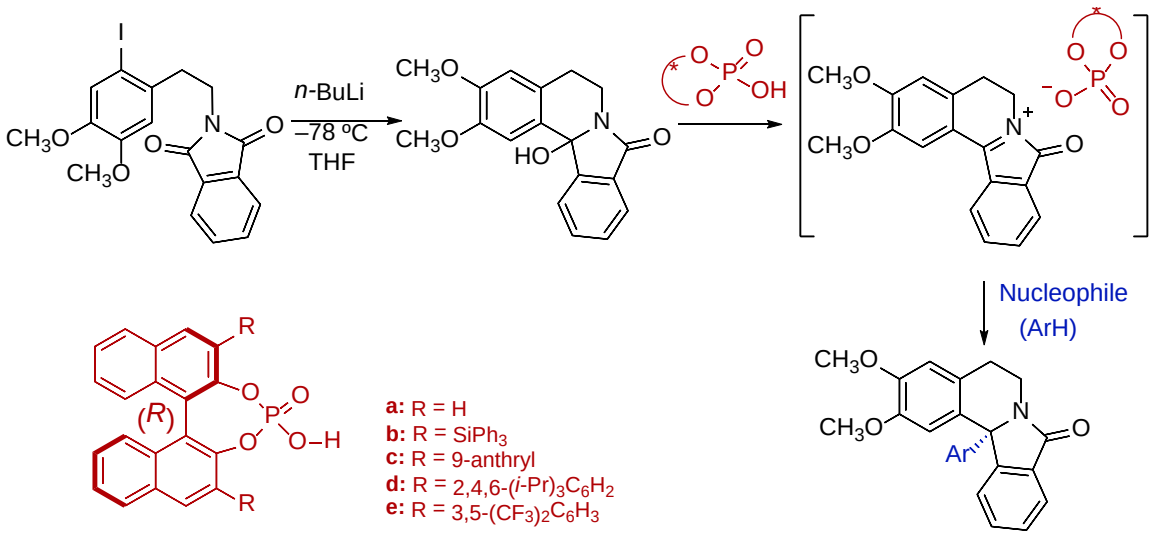
The α-amidoalkylation reaction of aromatic systems using *N*-acyliminium ions as electrophiles is one of the most attractive methods for C-C bond formation in heterocyclic chemistry and has found widespread application in natural products synthesis.[1] A significant progress in the application of enantioselective versions of α-amidoalkylation reactions has been marked by the development of chiral Brønsted acids (mainly BINOL derived phosphoric acids) and hydrogen bond donors (mainly ureas and thioureas). In this context, we have shown that BINOL-derived chiral Brønsted acids catalyze the intermolecular α-amidoalkylation of a bicyclic α-hydroxylactams, obtained by Parham cyclyzation of the corresponding *N*-phnethylimides, with indole derivatives. Thus, a convenient enantioselective synthesis of 12b-substituted isoindoloisoquinolines (*ee* up to 95 %) has been achieved.[2]



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[1] Martínez-Estibalez, U.; Gómez-SanJuan, A.; García-Calvo, O.; Lete, E.; Sotomayor, N. *Eur. J. Org. Chem.* **2011**, 3610
[2] Aranzamendi, E.; Sotomayor, N; Lete,. E. *J. Org. Chem.* **2012**, *77*, 2986.

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Enantioselective Synthesis of Chiral Proline Derivatives

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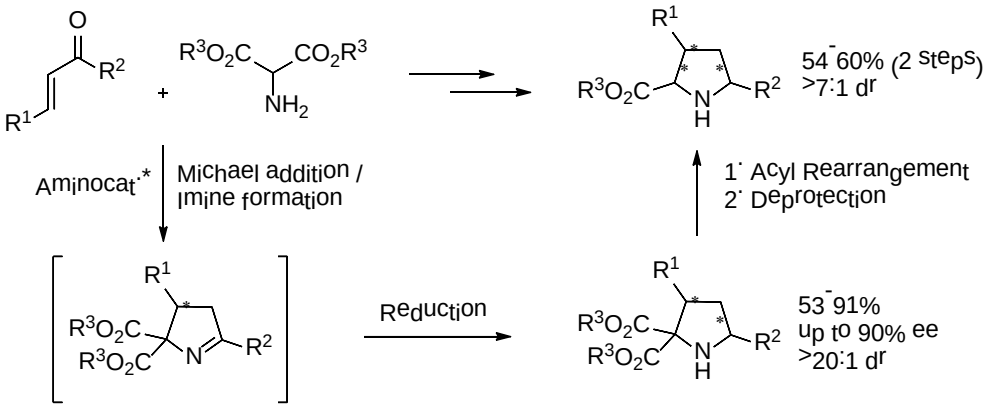
The pyrrolidine framework is present as key structure in many natural products with interesting biological and pharmaceutical activities.[1] It is also used in organic chemistry playing different roles such as ligand, organocatalyst or building block in chiral pool synthesis.[2] Furthermore, these properties are very often influenced by the configuration of the stereogenic center present in the molecule. For this reason, new and efficient routes are required to synthesize chiral proline derivatives in a stereocontrolled way. With this in mind, our group has established a good approach to this scaffold employing as key steps an organocatalytic cascade process based on a Michael addition/imine formation sequence and a novel base-promoted rearrangement reaction (Scheme 1). Therefore, the reaction between enones and aminomalonates has been studied using a chiral primary amine as catalyst, due to the known ability of the latter to activate α,β-unsaturated ketones as Michael acceptors under iminium ion formation.[3] A sequential diastereoselective reduction leads to enantiopure 1,3-disubstituted pyrrolidines in good yield and enantioselectivity, which are transformed into the desired trisubstituted proline derivatives through a base-promoted rearrangement/deprotection reactions under mild conditions.



Scheme 1

[1] For selected reviews, see: (a) Nair, V.; Suja, T. D. *Tetrahedron* **2007**, *63*, 12247; (b) Hanessian, S. *ChemMedChem* **2006**, *1*, 1300; (c) Pyne, S. G.; Tang, M.-Y. *Curr. Org. Chem.* **2005**, *9*, 1393; (d) Liddell, J. R. *Nat. Prod. Rep.* **2002**, *19*, 773; (e) Sardina, F. J.; Rapoport, H. *Chem. Rev.* **1996**, *96*, 1825.
[2] For selected reviews, see: (a) Mukherjee, S.; Yang, J. W.; Hoffmann, S.; List, B. *Chem. Rev.* **2007**, *107*, 5471; (b) Erkkilä, A.; Majander, I.; Pihko, P. M. *Chem. Rev.* **2007**, *107*, 5416; (c) Berkessel, A.; Gröger, H. *Asymmetric Organocatalysis*; Wiley-VCH: Weinheim, 2005.
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Catalytic Enantioselective Quick Entry to Aldol-Tethered 1,6- and 1,7-Enynes and Their Synthetic Application

Mikel Oiarbide, Jesús M. García, José M. Odriozola, Jesús Razkin, Irati Lapuerta, Iñaki Urruzuno, Silvia Vera and Claudio Palomo

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Herein we present an effective asymmetric route to functionalized 1,6- and 1,7- enynes based on a direct cross-aldol reaction between ω-unsaturated aldehydes and propargylic aldehydes (α,β-ynals) promoted by combined α,α-dialkylprolinol ether/Brønsted acid catalysis. This synergistic activation strategy is a key to access the corresponding aldol adducts with high enantio- and diastereoselectivity.[1] The aldol reaction also proceeds well with propargylic ketones (α,β-ynones) thus enabling a stereocontrolled access to the corresponding tertiary alcohols. The utility of these adducts, which are difficult to prepare through standard methodology, is demonstrated by their transformation into trisubstituted bicyclic enones using standard Pauson-Khand conditions.

1. E. Gomez-Bengoa, J. M. García. S. Jimenez, I. Lapuerta, A. Mielgo, J. M. Odriozola, M. Oiarbide, I. Otazo, I. Urruzuno, S. Vera, C. Palomo. *Chem. Sci. 2013, 4*, 3198-3204.

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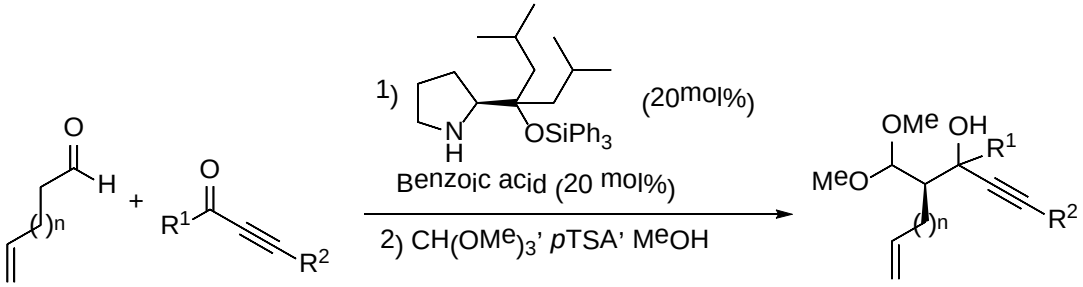

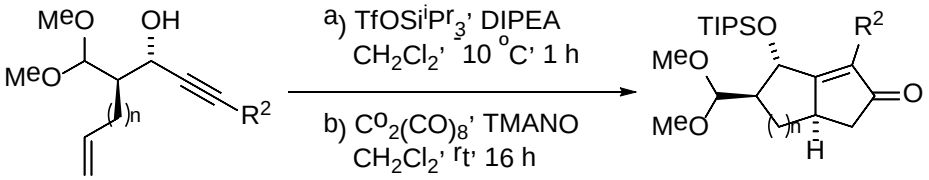

R1: H, CO_2Me
n = 1, 2

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New Methods For Stereocontrolled Cycloaddition/Dearomatization Reactions Under Catalytic Conditions

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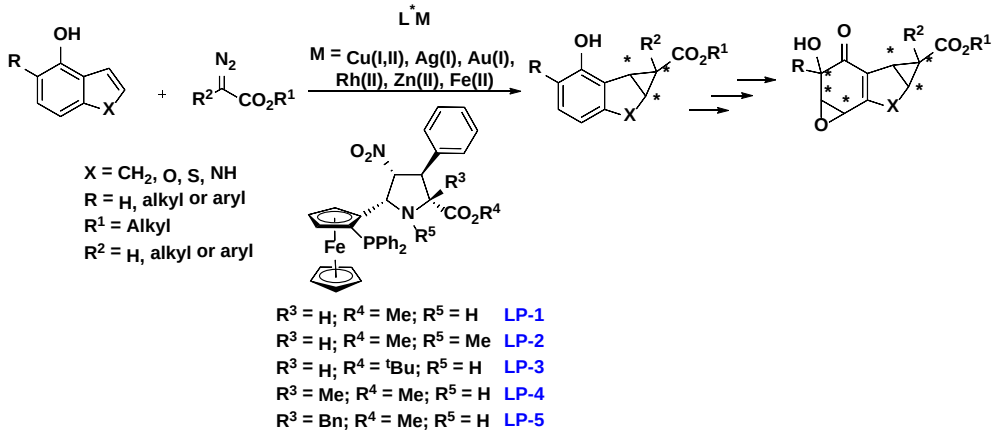
Our group has previously developed ferrocenyl-proline ligands that incorporate planar and central chirality. This feature makes them suitable for a particularly efficient simultaneously chiral induction. In fact, they have shown excellent diastereo- and enantioselectivity in [3+2] cycloaddition reactions between azomethine ylides and electron deficient alkenes.[1] Büchner[2] discovered in 1885 a route for the functionalization of benzene employing diazo compounds to provide a carbene moiety. Since then, the use of diazo compounds has been the most developed method for the metal mediated carbene transfer to C-C double bonds.[3] This reaction provides a very useful method for the convergent formation of cyclopropanes. These [2+1] cycloadducts constitute attractive target molecules in natural products and bioorganic chemistry.[4] In the present work stereoselective [2+1] reactions between fused hetero polyaromatic rings and different diazo compounds are described, in which ligands LP-1 to LP-5 constitute the source of chirality (**Scheme 1**).



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Scheme 1. Asymmetric cyclopropanation reaction catalyzed by ferrocenyl-proline-metal complexes followed by oxidative dearomatization.

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Searching for new applications of the hypervalent iodine reagents in the construction of nitrogen containing compounds

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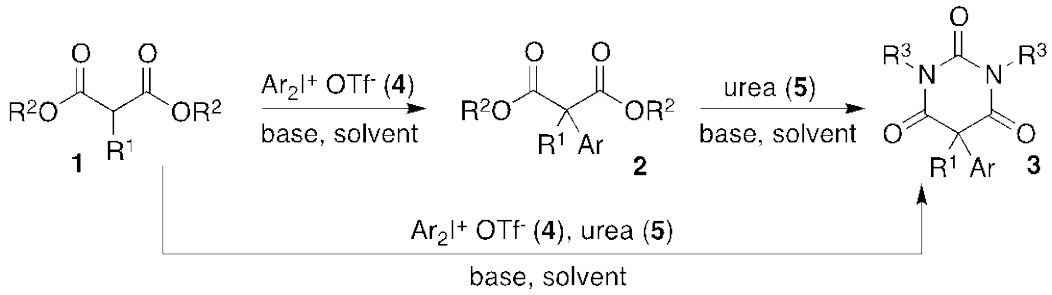
The search for novel applications of the hypervalent iodine reagents in organic synthesis has witnessed a huge development in the last years due to the fact that most of them shows activity in the presence of almost all types of functional groups.[1] Their high stability and ease of use make these reagents ideal candidates to be included in many synthetic designs. The two main aspects of the reactivity of this kind of reagents are (i) the high electrophility at the iodine atom, and (ii) the extremely high ability of iodobenzene (the resulting by-product of several of the most commonly used hypervalent iodine chemicals) to act as a "super-leaving group". Continuing our interest in this field, we are facing at the moment a new challenge.[2] The ability of some I(III) reagents, such as diaryliodonium salts **4**, to transfer one of their groups to the nucleophilic position of different substrates[3] has been employed, for example, in the metal-free arylation of malonates **1**.[4] The required conditions to accomplish this task (enolate formation under basic conditions) are similar to the ones required to perform a subsequent construction of a barbituric acid of type **3** in the presence of ureas **5**.[5] In other words, it is the aim of this project to perform the two-step synthesis of 5-aryl substituted barbituric acids **3** in a one-pot procedure (without isolation of **2**) or even in a multicomponent approach (Scheme 1).

Scheme 1. Two different strategies directed to the preparation of 5-arylsubstituted barbituric acids **3**.



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Intramolecular Palladium-catalyzed C-H activation reactions: Synthesis of substituted quinolones

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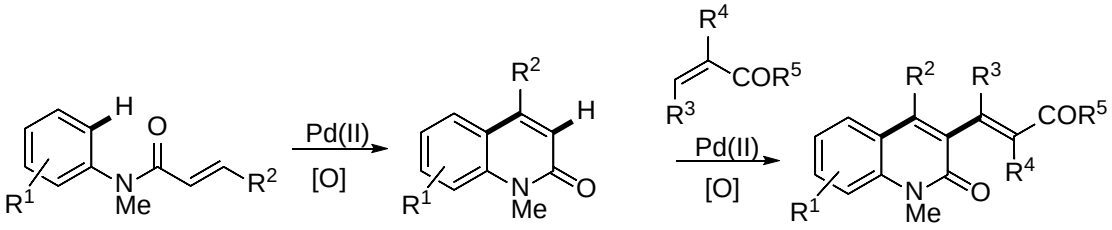
In the last years, the Palladium-catalyzed direct alkenylation of Csp^2-H bonds, an oxidative variant of the Heck reaction known as Fujiwara-Moritani reaction, has emerged as an efficient, atom-economical, and environmentally friendly synthetic tool for the preparation of highly functionalized aromatic molecules. In connection with our work in catalytic C-H activation chemistry,[1] we decided to apply this procedure to the synthesis of polysubstituted quinolone scaffolds, an important structural motif embedded in a wide variety of bioactive natural products and pharmaceuticals. An efficient approach to the synthesis of biologically active 3-alkenyl-4-substituted quinolin-2(1H)-ones that involves two sequential C-H alkenylation reactions has been developed. First, a Pd(II) catalyzed selective 6-*endo* intramolecular C-H alkenylation of *N*-phenylacrylamides has allowed the construction of the quinolone core, which could be further functionalized in C-3 through a second intermolecular C-H alkenylation reaction. This method is a significant advance over the existing procedures that require preactivatated reaction partners. Furthermore, these reactions can also be carried out in aqueous media at room temperature, using a 2% aqueous solution of PTS, or even in water, in good yields. Details of these transformations will be given.



R^1= H, OCH_3
R^2 = H, alkyl, aryl
R^3, R^4 = H, CH_3
R^5= O*t*Bu, OCH_3, NMe_2

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Favouring Trienamine Activation through Unconjugated Dienals

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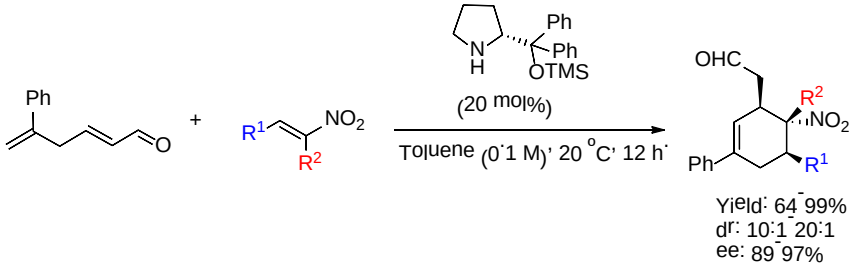
The possibility for primary or secondary amines to activate enolizable aldehydes or ketones towards a variety of reactions opened the way for their α-functionalization in a catalytic and enantioselective fashion. In the same line, the β-functionalization of enones and enals is possible through the catalytic formation of an α,β-unsaturated iminium ion. More recently, the combination of these two activation manifolds along with the principle of vinylogy has opened the possibility for the remote functionalization of unsaturated aldehydes and ketones, allowing the γ- and δ-functionalization through dienamine catalysis and vinylogous iminium ion activation respectively. Much more recently, it has also been shown that even a more remote ε-functionalization is also possible by the formation of trienamine intermediates[1] which, if conformationally locked, also allow the selective installation of ε-stereocenters with high level of stereochemical control.

The implementation of a reaction through dienamine or trienamine intermediates entails that the conjugation level of the starting material has to increase from a simple aldehyde or ketone to an α,β-unsaturated or α,β,γ,δ-polyunsaturated aldehyde or ketone respectively and this involves a progressive depletion of its reactivity towards condensation with the aminocatalyst.

In this work, unconjugated 2,5-dienals are proposed to constitute more reactive substrates than the corresponding fully conjugated α,β,γ,δ-unsaturated aldehydes towards organocatalytic activation through trienamine intermediates. This has been demonstrated in the Diels-Alder reaction with nitroalkenes,[2] which proceeds with clean β,ε-selectivity to afford the final products in high yields and stereoselectivities, while the related polyconjugated 2,4-dienals were found to be completely unreactive.



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New catalytic systems for oxygen-mediated oxidative processes

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The oxidation of alcohols to the corresponding carbonyl compounds is a current transformation in laboratory and industrial chemistry. Traditionally this reaction involves oxidants used in stoichiometric or overstoichiometric amounts so that relatively large quantities of waste are generated. Molecular oxygen is the ideal oxidant (readily available, safe, environmentally friendly, water as waste, etc.), but the need of metal catalysts to control the reaction outcome in relatively high amounts can become a serious drawback.[1,2] We wish to present two pallacyclic systems with remarkable catalytic properties in the aerobic oxidation of a number of alcohols.

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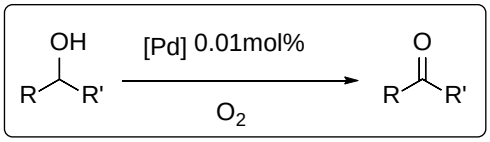
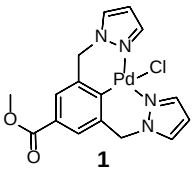
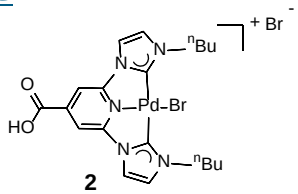
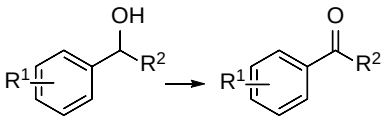




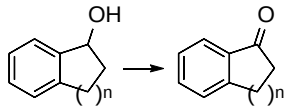
1



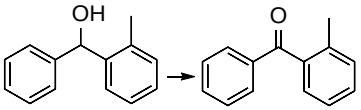
2



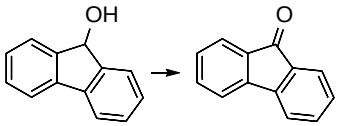
	1 (%)	**2** (%)
R^1= H; R^2= Me	99	97
R^1= H; R^2= CN	98*	99*
R^1= H; R^2= Ph	99	97
R^1= *p*-Me; R^2= Me	97	98
R^1= H; R^2= Et	97	98
R^1= *o*-OMe; R^2= Me	96*	97*
R^1= H; R^2= ^{t}Bu	98	94
R^1= *p*-Cl; R^2= Me	98	99
R^1= H; R^2= COOH	99*	98*
R^1= H; R^2= CHOHPh	97	98
R^1= H; R^2= Bn	91	92
R^1= H; R^2= Bz	96	94



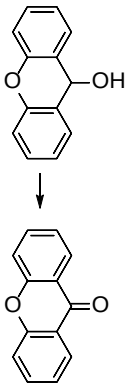
	1 (%)	**2** (%)
n= 1	95	97
n= 2	90	95



1 (%) **2** (%)
50 55



1 (%) **2** (%)
80 74



1 (%) **2** (%)
70 93

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Electronic properties of disordered functionalized carbon nanotubes

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Abstract: Carbon nanotubes (CNTs) present important electronic, mechanical and optical properties. These properties can be different compared to the pristine nanotube when its surface is functionalized. These changes can be explored in research and application areas such as building nanodevices that act like sensors and filters. Following this idea, in this work we made a systematic study of the influence of two type of functionalization groups −OH and −COOH at different concentrations (5, 10, 15, 20 and 25%) of the surface atoms (higher concentrations were avoided due to possible steric effects). The methodology used consists in create an script that add, randomly, the functionalization groups to the surface of a (10,0) single wall CNT. In this way, we generated 10.000 structures for each concentration and selected the representative ones according the disorder created. To accomplish this, the quasi-entropy of each generated structure was calculated using the USPEX software. All the calculations were performed using semiempirical PM7 method as implemented in the software MOPAC. For the representative structures, the highest occupied molecular orbital energy (∈H), the lowest unoccupied molecular orbital energy (∈L), the electronic gap (ΔE), the chemical potential (μ), the molecular hardness (η), the electrophilicity index (ω) and the electrostatic potential were calculated. Other parameters like heat of formation, entropy, area and volume were also determined. From the obtained results we were able to correlate each calculated property with the type and concentration of the functionalization groups. From these correlations it is possible to select a priori type of functionalization and its concentration accordingly the desired property.
Keywords: carbon nanotubes, semi-empirical, functionalization

1. Introduction

In recent decades, the computational chemistry has gained its place among the methods researching chemical phenomena as the results are reliable [1]. Thus, it was used computational chemistry to calculate the properties of functionalized nanotubes.

Since the functionalized carbon nanotubes have electronic, mechanical and optical properties different from those of pristine nanotubes, this phenomenon is exploited for use in sensors and has been explored in different areas of research and applications [2].

At the most fundamental level, a chemical sensor can be defined as a device that responds to changes in the local chemical environment. For a chemical sensor to be useful,

its response should be predictable in a way that depends on the magnitude of the changes in the chemical environment. Furthermore, it should be sensitive and selective. The development of chemical sensors is a well-established field. Nevertheless, in recent years we have witnessed an increase in sensor technologies for most sensitive and more specific systems with more complex architectures and reduced size due to advances in nanotechnology. Precisely, nanomaterials are strong candidates for analyte detection as the small size and large available area increment sensitivity.

2. Results and Discussion

To characterize the structural deformation of the nanotube due to functionalization's, the root-mean-square deviation (RMSD) was determined. The smaller this value is, the smaller will be the structural difference between the two structures indicating a smaller deformation of the nanotube.

The calculated RMSD from the optimized structure of the original nanotube and optimized structures of "naked" nanotubes (i.e. without functionalization) is shown in figure 1. As can be seen, concentrations of 5, 10 and 20% produced similar deformation for both systems. At concentrations 15 and 25%, the difference was more appreciable. The behavior of the entropy is the expected one since with increasing the concentration of functionalization, the degrees of freedom of the system increase (which increases the disorder thereof).

The energies of the frontier orbitals HOMO and LUMO, were calculated. Through the energy of these orbitals it is possible to know how each structure is reactive. The HOMO energy measures the electron-donor character of the compound [1], i.e., the higher the energy of the HOMO is the highest electron-donor ability. Since the energy of the LUMO, measures the electron-acceptor character, therefore, the lower the lower LUMO energy will be resistance to accepting electrons.

In figure 1 we can see that the energy of the molecular orbitals showed an oscillatory character with the concentration of functionalization in both cases (functionalization with -COOH group and -OH).

This was an unexpected behavior since the functionalization was done gradually and not correlated with structural changes or the number of electrons in the structures. Looking at figure 1, we have that the functionalized nanotubes with 0, 10 and 20% carboxyl and hydroxyl are those that have the highest HOMO energy, so these structures have a higher capacity to donate electrons.

The functionalized nanotubes with 5, 15 and 25% of carboxyl and hydroxyl, are those with less character electron donor, then these structures have greater resistance to receive electrons.

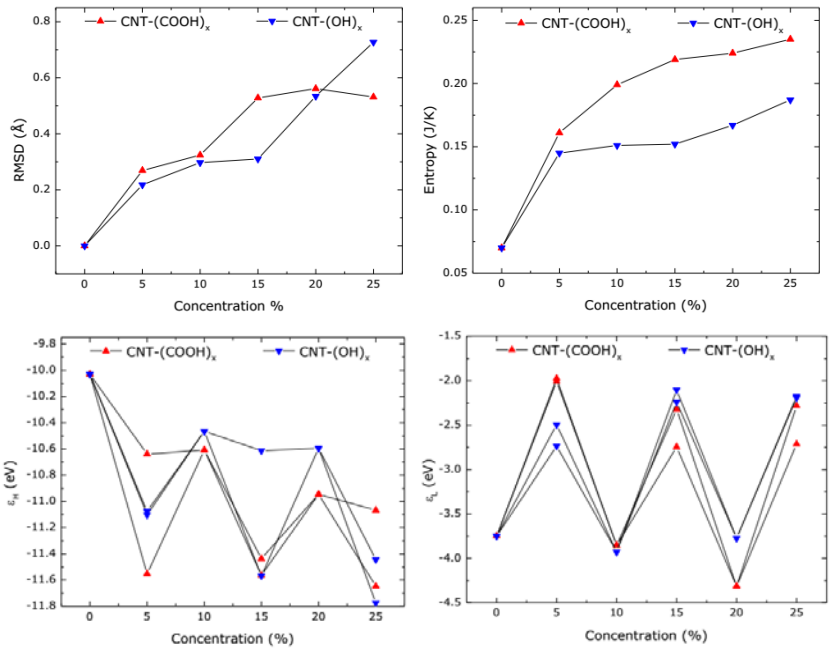

Figure 1. Some of the calculated properties: Deformation (top-left), entropy (top-right), HOMO energy (bottom-left), LUMO energy (bottom-right).

3. Materials and Methods

The computational study was designed to carry out a systematic study of the influence that has the variation in concentrations of surface functionalization on the nanotubes properties. For this, we started with a single-wall carbon nanotube (10,0) and the functional groups -OH and -COOH.

For each of the chosen concentrations (5, 10, 15, 20 and 25%), 10k CNT+OH and CNT+COOH complexes were stochastically generated in order to ensure greater diversity in the ensemble. In order to select a representative structure for each concentration/system, the entropy was used as a criterion: the structures with higher entropy (more disorder) were selected [3]. For each of the chosen complex, using the semi-empirical PM7 method [4] as implemented in the MOPAC2016 software [5], the structural and electronic properties have been calculated. Each representative structure was optimized to the lower energy structure. Then, the electronic properties (HOMO, LUMO, chemical potential, formation heat, dispersion energy and molecular hardness) and structural properties (deformation -RMSD-, volume, area and entropy).

4. Conclusions

Was obtained that structural properties such as volume and entropy had a monotonic behavior (increasing) with increasing concentration of functionalization. The values for the –COOH system are greater than for the –OH system. In the case of properties such as the area and the RMSD was obtained a different behavior.

In the case of the area, the curve showed a maximum value for different values for each concentration of functionalization. In the case of RMSD for the values 5, 10 and 20%, the deformations caused the nanotubes were of the same order. As for the concentrations of 15 and 25%, there was a significant variation.

In the case of electronic properties such as formation heat and dispersion energy, both properties exhibited decreasing behavior with increasing concentration of functionalization. For other properties (ionization potential, gap, HOMO energy, LUMO energy, dipole moment, chemical potential, molecular hardness and electrophilic index), the properties had an oscillatory behavior that we could not associate it with structural properties.

The results obtained allow us to correlate the desired properties for the system according to the type of functionalization and the concentration. Because of this, it is possible to design specific devices for use in applications with predetermined properties.

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Author Contributions

IC idealize the experiments and prepare the images and the final manuscript. MSR prepared the input files and run the simulations.

Conflicts of Interest

The authors declare no conflict of interest.

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QSRR Prediction of Parham reactions yield taking into consideration different reaction conditions

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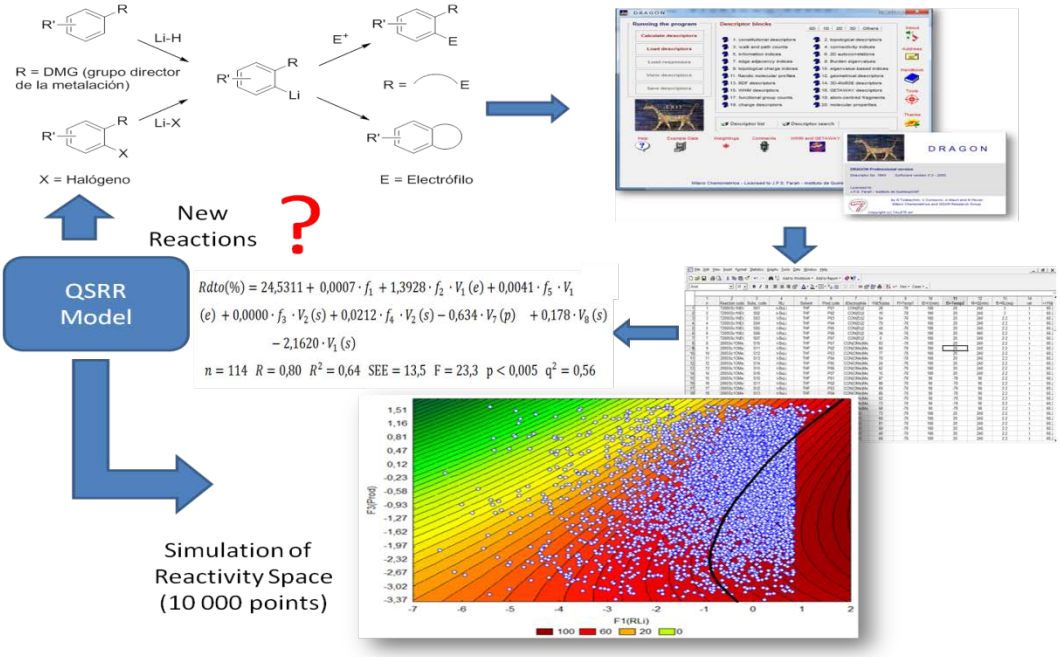
Abstract: Parham reaction is very important route for the synthesis of heterocycle compounds using organolithium reagents in the presence of Halogens and internal electrophiles. In this paper we collected a dataset of >100 reactions for many substrates and internal electrophiles with a wide range of yield of reaction (0 – 99%). The reactions have been carried out in many different experimental conditions with different values non-structural variables (δ_k) like: temperature of addition, addition time, organolithium equivalents, reaction times, and reaction temperature. Next, we calculated many structural and/or physiochemical variables (V_k) for the substrates, electrophiles, and products of the reaction. After that, we constructed a QSRR model able to predict the yield of reaction in many different conditions with acceptable accuracy. We also carried a 10 000-points simulation of the reaction conditions.



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Linear Indices Bob-Jenkins operators for development of multi-output models using multi-target inhibitors of ubiquitin-proteasome system

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Abstract: The ubiquitin-proteasome system (UPS) plays an important role in the degradation of cellular proteins and regulation of different cellular processes that include cell cycle control, proliferation, differentiation, and apoptosis. On other hand, the ChEMBL database contains >5000 experimental outcomes for >2000 compounds tested as possible proteasome inhibitors using a large number of pharmacological assay protocols. All these assays report a large number of experimental parameters of biological activity like EC50, IC50, percent of inhibition, and many others that have been determined under many different conditions, targets, organisms, etc. Although this large amount of data offers new opportunities for the computational discovery of proteasome inhibitors, the complexity of these data represents a bottleneck for the development of predictive models. In this work, we used linear molecular indices calculated with the software TOMOCOMD-CARDD (TC) and Bob-Jenkins moving average operators to develop a multi-output model that can predict outcomes for 20 experimental parameters in >450 assays carried out under different conditions. This generated multi-output model showed values of accuracy, sensitivity, and specificity above 70% for training and validation series. Finally, this model is considered multi-target and multi-scale, because it predicts the inhibition of the UPP for drugs against 22 molecular or cellular targets of different organisms contained in the ChEMBL database

Keywords: Ubiquitin-proteasome pathway inhibitors; CHEMBL; Multi-target, Multiscale and Multi-output models; Moving averages, QSAR

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QSTR modeling based on multiple linear regression for acute toxicity prediction of phenol derivatives against Tetrahymena pyriformis

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Abstract: In this work, the modeling of inhibitory grown activity against Tetrahymena pyriformis is described. The 0-2D Dragon descriptors based on structural aspects to gain some knowledge of factors influencing aquatic toxicity are mainly used. Besides, it is done by an enlarged data of phenol derivatives describe for the first time. It overcomes the previous datasets with about one hundred compounds. Moreover, the results of the model evaluation by the parameters in the training, prediction and validation provide adequate results comparable with those of the previous works. The more influential descriptors involved in the model are: X3A, MWC02, MWC10 and piPC03 with positive contributions to the dependent variable; and MWC09, piPC02 and TPC with negative influences. In a next step, a median-size database of nearly 8,000 phenolic compounds extracted from ChEMBl was evaluated with the quantitative-structure toxicity relationship (QSTR) model developed providing some clues (SARs) for identification of ecotoxicological compounds. The outcome of this report are very useful to screen chemical databases in use for finding the compounds responsible of aquatic contamination in the biomarker used in the current work.

Keywords: Tetrahymena pyriformis, Dragon descriptors, multiple linear regression

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Computer aided design of new inhibitors of acetylcholinesterase

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Abstract: It is well known that the cholinergic deficiency contributes to the cognitive deterioration of Alzheimer's Disease (AD) patients and that its pathways in the cerebral cortex are also compromised. In this work we use computational tools to design new and more powerful inhibitors of acetylcholinesterase (AChe). We made use of the de novo design and fragment-based drug design (FBDD). In the former approach, we started from reference drugs used in the AD treatment. These drugs were break into small pieces (fragments). These fragment were used as seed to grown new molecules or to be linked with other new fragments. In the latter approach, a library of fragments is docked in the active site of the enzyme. The interaction of each fragment is measured and they are organized by their affinity. The best ranked fragment are them linked between them to form new molecules with high degree of interaction with the active site of the enzyme. Using this strategy, we were able to produce a library of 2M new molecules. This library was filtered using as criterion the adsorption, distribution, metabolism and excretion (ADME) properties. The resulting library with around 6k molecules is filtered again using the Tanimoto similarity coefficient (structures with values greater than 0.85 were eliminated). The final library with 1.5k compound was submitted to docking studies. Finally, 10 compounds with better interaction energies than the reference compounds were obtained.

Keywords: Alzheimer's Disease, acetylcholinesterase, de novo design, fragment docking

1. Introduction

The international organization Alzheimer's Disease International (AID) had published several technical reports to disseminate aspects of the disease as the prevalence of dementia, disability and mortality, the impact that the AD has in the global economic; the effects and benefits of early diagnosis and about the overcoming the stigma of dementia.

To date, AD has no cure and the disease is caused by the consequence of dementia caused by the decrease in the transmission of nerve impulses caused by neurodegeneration. Therefore, the actual treatments are based on drugs which leverage the transmission of electric impulses.

One way to improve nerve transmission is reducing or inhibiting the activity of the acetylcholinesterase (AChE)enzyme. This enzyme is responsible by decreasing the levels of acetylcholine (ACh) in the synaptic cleft. Decreasing the activity of AChE, keeps higher ACh levels in cleft, thus enhancing the transmission of the electric impulses. In this way, the anticholinesterase inhibition drugs are capable of modifying the progress of AD, improving the

cognitive ability of the patient, increasing its quality life, without, however, preventing the development of disease.

Currently, there are only 5 drugs approved for clinical treatment of AD, and 4 of them are AChE inhibitors and only one with a different action mechanism by acting as an antagonist of N-methyl-D-aspartate (NMDA) receptors, avoiding excessive release of glutamate, responsible for neurotoxicity.

2. Results and Discussion

To evaluate the quality of docking simulations (best poses) among the ligands and the proteins we used the Glide Score function (GScore). In table 1, the GScore of the best 5 molecules is shown together with the reference drugs.

Comparing the score of the reference drugs with the generated molecules presented in table 1, we can see that all these five molecules have better scores, suggesting potentials acetylcholinesterase inhibitor stronger than the reference drugs.

In figure 1 the 2D interaction diagrams between the protein and the best molecule and the reference drugs are shown. From these diagrams, it is possible to identify the atoms involved in the hydrogen interactions together with the amino acid involved in the $\pi - \pi$ and π-cation interactions. In all cases, the interactions are spread through the molecule structure given to the complexes good stability.

In the 2D diagram for molecule **798** (figure 1(d)), we can see that not only the interactions are distributed through the molecule, also this molecule has much more interactions with the protein than the reference drugs. This is translated in a better docking score.

Table 1. Docking score (GScore) for the best molecules and for the references drugs.

Molecule	798	746	671	695	720
GScore	−17.352	−17.043	−16.313	−16.192	−15.881
Reference	Donepezil	Galantamine	Rivastigmine		
GScore	−11.676	−12.075	−8.437		

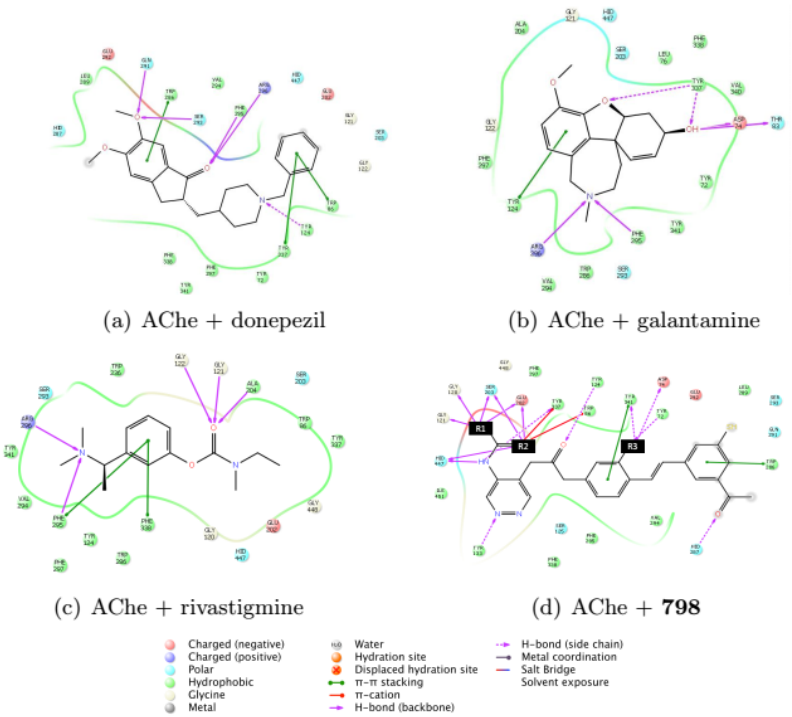

Figure 1. 2D interaction diagram.

3. Materials and Methods

To obtain the structure of the acetylcholinesterase we used the search resources of the Protein Data Bank (PDB) [1]. The search results were then filtered by organism and using the x-ray resolution as criterion. From this, we get the structure for a human AChE with code 4M0E and with the resolution equal to 2.00 Å [2]. To correct the proteins structure is then used the Protein Preparation Wizard protocol as implemented in the Schrödinger Suite [3] using the Maestro interface [4]. This protocol adds hydrogen atoms, corrects bonds, complete chains, etc.

The computational modeling of new inhibitors for the acetylcholinesterase was divided into four steps.

In the first step, using the LigBuilder software [5; 6], the fragment-based drug design approach was used together with the de novo design to generate new molecules 1st library (1LB) with around 2.5M molecules.

In the second step, the 1LB was filtered using physicochemical descriptors related to the Absorption, Distribution, Metabolism and Excretion (ADME) properties, producing a second library (2LB) with 6k molecules.

In the third step, the 2LB was filtered using the similarity as criterion given a third library -3LB- with 1.5k molecules. We used the Tanimoto metric to compute the similarity among all the molecules i.e. if the Tanimoto coefficient of two structures is greater than 0.85, the structures are considered similar and removed from library.

Finally, the 3LB was using in the docking studies. The docking studies were carried out using the Glide software [7] and the Induced Fit Protocol [8] from the Schrödinger Suite [3]. This software starts from a population of conformers and apply the genetic algorism to obtain the best docking poses. To evaluate each pose, it uses a scoring function called GlideScore that consider the van der Waals energy, the Coulomb energy, a lipophilic contact term, an hydrogen-bonding term among other terms [9; 10]. The GlideScore (or GScore) has units of kcal/mol and the lower it is, the better the interaction is. In the first step, the molecules from 3LB were used in a docking performed considering the protein as a rigid structure and the ligand as flexible. From this docking steps, the best 100 docked molecules were used as input ligand in a flexible-flexible docking procedure.

4. Conclusions

The fragment based drug design method together with the de novo design used in this work turns to be a good alternative to create/design new molecules that can inhibit the acetylcholinesterase. Based on the calculated GScore, the de novo designed molecules have better inhibitor capacity than the drugs most used in the market.

Acknowledgments

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Author Contributions

IC idealize the experiments and prepare the images and the final manuscript. AP, LF, ALFA and BAV search for the protein structure, prepare the input files and run the simulations.

Conflicts of Interest

The authors declare no conflict of interest.

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The combination of complementary metabolomic platforms to unravel Alzheimer's disease pathogenesis

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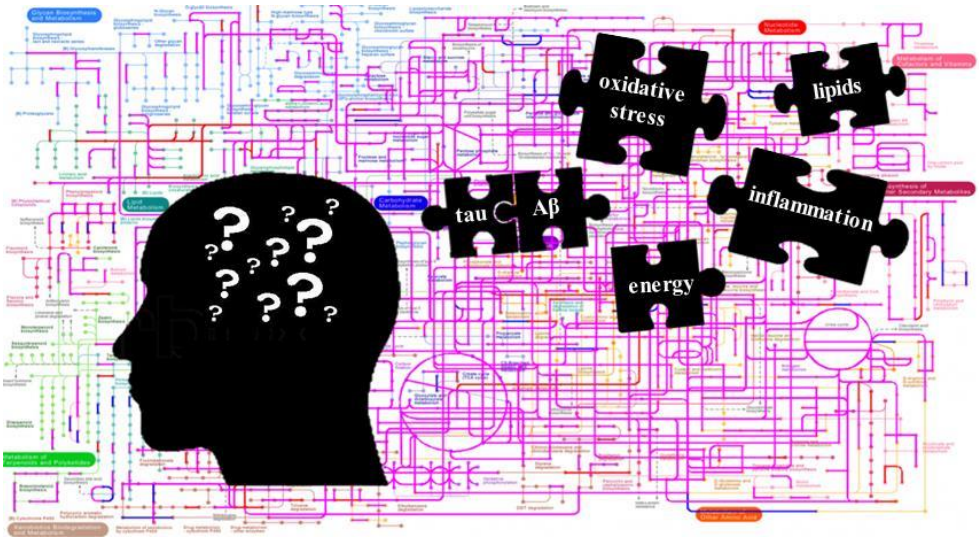
Abstract: *Alzheimer's disease (AD) is the most common neurodegenerative disorder among older people, characterized by an insidious onset and a progressive decline of cognitive functions. Nowadays, there is no cure for AD mainly because its etiology is still unclear and current diagnostic tests show great limitations, including low sensitivity and specificity, as well as the impossibility to detect characteristic symptoms at early stages of disease. Thus, the main objective of this work was the optimization of complementary metabolomic approaches based on mass spectrometry in order to investigate AD pathogenesis and discover potential biomarkers for diagnosis. With the aim to get a comprehensive metabolome coverage, multiple analytical platforms were developed, including screening procedures based on direct mass spectrometry analysis and hyphenated approaches with orthogonal separation mechanisms such as liquid chromatography, gas chromatography and capillary electrophoresis. The application of these techniques to serum samples from patients suffering from Alzheimer's disease and mild cognitive impairment enabled the identification of numerous metabolic alterations linked to pathogenesis of this disorder and its progression from pre-clinical stages, including abnormalities in the composition of membrane lipids, deficits in energy metabolism and neurotransmission, and oxidative stress, among others. Accordingly, it could be concluded that the combination of complementary metabolomic platforms allows studying etiology associated with Alzheimer's disease in a deeper manner.*

Keywords: *metabolomics; Alzheimer's disease; mass spectrometry*

Slides presentation*:* http://sciforum.net/file/download/mol2net-02/Mol2Net_02_Raul_gonzalez_slides.pdf

Graphical Abstract:



Introduction:

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by a multifactorial pathogenesis, still not completely understood, in which numerous pathological processes are involved, including the deposition of β-amyloid plaques and neurofibrillary tangles, inflammation, oxidative stress, and abnormal metal homeostasis, among many others. Furthermore, current diagnostic tests for AD show great limitations, including low sensitivity and specificity, as well as the impossibility to detect characteristic symptoms at early stages of disease. For these reasons, the discovery of novel AD biomarkers is crucial in order to identify key features of the underlying pathology and develop accurate diagnostic methods. In this context, metabolomics plays a prominent role because of its potential to provide a global overview of altered biochemical pathways in response to genetic or environmental factors.

Materials and Methods:

Blood serum samples from AD patients and healthy controls were subjected to multiple complementary metabolomic platforms based on mass spectrometry, including direct infusion electrospray mass spectrometry (DI-ESI-MS) [1], flow injection atmospheric pressure photoionization mass spectrometry (FI-APPI-MS) [2], reversed phase ultra-high performance liquid chromatography mass spectrometry (RP-UHPLC-MS) [3], gas chromatography mass spectrometry (GC-MS) [4] and capillary electrophoresis mass spectrometry (CE-MS) [5]. Data were then submitted to multivariate and univariate statistical analysis in order to find significant metabolic perturbations between groups.

Results and Discussion:

Metabolomics based on direct mass spectrometry analysis showed a great potential to perform a preliminary metabolic screening due to its reduced analysis time and instrumental simplicity. For this purpose, serum samples were treated according to a two-step extraction protocol and then analyzed by high resolution mass spectrometry with combined electrospray (ESI) and atmospheric pressure photoionization (APPI) sources, in both positive and negative ionization modes, in order to maximize metabolome coverage [1,2]. Complementarily, these samples were also fingerprinted with three complementary metabolomic platforms based on the coupling of orthogonal separation techniques with mass spectrometry. Analysis by RP-UHPLC-MS revealed significant alterations in serum levels of numerous lipids, such as phospholipids, sphingolipids, acyl-glycerides and acyl-carnitines, thus demonstrating the potential of this platform for the investigation of low polarity compounds [3]. Alternatively, the use of GC-MS enabled the detection of several low molecular weight metabolite classes, including carbohydrates, amino acids, fatty acids and organic acids [4]. To conclude, CE-MS was proposed for studying the polar fraction of the serum metabolome [5]. The application of these metabolomic tools to serum samples from patients with Alzheimer's disease and healthy

controls allowed the identification of numerous pathological mechanisms associated with the pathogenesis of this disorder. Thus, some of the most important findings of this study were the detection of significant changes in the composition of membrane lipids, deficits in energy metabolism and neurotransmitter systems, oxidative stress, hyperammonemia or hyperlipidemia, among others.

Conclusions:
The combination of multiple complementary metabolomic approaches allows investigating in depth the etiology associated with Alzheimer's disease, as well as the discovery of potential biomarkers for diagnosis.

Conflicts of Interest:
The author declares no conflict of interest

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Artificial Neural Networks and Multilinear Least Squares to Model Physicochemical Properties of Organic Solvents

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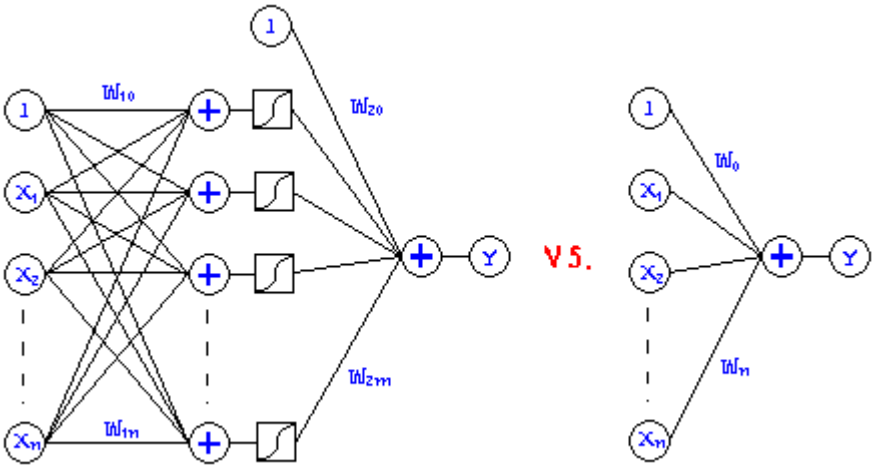
Abstract: *The mean molecular connectivity indices (MMCI) proposed and used in previous studies are used here in conjunction with the well-known molecular connectivity indices (MCI) to remodel six properties of organic solvents. The MMCI and MCI descriptors of the Multilinear relationships for the six properties, obtained with the multilinear least - squares (MLS) procedure, were used to perform the artificial neural network (ANN) computations. The aim is to detect advantages and underline the limits of the ANN approach that, even if it improved the model, it is somewhat 'fuzzy' concerning the stability of the modeling. The MLS procedure replicates the obtained results as long as one wishes, a characteristic not shared by the ANN methodology, which, if on one side increases the quality of a description on the other increases also its overfitting. The present study reveals also how ANN methods prefer MCI relatively to MMCI descriptors. Four different types of ANN computations show that (i) MMCI descriptors are preferred with properties with poor number of points. MLS (ii) is to be preferred over ANN statistical results, with some exceptions, when the number of ANN weights is similar to the number of correlation coefficients of MLS. Furthermore, in (iii) some cases MLS modeling quality is quite similar to the modeling quality of ANN computations.*

Keywords: *Artificial Neural Networks, Multilinear Least Squares, physicochemical properties modeling, QSPR, molecular connectivity indices, mean molecular connectivity indices, boiling point, density, flash point, viscosity, surface tension, elutropic value.*

Graphical Abstract:



Introduction:

Recently [1] the mean molecular connectivity indices, MMCI, were introduced and used to model eleven properties of organic solvents. The multlinear least squares, MLS, used to derive the quantitative structure-property relationships (QSPR) showed that three out of six properties, the refractive index, *RI*, the flash points, *FP*, and the *UV* cutoff values, were modeled with the mean molecular connectivity indices, MMCI, while the remaining properties were modeled with the well-known molecular connectivity indices, MCI. The MMCI indices are centered on the basic concepts of the delta, valence delta, *I*- and *S*-indices that go back to the origins of the molecular connectivity theory. [2-7] Results from two other recent studies that used semiempirical sets of descriptors [8, 9] showed that the artificial neural network (ANN) procedure with variable number of hidden neurons, chosen by the software, normally improves the quality of a QSPR obtained by the aid of the MLS methodology. Nevertheless, this improvement is somewhat artificial as the ANN computations for the eleven properties employed a number of weights, due to the presence of more than one hidden neuron, much greater than the number correlation coefficients in the MLS procedure.

The present work aims are to pin down the real advantages but also the drawbacks of the ANN methodology applying it to the model of six properties of Ref.1 with only either MCI or MMCI used as descriptors. Four different types of ANN computations are here performed to detect the level of the achieved improvement, if any, (*a*) with one hidden neuron, (*b*) with a pre-fixed number of hidden neurons, (*c*) with a variable number of hidden neurons chosen by the software, and (*d*) with a minor number of descriptors, for the one hidden neuron case. This last case was tried to render the number of ANN weights equal to the number of correlation coefficients of the MLS case. Furthermore, it was monitored if ANN computations prefer either MCIs or MMCIs for modeling purposes. The descriptors for the six properties are those of ref. 1 but whenever a property was not satisfactorily

modeled by the given MCI (or MMCI) the second or third best MCI (or MMCI) were chosen.

Materials and Methods:

Table 1 shows the molecular connectivity χ indices, the molecular pseudoconnectivity ψ indices (*pseudo-MCI*), the dual connectivity and pseudoconnectivity indices (*Dual MCI, pseudo-MCI*) used in this study. Three new indices are also defined: $\Delta = \Sigma_{EA} n_{EA}$, $\Sigma = \Sigma_{EA} <S_{EA}>$, and $T_{\Sigma/M} = \Sigma^3/M$ [1.7] (M = molar mass); Δ encodes the number of electronegative atoms (n_{EA}), Σ encodes the sum of the *S*-State index for the electronegative atoms, *N*, *O*, *F*, *Cl*, *Br* ($<S_{EA}>$ is the average value for a specific type of atom).

Table 2 shows the definitions of the MMCI (the first M stands for 'mean').

In Tables 1, and 2 i = 1-N denotes the atoms of a molecule, ij denotes directly σ-bonded atoms, and in Table 2, $p = N$. The Lehmer mean, ${}^{L}M$, for p = 2, equals the symmetrical mean, ${}^{S}M$. Replacing in Table 1 δ with the valence delta, δ^v, allows to obtain the corresponding valence MCI, $\{D^v, {}^0\chi^v, {}^1\chi^v, \chi^v{}_t, {}^0\chi_d{}^v, {}^1\chi_d{}^v, {}^1\chi_s{}^v\}$, while replacing the Intrinsic-*I*-State with the Electrotopological *S*-State index the corresponding pseudoconnectivity electro-topological indices are obtained, $\{{}^S\psi_E, {}^0\psi_E, {}^1\psi_E, {}^T\psi_E, {}^0\psi_{Ed}, {}^1\psi_{Ed}, {}^1\psi_{Es}\}$. [3-9] Replacing in Table 2 δ, with δ^v, *I* and *S* three other subsets of MMCI are obtained: the valence, $\{{}^AM^v, {}^GM^v, {}^HM^v, {}^RM^v, {}^SM^v, {}^UM^v, {}^{Ho}M^v, {}^LM^v, {}^{St}M^v\}$, the *I-State*, $\{{}^AM_I, {}^GM_I, {}^HM_I, {}^RM_I, {}^SM_I, {}^UM_I, {}^{Ho}M_I, {}^LM_I, {}^{St}M_I\}$, and the *E*-State $\{{}^AM_E, {}^GM_E, {}^HM_E, {}^RM_E, {}^SM_E, {}^UM_E, {}^{Ho}M_E, {}^LM_E, {}^{St}M_E\}$ MMCI, respectively. Because some *S* values can be negative (highly electropositive atoms) to avoid imaginary *S*-State MMCI values, a rescaling of the *S* value is undertaken as it is explained in ref. 7. Summing up, we have thirty-one MCI and thirty-six MMCI. Every index was obtained with a visual basic home - made program that runs on a normal PC that uses both adjacency and distance matrices [6].

The multilinear least squares procedure of Statistica 8 was used to find the best MCI and MMCI set of descriptors for the training set of Table 3, which is then used to evaluate the left-out compounds [those with (°) in Table 2, ~ 30% of all compounds, 25% for *El*]. The overall quality of each model was obtained with the Excel spreadsheet by plotting the observed versus the calculated property for the training and for the training plus evaluated points. The choice for the number of indices of a relationship has been done having in mind the *Topliss-Costello* rule: [10] the ratio of data points to the number of variables should be higher or equal to five, and should provide a correlation coefficient factor $r > 0.84$, i.e., $r^2 > 0.70$. The source for the properties of organic solvents listed in Table 3 is in ref. 7.

ANN methods perform regression and data validation, and carry out both tasks in a non-parametric way that makes no assumption regarding the relationship between y and x, where $y = f(x)$. This means that the function

Property = *f*(*indices*) is not known *a priori*. This non-parametric model is a kind of *black box* that tries to discover the mathematical function that can approximate the relationship between the *indices* and the *property* well enough. It uses highly flexible transfer functions with adaptable parameters that can model a wide spectrum of functional relationships.[11] The activation functions for both hidden and output nodes used in Statistica 8 are: identity (*i*), logistic sigmoid (*l*), hyperbolic tangent (*t*), sine (*s*), and exponential (*e*).

ANN results were obtained with the built-in utility of Statistica 8, the multilayer perceptron neural network (MLP). The used network has three-layer feedforward architecture with unidirectional full connections between successive layers and with error backpropagation (or backprop). The three layers are: *input units* → *hidden units* → *output units* (units are also known as neurons or nodes), and they correspond to: *variables* → *hidden units* → *P*, where the only output unit is the targeted property, *P*. In present study the number of variables corresponds to the number of MCI or MMCI descriptors. Each neuron (or node) in a particular layer is connected to every neuron in the next layer. The connections between neurons are practically the weights that determine the values assigned to the nodes. There exist additional weights assigned to the bias values that act as node value offsets, i.e., the weights of the connections: *input bias* → *hidden neuron*, and *hidden bias* → *P*. The number of weights is thus: [(*No. input nodes* + *2*)·(*No. hidden nodes*) + *1*]. The weights adjusted by the training process are initially random and are passed to all nodes of the following layer. The training process is iterative and each iteration is called an epoch. The weights are slightly varied in each epoch to minimize the sum-of-squares error function: $SOS = \sum_{i=1\text{-}N} (P_{iclc} - P_i)^2$, where P_{iclc} (*clc* = calculated) is the i^{th} predicted value (network outputs) of the property, and P_i is the target value. This function is the sum of differences between the prediction outputs and the target defined over the entire training set of points (compounds) *N*. Statistica 8 allows setting the number of networks to train and retain (*Ntr*/*Nre*). Here, two sets of values are chosen: *Ntr/Nre* = 10^3/200 and *Ntr/Nre* = 10^5/200. In the corresponding tables only *Ntr* is shown as *Nre* is constant. The ANN network of Statistica 8 optimized with the BFGS (Broyden-Fletcher-Goldfarb-Shanno) algorithm ensures a fast convergence rate.[12]

Statistica 8 initially sets by defect the number of hidden nodes between 3 and 11. Nevertheless, we will here impose four procedures (for the 4th procedure see later on): (*i*) first a single hidden node, then (*ii*) hidden nodes from two to twelve will sequentially be tried ‘by hand’ (i.e., program does not allow the imposed number of hidden nodes to be changed), and, finally, (*iii*) the program chooses the number of hidden nodes. To come as close as possible to the MLS results it was decided (*iv*), to compute again the one hidden neuron case where either one or two indices with the lowest sensibility value have

been deleted. In this case, for instance, the number of weights for the 4-1-1 case of T_b is 7, and it equals the number of correlations coefficients from the MLS calculations with six indices.

The results of the five procedures, one MLS and four ANN, given in separate tables, allow a meaningful comparison among them. The MLS procedure optimizes a number of regression parameters equal to the number of variables plus one (the *bias* parameter), which means that a practical comparison between the two methods should only be performed when ANN uses only one hidden neuron. In this case, due to the previous relation, the number of ANN weights equals the number of MLS correlation coefficients plus two. One should expect that with growing number of hidden neurons the model of a property should constantly improve due to the growing number of weights for each variable (akin having a variable with many different regression coefficients). With ANN it is not rare the case that as the model becomes exceedingly good with growing number of weight parameters, and this frequently results in *overfitting* with exceedingly poor externally evaluated values. The choice of training (TR = 80% of the values in Table 3) and test sets (TE = 20% of the values, i.e., the underlined bold values in this same Table) usually avoids overfitting as the network is repeatedly trained for a number of cycles so long as the test error is decreasing, otherwise the training is halted. This method, also known as 'early stopping' procedure [13] avoids the trap that the program will always choose the maximum number of hidden nodes. Actually each property shows an optimal number of nodes which rarely corresponds with its maximum possible.

Results:

Table 4 from ref. 1, collects the MLS results for the six properties. The training set for the *El* property includes pentane and tetrahidrofurane.

Table 5 through 8 collect the various ANN - MLP results for the set of variables (descriptors) of Table 4 or for the alternative (either MMCI or MCI) set of variables obtained with the MLS method. Throughout the Tables 5-8, in the first column are given, as in Table 4, the δ^v type (see Appendix), and the number of networks to train, *Ntr* = 10^3 or 10^5 (when the two numbers rose to similar results *Ntr* = 10^3 was preferred), while the number of networks to retain is always 200. It was not always possible to achieve improvement in modeling with *Ntr* = 10^5 even after three runs (more were not tried), and when there was improvement, it was never sharp excepting two cases as we shall see. The activation functions together with the neuronal architecture are in the second column of Tables 5 – 8. In this column, 3rd line, are given for each property the *number of epochs* for which the ANN-MLP calculation runs even if the actual number of cycles used to train the model might be greater. In fact, as the *number of epochs* is not definitive it cannot be held as an unfailing parameter (it can exceed the given number). In

the third column is the set of variables together with their statistics. Throughout this column in the second line are the sensitivity values, which are the values that are due to the sensitivity analysis that rates the importance of the models' input variables. These r^2 and *s*, statistics were obtained with the EXCEL spreadsheet plotting the observed *property*, *P*, vs. the calculated one, P_{clc}, once for the training and test set compounds, *N*(*a*TR + *b*TE), and the other time for the training {TR} + test {TE} + external evaluation {EV} sets, *N*(*a*TR + *b*TE + *c*EV), where *a*, *b*, and *c* are their number. We remind the reader that the MLS procedure has no test compounds, only training compounds, *N*(TR). No ANN weights are shown as, for instance, a [5-7-1] network has fifty weights. Furthermore, it is to notice that every time an ANN-MLP runs different weights and sensitivity values are obtained a first non-minor drawback avoided by the MLS procedure.

For comparison purposes it was decided to maintain in the ANN calculations (see Tables 5-8) the same number of outliers excluded throughout the MLS procedure and given in Table 4,where the exclusion was done for residuals greater than 3*s*. Clearly, such restriction is no more valid throughout ANN tables 5 - 8. In Table 5 are given the ANN results obtained with a single hidden neuron. Following Tables 6 and 7 display the multiple neuron cases: Table 6 with an externally imposed number of hidden neurons that was cycled from 2 to 12, and Table 7 with the number of hidden neurons chosen automatically by the program (between 3 and 11). For *El*, the program sets this number between 3 and 10. For those cases where similar good models are achieved with different sets of hidden nodes, the set with minimal number of nodes was chosen.

Discussion:

For an easier lecture and interpretation the detailed and most important statistical results collected through Tables 4 – 7 are summarized in Table 9. Table 8, illustrates a special case that will be discussed later on. While Tables 4 – 7 collect the detailed information about the modeling of the six properties, and especially about the type of indices, valence deltas, and structure of the ANN computations, Table 9 gives direct information about of the various models. MMCI indices throughout the ANN computations with one hidden neuron, (*ANN* ***1****HN*, Table 5), are important descriptors for flash point, *FP*, and elutropic values, *El*.

As soon as the number of hidden neurons grows either by external choice, *enHN* (Table 6), or by software choice, *snHN* (table 7), MMCIs are optimal descriptors only for Elutropic values (silica), *El*, the property with the lowest number of points. The second thing we notice is that for an optimal modeling the number of hidden neurons (number in bold, 2nd line of the statistical values for each property) that are externally chosen (*ANN enHN*, Table 6) is smaller relatively to the number of hidden neurons chosen by the software (last column, *ANN snHN*, Table 7). In some cases it is much smaller, like for $\boldsymbol{T_b}$ (an

extreme case), ***d***, and ***γ***. Concerning the statistical results we see that *ANN 1HN* (Table 5) improve at the training level (first line) over MLS (Table 4) for T_b, and ***El*** properties, while it stays behind with ***FP***, otherwise results are rather similar. With the whole set of compounds (second line), i.e., with training (and test with ANN) plus evaluated compound *ANN 1HN* (Table 5) calculations improve again for T_b, and ***El***, while they stay behind with ***γ***.

The multiple hidden neuron case shows that, at the training level *ANN enHN* (Table 6), things improve consistently over the two previous cases (*MLS* and *ANN 1HN*) for T_b, ***d***, ***γ***, and ***FP***. For ***El*** there is improvement only relative to the *MLS* (Table 4) case. Results for viscosity, ***η***, are rather similar throughout the three cases. In most cases improvement concerns both the r^2 and the *s* statistics. Concerning the whole set of compounds (tranining plus test and evaluated) statistics improve relatively to the two previous cases (*MLS* and *ANN 1HN*) for T_b, ***γ***, and ***FP***. The advantage of *ANN* over the *MLS* procedure is usually not drastic throughout the six properties. The *ANN snHN* (Table 7, and Table 9, last column) procedure with software chosen number of hidden neurons normally uses more hidden neurons that the previous *ANN enHN* (Table 6, and Table 9, before the last column) one. Actually, it does not achieve any practical improvement. Normally, its statistics are either worse or similar to the *ANN enHN*. This means that if you intend to let the software choose the number of hidden neurons then better you stick to the *MLS* modeling.

In those cases where deletion of two indices resulted in a poor modeling, only an index is deleted. In this last case the number of weights is no more equal (actually is bigger by one) to the number of correlation coefficients of the MLS case. Results are shown in Table 8, and, as the reader can notice, two properties, ***γ***, and ***El***, due to poor modeling do not show up, while for properties ***d***, and ***FP***, only one index has been deleted.

Remind that sensibility values are no absolute values as they normally change from run to run, like the weights, and they are no last word about the importance of an index. The statistics here are usually not as good as in the MLS case (Table 4).

Tables 5 - 8 tell us that there is no fixed preferential value for the parameter *Ntr* (numbers of networks to train), sometimes quite different *Ntr* values give rise to similar statistical values and some other times they give rise to completely different ones. Thus, it is always worth trying several *Ntr* values. Concerning the most used values for δ^v Tables 4 through 7 show that the $\delta^v{}_{ppo}$ configuration is the most used, especially in both *nHN* cases (Tables 6, and 7), which means a strong dependence on the core electrons for heavier atoms (see Appendix). For what concerns the exponent of the fractional term in δ^v (see Appendix) the most used values are 1, -0.5 (strong hydrogen atom dependence), and 50 (no hydrogen atom dependence). The strong

hydrogen dependence of δ^{v} reveals that the hydrogen atoms should not be underestimated.

Table 1. Definition of the Molecular Connectivity Indices (MCI). Replacing δ with δ^{v} and I with S the corresponding valence, χ^{v}, I-State, ψ_I, and E-State, ψ_E, MCIs are obtained.

MCI	*Pseudo-MCI*	*Dual MCI + Δ + Σ*	*Dual pseudo-MCI + $T_{\Sigma/M}$*
$D = \Sigma_i \delta_i$	${}^{S}\psi_I = \Sigma_i I_i$	${}^{0}\chi_d = (-0.5)^N \Pi_i(\delta_i)$	${}^{0}\psi_{Id} = (-0.5)^N \Pi_i(I_i)$
${}^{0}\chi = \Sigma(\delta_i)^{-0.5}$	${}^{0}\psi_I = \Sigma(I_i)^{-0.5}$	${}^{1}\chi_d = (-0.5)^{(N+\mu-1)} \Pi(\delta_i +$	${}^{1}\psi_{Id} = (-0.5)^{(N+\mu-1)} \Pi(I_i +$
${}^{1}\chi = \Sigma(\delta_i\delta_j)^{-0.5}$	${}^{1}\psi_I = \Sigma(I_i I_j)^{-0.5}$	${}^{1}\chi_s = \Pi(\delta_i + \delta_j)^{-0.5}$	${}^{1}\psi_{Is} = \Pi(I_i + I_j)^{-0.5}$
$\chi_t = (\Pi \delta_i)^{-0.5}$	${}^{T}\psi_I = (\Pi I_i)^{-0.5}$	$\Delta = \Sigma_{EA} n_{EA}$, $\Sigma = \Sigma_{EA} \langle S_{EA} \rangle$	$T_{\Sigma/M} = \Sigma^3 / M^{1.7}$

N is the number of atoms, ij means corresponds to σ bond, μ is the cyclomatic number.

Table 2. Definition of the Mean Molecular Connectivity Indices (MMCI). Replacing δ with δ^{v}, I, and with S the corresponding valence, M^{v}, I-State, M_I, and E-State, M_E, MMCIs are obtained.

${}^{A}M = \Sigma_i \delta_i / n$	${}^{G}M = \Sigma_{ij}(\delta_i\delta_j)^{1/2}$	${}^{H}M = 2\Sigma_{ij} (\delta_i^{-1} + \delta_j^{-1})^{-1}$
${}^{R}M = \Sigma_{ij}[(\delta_i^2 + \delta_j^2)/2]^{1/2}$	${}^{S}M = \Sigma_{ij} (\delta_i^2 + \delta_j^2) / (\delta_i + \delta_j)$	${}^{U}M = \Sigma_{ij}[\delta_i - \delta_j + (\delta_i^2 - 2\delta_i\delta_j + 5\delta_j^2)^{0.5}]/2$
${}^{Ho}M = \Sigma_{ij}(\delta_i^{p} + \delta_j^{p})^{1/p} / 2$	${}^{L}M = \Sigma_{ij}(\delta_i^{p} + \delta_j^{p}) / (\delta_i^{p-1} + \delta_j^{p-1})$	${}^{St}M = \Sigma_{ij}[(\delta_i^{p} - \delta_j^{p}) / (p\delta_i + p\delta_j)]^{1/(p-1)}$

A: Arithmetic; G: geometric; *H*: harmonic; *R*: root mean square; *S*: symmetric; *U*: unsymmetric; *Ho*: Hölder; *L*: Lehmer; *St*: pseudo-Stolarsky

Table 3. Six properties of organic solvents plus their molar mass M (g·mol^{-1}): ***T_b***, boiling point (K); ***d***, density (at 20°C±5°C relative to water at 4°C, g/cc); ***FP***, flash point (K); ***η***, viscosity (Cpoise, 20°C; [1]at 25°C, [2] at 15°C); ***γ***, surface tension (mN/m at 25°C); and ***El***, Elutropic value (silica).

Solvent	*M*	T_b	*d*	*FP*	*η*	*γ*	*El*
(°)Acetone	58.1	329	0.791	256	0.32	23.46	0.43
(°)Acetonitrile	41.05	355	0.786	278	0.37	28.66	0.50
Benzene	78.1	353	0.84	262	**0.65**	28.22	0.27
Benzonitrile	103.1	461	1.010	344	1.24[1]	38.79	
1-Butanol	74.1	391	0.810	308	2.95	**24.93**	
(°)2-Butanone	72.1	353	0.805	270	0.40	23.97	0.39
Butyl Acetate	116.2	398	0.882	**295**	**0.73**	**24.88**	
CS_2	76.1	319	1.266	**240**	0.37	**31.58**	
CCl_4	153.8	350	1.594		**0.97**	**26.43**	0.14
Cl-Benzene	112.6	405	1.107	296	0.80	32.99	
1Cl-Butane	92.6	351	0.886	267	0.35	23.18	
$CHCl_3$	119.4	334	1.492		0.57	26.67	**0.31**
Cyclohexane	84.2	354	0.779	255	1.00	24.65	0.03
(°)Cyclopentane	70.1	323	0.751	236	0.47	21.88	
1,2-diCl-Benzene	147.0	453	1.306	**338**	1.32		
1,2-diCl-Ethane	98.95	356	1.256	288	0.79	31.86	

diCl-Methane	84.9	313	1.325		0.44	27.20	0.32
N,*N*-diM-Acetamide	87.1	**438**	**0.937**	343			
N,*N*-diM-Formamide	73.1	**426**	**0.944**	**330**	0.92		
1,4-Dioxane	88.1	374	1.034	285	**1.54**	**32.75**	
Ether	74.1	308	0.708	**233**	0.24	16.95	0.29
Ethyl acetate	88.1	350	0.902	270	0.45	23.39	**0.45**
(°)Ethyl alcohol	46.1	351	0.785	281	1.20	21.97	
Heptane	100.2	371	0.684	272		19.65	0.00
Hexane	86.2	**342**	**0.659**	**250**	0.33	17.89	0.00
2-Methoxyethanol	76.1	398	0.965	319	**1.72**	30.84	
(°)Methyl alcohol	32.0	338	0.791	284	0.60	22.07	0.73
4-Me-2-Pentanone	100.2	391	0.800	286			
2-Me-1-Propanol	74.1	381	0.803	310			
2-Me-2-Propanol	74.1	356	0.786	277		19.96	
DMSO	78.1	462	1.101	368	2.24	**42.92**	
(°)Nitromethane	61.0	374	1.127	308	0.67	36.53	
1-Octanol	130.2	469	0.827	354	10.6[2]	27.10	
(°)Pentane	72.15	309	0.626	224	0.23	15.49	0.00*
3-Pentanone	86.1	375	0.853	279		24.74	
(°)1-Propanol	60.1	370	0.804	288	2.26	23.32	
(°)2-Propanol	60.1	356	0.785	295	2.30	20.93	0.63
Pyridine	79.1	388	0.978	293	0.94	36.56	0.55
tetraCl-Ethylene	165.8	394	1.623		0.90		
(°)tetra-Hydrofuran	72.1	340	0.886	256	0.55		0.35*
Toluene	92.1	**384**	**0.867**	277	0.59	27.93	0.22
1,1,2triCl,triFEthane	187.4	**321**	**1.575**		**0.69**		**0.02**
2,2,4-triMe-Pentane	114.2	372	0.692	266	0.50		0.01
o-Xylene	106.2	**417**	**0.870**	**305**	0.81	29.76	
p-Xylene	106.2	411	0.866	300	0.65	28.01	
(°)Acetic acid	60.05	391	1.049			27.10	
Decaline	138.2	**465**	**0.879**				
diBr-Methane	173.8	370	1.542			**39.05**	
1,2-diCl-Ethylen(Z)	96.9	334	1.284				
(°)1,2-diCl-Ethylen(E)	96.9	321	1.255				
1,1-diCl-Ethylen	96.9	**305**	**1.213**				
Dimethoxymethane	76.1	315	0.866				
(°)Dimethylether	46.1	249					
Ethylen Carbonate	88.1	511	1.321				
(°)Formamide	45.0	484	1.133			57.03	
(°)Methylchloride	50.5	249	0.916				
Morpholine	87.1	402	1.005				

Quinoline	129.2	510	1.098	42.59
(°)SO_2	64.1	263	1.434	
2,2-tetraCl-Ethane	167.8	**419**	**1.578**	35.58
tetraMe-Urea	116.2	450	0.969	
triCl-Ethylen	131.4	360	1.476	

(°) externally validated compounds; underlined **bold** values: test compounds used in ANN-MLP calculations, * for this property these two compounds are included in the training set {TR} (Table 4) and training + test sets {TR + TE} (Table 5).

Table 4. Best set of descriptors for the properties of Table 3 with MLS methodology. 1st column: δ^v type for the valence-dependent indices. 2nd column: set of descriptors and their statistical quality.

δ^v - type	Regression equations
$\delta^v_{po}(1)$	$\boldsymbol{T_b} = 237.5 + 139.1\,{}^0\chi + 24.69 D^v + 527.7\,{}^0\psi_I - 25.91\,{}^1\psi_I - 1500\,{}^0\psi_E + 41.53\,T_{\Sigma/M}$ (24, 31, 3.5, 69, **21**, 222, 10) N(TR) = 45, r^2 = 0.821, s = 22; N(+16EV) = 61, r^2 = 0.792, s = 25
$\delta^v_{ppo}(-0.5)$	$\boldsymbol{d} = 0.733 + 0.024 D^v + 0.211\,{}^0\chi^v + 1.463\,{}^1\chi^v_s - 0.022\,{}^S\psi_E + 0.148\,\Delta$ (0.06, 0.002, 0.02, 0.3, 0.002, 0.01) N(TR) = 45, r^2 = 0.939, s = 0.07; N(+15EV) = 60, r^2 = 0.914, s = 0.08
$\delta^v_{po}(-0.5)$	$\boldsymbol{\gamma} = 8.683 + 0.386 D^v + 397.6\,{}^1\chi^v_s + 151.9\,{}^T\psi_I - 502.4\,{}^1\psi_{Is} + 3.347\,\Delta$ (2.3, 0.05, 57, 36, 90, 0.7) N(TR) = 29, r^2 = 0.835, s = 3.1; N(+10EV) = 39, r^2 = 0.792, s = 3.1
$\delta^v_{ppo}(0.5)$	$\boldsymbol{FP} = 387.1 + 26.99\,{}^HM - 94.38\,{}^HM_I + 33.03\,{}^GM_E + 114.5\,{}^UM_I - 83.10\,{}^{Ho}M_E$ (26, 6.2, 12, 5.2, 13, 11) N(TR) = 29, r^2 = 0.829, s = 16; N(+11EV)= 40, r^2 = 0.764, s = 17
$\delta^v_{po}(-0.5)$	$\boldsymbol{\eta} = -0.216 + 0.001\,{}^1\chi_d + 0.486\,{}^1\psi_I + 2.20\cdot10^{-5}\,{}^1\psi_{Id} - 3.83\cdot10^{-6}\,{}^0\psi_{Ed} + 0.098\,\Sigma$ (0.2, 0.0003, 0.1, $7\cdot10^{-6}$, 10^{-7}, 0.01) N(TR) = 28, r^2 = 0.969, s = 0.4; N(+10EV) = 38, r^2 = 0.939, s = 0.4
$\delta^v_{ppo}(1)$	$\boldsymbol{EI} = 0.018 + 0.181\cdot10^{-3}\,{}^1\chi_d - 0.675\cdot10^{-6}\,{}^1\chi^v_d + 0.003\,{}^0\psi_{Id} + 140.8\,T_{\Sigma/M}$ (0.02, 0.00006, 10^{-7}, 0.0004, 14) N(TR) = 15, r^2 = 0.934, s = 0.06; N(+3EV) = 18, r^2 = 0.931, s = 0.06

*Me = Methyl, THF = tetrahydrofuran, Et = Ethyl.

Table 5. ANN results for the set of descriptors of Table 4 with one hidden neuron. 1st column: the δ^{v}-*type* and the *Ntr* value; 2nd column: ANN-MLP architecture, the abbreviation for the activation functions for the hidden and output layers, the number of epochs, and training and test errors; 3rd column: input indices, their sensitivity value, and statistical parameters for the training plus test, a[*N*(*a*TR + *b*TE)], and plus the evaluation compounds: [*N*(*a*TR + *b*TE + *c*EV)].

δ^{v}-*type*	*ANN-MLP*	(***Variables***) → ***Property***
$\delta^{v}_{po}(1)$ $Ntr = 10^5$	**6 - 1 - 1** (*e*, *l*)* 41 0.005/0.003	$(^{0}\chi, D^{v}, {}^{0}\psi_{I}, {}^{1}\psi_{I}, {}^{0}\psi_{E}, T_{\Sigma/M}) \rightarrow T_b$ (30.67, 34.22, 41.80, 1.111, 15.76, 2.291) *N*(36TR + 9TE) = 45, r^2 = 0.850, *s* = 21; *N*(+ 16EV) = 61 r^2 = 0.820, *s* = 23 Excluded outlier: dMe-Ether & SO_2 ϵ {EV}
$\delta^{v}_{ppo}(-0.5)$ $Ntr = 10^3$	**5 - 1 - 1** (*t*, *t*) 33 0.002/0.0006	$(D^{v}, {}^{0}\chi^{v}, {}^{1}\chi^{v}_{s}, {}^{S}\psi_{E}, \Delta) \rightarrow d$ (17.99, 8.653, 2.953, 41.31, 12.37) *N*(36TR + 9TE) = 45, r^2 = 0.956, *s* = 0.1; *N*(+ 15EV) = 60, r^2 = 0.930, *s* = 0.1 Excluded outliers: MeCl & MeOH ϵ {EV}
$\delta^{v}_{po}(-0.5)$ $Ntr = 10^5$	**5 - 1 - 1** (*e*, *t*) 27 0.005/0.006	$(D^{v}, {}^{1}\chi^{v}_{s}, {}^{T}\psi_{I}, {}^{1}\psi_{Is}, \Delta) \rightarrow \gamma$ (9.086, 34.48, 34.44, 45.45, 2.328) *N*(22TR + 7TE) = 29, r^2 = 0.841, *s* = 2.8; *N*(+ 10EV) = 39, r^2 = 0.705, *s* = 3.7 Excluded outlier: nitromethane & formamide ϵ {EV}
$\delta^{v}_{ppo}(0.5)$ $Ntr = 10^3$	**5 - 1 - 1** (*e*, *e*) 39 0.009/0.009	$({}^{H}M, {}^{H}M_{I}, {}^{G}M_{E}, {}^{U}M_{I}, {}^{Ho}M_{E}) \rightarrow FP$ (445.1, $1.44\cdot10^6$, $2.65\cdot10^6$, $4.22\cdot10^6$, $17\cdot10^6$) *N*(22TR + 7TE) = 29, r^2 = 0.801, *s* = 16; *N*(+ 11EV) = 40, r^2 = 0.769, *s* = 16 Excluded outliers: 2Me-Butane ϵ {EV}
$\delta^{v}_{po}(-0.5)$ $Ntr = 10^3$	**5 - 1 - 1** (*e*, *l*) 17 0.0006/0.0004	$({}^{1}\chi_{d}, {}^{1}\psi_{I}, {}^{1}\psi_{Id}, {}^{0}\psi_{Ed}, \Sigma) \rightarrow \eta$ (1.982, 1.509, 1.060, 12.04, 3.824) *N*(22TR + 6TE) = 28, r^2 = 0.972, *s* = 0.3; *N*(+ 10EV) = 38, r^2 = 0.942, *s* = 0.4 Excluded outlier: MeOH ϵ {EV}
$\delta^{v}_{ppo}(1)$ $Ntr = 10^3$	**4 - 1 - 1** (*i*, *i*) 20 0.002/0.0003	$({}^{A}M^{v}, {}^{H}M_{E}, {}^{G}M_{E}, {}^{St}M_{I}) \rightarrow EI$ (52.93, 3072, 3020, 27.81) *N*(12TR + 3TE) = 15, r^2 = 0.966, *s* = 0.04; *N*(+ 3EV) = 18, r^2 = 0.955, *s* = 0.04 pentane and THF ϵ {TR}; Excluded. outlier.: MeOH & 2-propanol ϵ {EV}

* Activation functions: *e* = exponential, *i* = identity, *l* = logistic, *t* = tanh. *s* = sin.

Table 6. ANN - MLP results for the set of descriptors of Table 4 with externally imposed number of hidden neurons. 1st column: the δ^v-*type* and the *Ntr* value; 2nd column: ANN-MLP architecture, the abbreviation for the activation functions for the hidden and output layers, the number of epochs, and training and test errors; 3rd column: input indices, their sensitivity value, and statistical parameters for the training plus test, a[*N*(*a*TR + *b*TE)], and plus the evaluation compounds: [*N*(*a*TR + *b*TE + *c*EV)].

δ^v-*type*	*ANN-MLP*	(***Variables***) → ***Property***
	6 - 2 - 1	$({}^0\chi, D^v, {}^0\psi_I, {}^1\psi_I, {}^0\psi_E, T_{\Sigma/M}) \rightarrow T_b$
$\delta^v_{po}(1)$	(*t*, *t*)	(18.17, 50.17, 138.5, 6.414, 93.87, 4.392)
$Ntr = 10^3$	73	*N*(36TR + 9TE) = 45, r^2 = 0.891, *s* = 17; *N*(+ 16EV) = 61 r^2 = 0.871, *s* = 20
	0.004/0.002	Excluded outlier: SO_2 & MeOH ϵ {EV}
	5 - 4 - 1	$(D^v, {}^0\chi^v, {}^1\chi^v_s, {}^S\psi_E, \Delta) \rightarrow d$
$\delta^v_{ppo}(-0.5)$	(*t*, *l*)	(41.54, 29.37, 9.057, 47.73, 29.59)
$Ntr = 10^3$	58	*N*(36TR + 9TE) = 45, r^2 = 0.990, *s* = 0.04; *N*(+ 15EV) = 60, r^2 = 0.966, *s* = 0.1
	0.0004/0.000	Excluded outliers: formamide & MeCl ϵ {EV}.
	5 - 4 - 1	$(D^v, {}^1\chi^v_s, {}^T\psi_I, {}^1\psi_{Is}, \Delta) \rightarrow \gamma$
$\delta^v_{po}(-0.5)$	(*t*, *e*)	(1285, 21.98, 2093, 62687, 5.853)
$Ntr = 10^5$	36	*N*(22TR + 7TE) = 29, r^2 = 0.908, *s* = 2.1; *N*(+ 10EV) = 39, r^2 = 0.871, *s* = 2.4
	0.004/0.002	Excluded outlier: nitromethane & formamide ϵ {EV}
	5 - 5 - 1	$(D, {}^1\psi_{Is}, {}^0\psi_{Ed}, \Delta, T_{\Sigma/M}) \rightarrow FP$
$\delta^v_{po}(1)$	(*t*, *l*)	(8.683, 2.965, 1.212, 5.431, 5.439)
$Ntr = 10^5$	35	*N*(22TR + 7TE) = 29, r^2 = 0.919, *s* = 10; *N*(+ 11EV) = 40, r^2 = 0.860, *s* = 13
	0.003/0.009	Excluded outliers: nitromethane ϵ {EV}
	5 - 3 - 1	$({}^1\chi_d, {}^1\psi_I, {}^1\psi_{Id}, {}^0\psi_{Ed}, \Sigma) \rightarrow \eta$
$\delta^v_{po}(-0.5)$	(*e*, *l*)	(4.609, 5.914, 1.286, 15.86, 6.803)
$Ntr = 10^5$	35	*N*(22TR + 6TE) = 28, r^2 = 0.982, *s* = 0.3; *N*(+ 10EV) = 38, r^2 = 0.975, *s* = 0.3
	0.0003/0.000	Excluded outlier: 2-butanone ϵ {EV}
$\delta^v_{ppo}(1)$	**4 - 2 - 1**	$({}^AM^v, {}^HM_E, {}^GM_E, {}^{St}M_I) \rightarrow EI$
	(*t*, *s*)	(80.08, 3075, 2819, 34.79)
$Ntr = 10^3$	22	*N*(12TR + 3TE) = 15, r^2 = 0.973, *s* = 0.03; *N*(+ 3EV) = 18, r^2 = 0.976, *s* = 0.03
	0.001/0.003	pentane and THF ϵ {TR}; excluded outliers: acetonitrile & 2-propanol ϵ {EV}

Table 7. ANN - MLP results with the number of hidden neurons chosen by Statistica 8. Descriptors are those of Table 4. 1st column: the δ^v-*type* and the *Ntr* value; 2nd column: ANN-MLP architecture, the abbreviation for the activation functions for the hidden and output layers, the number of epochs, and training and test errors; 3rd column: input indices, their sensitivity value, and statistical parameters for the training plus test, a[*N*(*a*TR + *b*TE)], and plus the evaluation compounds: [*N*(*a*TR + *b*TE + *c*EV)].

δ^v(*type*)	*ANN-MLP*	(***Variables***) → ***Property***
	6 - 11 - 1	$({}^0\chi, D^v, {}^0\psi_I, {}^1\psi_I, {}^0\psi_E, T_{\Sigma/M}) \rightarrow T_b$
$\delta^v_{po}(1)$	(*t*, *t*)	(17.98, 45.18, 106.2, 2.556, 72.23, 3.579)
$Ntr = 10^3$	39	*N*(36TR + 9TE) = 45, r^2 = 0.846, *s* = 21; *N*(+ 16EV) = 61 r^2 = 0.826, *s* = 24
	0.005/0.005	Excluded outlier: MeOH & SO_2 ∈ {EV}
	5 - 8 - 1	$(D^v, {}^0\chi^v, {}^1\chi^v_s, {}^S\psi_E, \Delta) \rightarrow d$
$\delta^v_{ppo}(-0.5)$	(*t*, *l*)	(20.47, 8.414, 4.606, 49.56, 19.77)
$Ntr = 10^5$	18	*N*(36TR + 9TE) = 45, r^2 = 0.970, *s* = 0.05; *N*(+ 15EV) = 60, r^2 = 0.938, *s* = 0.07
	0.001/0.001	Excluded outliers: MeCl & MeOH ∈ {EV}
	5 - 10 - 1	$(D^v, {}^1\chi^v_s, {}^T\psi_I, {}^1\psi_{Is}, \Delta) \rightarrow \gamma$
$\delta^v_{po}(-0.5)$	(*l*, *s*)	(18.16, 81.96, 74.19, 173.8, 2.809)
$Ntr = 10^3$	42	*N*(22TR + 7TE) = 29, r^2 = 0.890, *s* = 2.3; *N*(+ 10EV) = 39, r^2 = 0.851, *s* = 2.6
	0.004/0.002	Excluded outlier: nitromethane & formamide ∈ {EV}
	5 - 4 - 1	$(D, {}^1\psi_{Is}, {}^0\psi_{Ed}, \Delta, T_{\Sigma/M}) \rightarrow FP$
$\delta^v_{po}(1)$	(*l*, *l*)	(6.663, 2.542, 1.105, 4.616, 3.220)
$Ntr = 10^5$	81	*N*(22TR + 7TE) = 29, r^2 = 0.899, *s* = 11; *N*(+ 11EV) = 40, r^2 = 0.840, *s* = 14
	0.003/0.01	Excluded outliers: 2Me-Butane ∈ {EV}
	5 - 3 - 1	$({}^1\chi_d, {}^1\psi_I, {}^1\psi_{Id}, {}^0\psi_{Ed}, \Sigma) \rightarrow \eta$
$\delta^v_{po}(-0.5)$	(*e*, *l*)	(6.071, 4.640, 1.164, 14.16, 7.089)
$Ntr = 10^3$	26	*N*(22TR + 6TE) = 28, r^2 = 0.981, *s* = 0.3; *N*(+ 10EV) = 38, r^2 = 0.974, *s* = 0.3
	0.0003/0.0003	Excluded outlier: 2-butanone ∈ {EV}
	4 - 5 - 1	$({}^AM^v, {}^HM_E, {}^GM_E, {}^{St}M_I) \rightarrow EI$
$\delta^v_{ppo}(1)$	(*t*, *t*)	(66.31, 355.9, 331.7, 27.55)
$Ntr = 10^5$	49	*N*(12TR + 3TE) = 15, r^2 = 0.973, *s* = 0.03; *N*(+ 3EV) = 18, r^2 = 0.973, *s* = 0.03
	0.002/0.001	pentane and THF ∈ {TR} and excluded MeOH & 2-propanol ∈ {EV}

Table 8. ANN results for the set of descriptors of Table 4 with only one hidden neuron but where either one or two indices have been left out, usually, those with lowest sensitivity values in Table 5. For the structure of this table see Table 5. Only satisfactory results are shown here.

δ^v-type	ANN-MLP	(***Variables***) → ***Property***
	4 - 1 - 1	$({}^0\chi, D^v, {}^0\psi_I, {}^0\psi_E) \rightarrow T_b$
$\delta^v_{po}(1)$	(*e*, e)	(816.3, 863.6, 110900, 7016972)
$Ntr = 10^5$	25	N(36TR + 9TE) = 45, r^2 = 0.758, s = 26; N(+ 16EV) = 61 r^2 = 0.714, s = 29
	0.008/0.008	Excluded outlier: dMe-Ether & SO_2 ϵ {EV}
	4 - 1 - 1	$(D^v, {}^0\chi^v, {}^s\psi_E, \Delta) \rightarrow d$
$\delta^v_{ppo}(-0.5)$	(*I*, *t*)	(11.01, 7.934, 28.40, 4.905)
$Ntr = 10^3$	17	N(36TR + 9TE) = 45, r^2 = 0.917, s = 0.1; N(+ 15EV) = 60, r^2 = 0.895, s = 0.1
	0.004/0.002	Excluded outliers: SO_2 & Formamide ϵ {EV}
	4 - 1 - 1	$({}^HM_I, {}^GM_E, {}^UM_I, {}^{Ho}M_E) \rightarrow FP$
$\delta^v_{ppo}(0.5)$	(*i*, *I*)	(10.65, 14.68, 15.90, 12.16)
$Ntr = 10^5$	26	N(22TR + 7TE) = 29, r^2 = 0.719, s = 19; N(+ 11EV) = 40, r^2 = 0.702, s = 18
	0.01/0.02	Excluded outliers: 2Me-Butane ϵ {EV}
	3 - 1 - 1	$({}^1\chi_d, {}^0\psi_{Ed}, \Sigma) \rightarrow \eta$
$\delta^v_{po}(- 0.5)$	(*t*, *i*)	(1.603, 15.54, 10.70)
$Ntr = 10^3$	67	N(22TR + 6TE) = 28, r^2 = 0.965, s = 0.4; N(+ 10EV) = 38, r^2 = 0.917, s = 0.5
	0.0007/0.0003	Excluded outlier: MeOH ϵ {EV}

Table 9. Statistical, N/r^2(2nd decimal figure)*/s*, results for the six properties from Tables 4 to 7. **2nd column:** *MLS*, results, **3rd column**: *ANN* with one hidden neuron (*ANN **1**HN*) results, **4th column**: *ANN* with externally chosen number of hidden neurons (*ANN enHN*) results, **5th column**: *ANN* with software chosen number of hidden neurons (*ANN snHN*) results. First line shows the statistical results for the training (MLS) and train plus test (ANN) compounds, the second line shows the overall statistical results inclusive the evaluated compounds. ***M*** (bold and italics) stands for MMCIs (otherwise they are MCIs). In the last two columns are also given the number of hidden neurons (second line, italics and bold).

P	*MLS* (Table 4)	*ANN **1**HN* (Table 5)	*ANN enHN* (Table 6)	*ANN snHN* (Table 7)
T_b	45 / 0.82 / 22	45 / 0.85 / 21	45 / 0.89 / 17	45 / 0.85 / 21
	61/ 0.79 / 25	61 / 82 / 23	***2*** / 61 / 87 / 20	***11*** / 61 / 0.83 / 24
d	45/ 0.94 / 0.07	45 / 0.96 / 0.1	45 / 0.99 / 0.04	45 / 0.97 / 0.05
	60 / 0.91 / 0.08	60/ 0.93 / 0.1	*4* / 60 / 0.97 / 0.1	*8* / 60 / / 0.94 / 0.07
γ	29 / 0.84 / 3.1	29 / 0.84 / 2.8	29 / 0.91 / 2.1	29 / 0.89 / 2.3
	39 / 0.79 / 3.1	39 / 0.71 / 3.7	***4*** / 39 / 0.87 / 2.4	***10*** / 39 / 0.85 / 2.6
FP	***M*** / 29 / 0.83 / 16	***M*** / 29 / 0.80 / 16	29 / 0.92 / 10	29 / 0.90 / 11
	40 / 0.76 / 17	40 / 0.77 / 16	***5*** / 40 / 0.86 / 13	***4*** / 40 / 0.84 / 14
η	28 / 0.97 / 0.4	28 / 0.97 / 0.3	28 / 0.98 / 0.3	28 / 0.98 / 0.3
	38 / 0.94 / 0.4	38 / 0.94 / 0.4	***3*** / 38 / 0.98 / 0.3	***3***/ 38 / 0.97 / 0.3
El	15 / 0.93 / 0.06	***M*** / 15 / 0.97 / 0.04	***M*** / 15 / 0.97 / 0.03	***M*** / 15 / 0.97 / 0.03
	18 / 0.93 / 0.06	18 / 0.96 / 0.04	***2*** / 18 / 0.98 / 0.03	***5*** / 18 / 0.97 / 0.03

Conclusions:

The first interesting result of the present ANN computations is that they prefer MCIs instead of MMCIs, especially with properties with relatively large number of points. In fact, only *El*, with minimal number of points is advantageously described by MMCI when ANN with more than one hidden neuron is used. The second result being that it is better to impose from outside the number of hidden neurons. The third result being that it is better to run ANNs using quite different numbers of networks to train, *Ntr*. The fourth and the more interesting result being that normally ANN improve over the MLS calculations, but also that in many cases this improvement is not striking.

It should be remembered that MLS is anyway used to derive the best set of descriptors that are passed over to the ANNs, and that its statistical results are definitive, i.e., no matter how many times you repeat the calculations with the same indices you will obtain always the same results at every statistical level. ANN results are instead unsystematic and non-reproducible as the weights of the ANN

computations start from random values and the minimization procedure usually ends up with different values from run to run. This fuzzy character has nevertheless a positive side as if ANN computations are run over and over again the probability to end up with a quite good result is increasing. *ANN* results obtained with one hidden neuron either with the full set of descriptors (Table 5), or with a reduced set of descriptors, like in Table 8, if on one side they confirm the validity of the MLS calculations on the other side they leave open the possibility that somewhere there are ANN calculations that improve over them.

Anyway, (*i*) before throwing away a bad model for the training plus test compounds with ANN computations think it twice because they could hide a very good model for the evaluated compounds, and (*ii*) do not throw away the hydrogen atoms in calculations with MCIs or MMCIs as in many cases they are of good help.

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S2SNET Model for prediction of epitopes in vaccine design

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The prediction of immunogenic peptides that can be used for production of antipeptide antibodies is of great importance for design of vaccines however a problem in immunology is the impact on the immunological response after of a perturbation or variation in the sequence of a known peptide and/or other boundary conditions. Methods that establish mathematical models to identify the structure-Activity/Property (QSAR/QSPR) relationships have been developed in the past. On the other hand, Epitope Data- base (IEDB) http://www.iedb.org/, released public data useful for these studies. Specifically, Perturbation Theory QSAR method (PT-QSAR) has been used to predict B-epitopes from IEDB database. This method adds variation terms to a known experimental solution of one problem to approach a solution for a related problem without known exact solution. In this specific case, the method predicts the epitope activity $E_q(c_j)$ of one query peptide (q) in a set of experimental conditions (c_j). In so doing, the method uses as input the epitope activity $E_r('c_j)$ of one similar peptide already known that is used as peptide of reference (r); which have been assayed on the same or a different set of experimental conditions ($'c_j$). The method also uses as input the information about the sequences and conditions of assay of both peptides in the pair. In the present study we developed a model able to classify 500000 cases of perturbations with accuracy, sensitivity to 99%, and specificity 100% for training validation series. The perturbations include structural changes in 83683 peptides determined in experimental assays with boundary conditions involving 1448 epitope organism name, 2283 host organisms, 15 biological process, 28 experimental techniques and 505 possible adjuvants. The model may be useful for the prediction and optimization in silico of new epitopes under different boundary conditions for vaccine development [1–4].

Keywords: Vaccine design, QSAR, Cheminformatics

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Modeling the association between the seasonal asthma prevalence and upper respiratory infections.

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Abstract: Asthma affects a considerable amount of people worldwide from pediatric to elderly age group. This communication addresses the association between the seasonality of asthma in South and Central Florida with the occurrence of Upper Respiratory Track Infections (URTIs). Motivated by a statistical analysis of the Emergency Department visits due to both, asthma as the primary diagnosis and URTIs excluding asthma for eight consecutive years, a compartmental model, that extends the SEIR model is analyzed and contrasted with health data. It is hypothesized that asthma seasonality is likely to be associated with the thermal stress generated during the process of respiration in winter months, predisposing the lining of the epithelial cells due to the lack of humidity along the respiratory tract. Such a situation exposes the body to infections and exacerbates the response of the immune system accordingly. At the end, an inflammatory process results and asthma develops.

Keywords: *asthma, upper respiratory tract infections, thermal stress, SEIR dynamical systems, and seasonality.*

Introduction

Asthma is a condition affecting a considerable body of people worldwide and contributes to both, the economic and social burden of many families. In a previous communication by one of the authors [1], a statistical analysis of the number of asthma cases reported at Emergency Departments (ED) versus different weather parameters (temperature, humidity, pressure, ozone levels, and particulate matter) was done and resulting in a low correlation between potential predictors and the number of cases. The analysis was performed using two different schemes and including lagging effects: Generalized Additive Linear and Poisson models. The percentage of variability described by these models did not exceed the 25 %. It motivated the authors to look at an indirect effect of weather conditions on asthma prevalence and exacerbation through thermal stress –induced vulnerability to upper respiratory tract infections.

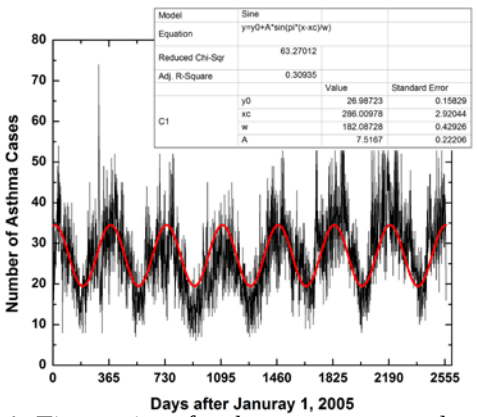

Fig. 1: Time series of asthma cases reported at ED from January 1, 2005 to December 31, 2011. The seasonal component appears in the inset.

Model and Results

The upper respiratory tract plays a very important role in the acclimatization of the human body. Air entering through the nose exchanges humidity and heat with the tract. In winter times, this process leads to sufficient heat losses and therefore, to a considerable thermal stress. As a result, the lining of the upper respiratory tract is affected and immunity depleted. It creates the perfect conditions for infections to penetrate and propagate. Both, asthma and URTIs as a primary diagnosis excluding one another respectively were recorded from counties in the southeast and center of Florida from January 1, 2005 to December 31, 2012. Overall, exacerbations

across South Florida show a seasonal pattern, which peaks between January and February. Time series decomposition is shown in Fig. 1, where the sine function accounts for the periodicity of the events. In standardized variable format (z-variable), the effect goes beyond the standard deviation around the mean number of cases per year, thus it is a measurable condition.

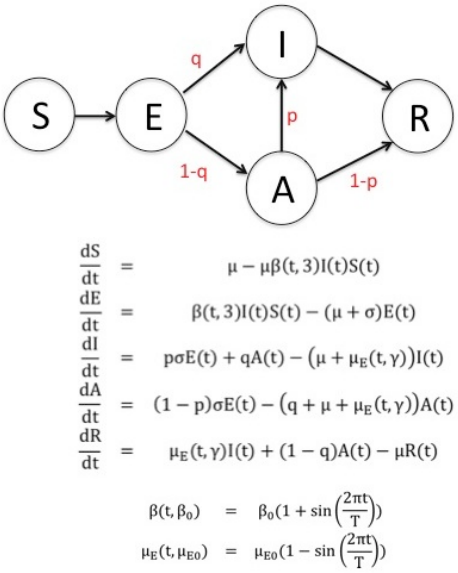

Fig. 2: Scheme of the SEIAR model used in this communication along with the system of ODE resulting from the compartmental representation.

Following the scheme of the SEIR dynamical model, a new one with asymptomatic patients (new compartment, see Fig. 2) is developed in fair agreement with ideas discussed in Ref. [2]. It was labeled as SEIAR model and it was solved with Mathematica. The model was solved for an ensemble of parameters, however after a chi-square test, the values that better fit the recorded number of cases are as follows: $\beta_0 = 3$, $\mu_{E0} = \gamma = 0.24$, $p = 0.7$, $q = 0.3$, $\mu = 0.35$, $\sigma = 0.21$, and $T = 52$.

The solutions for all five variables are depicted in Fig. 3, where the periodicity (seasonality) in the number of infected and asymptomatic individuals is clear. Such a result is also in agreement with results obtained in Refs [3 – 5]. A further analysis of residuals shows that deviations are randomly distributed on both sides of the recorded values with no apparent structure, therefore, it seems to be indicative of epidemic-based triggering channel.

Conclusions

Results of statistical analysis and mathematical modeling support the idea of the weather-induced upper respiratory tract infection for triggering many of the asthma reported cases at ED. A more systematic sensitivity analysis including other triggering options is needed in order to assess how far one option overwhelms the rest. Likewise, it might happen that options that are dominant in some areas might become second hand in others. An ecological epidemiology approach along with mathematical modeling will eventually lead to the understanding of the inner working of asthma episodes.

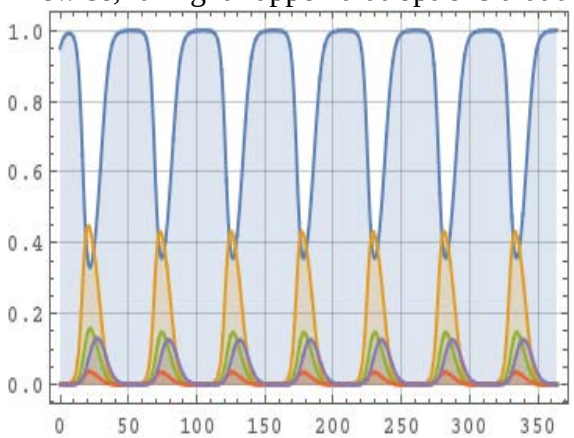

Fig. 3: Solutions of the SEIAR model obtained with Mathematica and showing the seasonality observed in health records.

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 2016, as well as the STEM-TRAC grant PO3C110190.

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Modeling the influence of the dot size distribution on the line shape of the quantum dot based solar cell.

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Abstract: Photovoltaic technologies have been improving over the last years mainly due to advances in the area of nanotechnologies. Multi-junctions solar cells have reached the largest efficiencies of light conversion (around 40 %) under laboratory conditions due to the fact that junction materials might be tuned to work for wider solar bands than single junction cells. At the same time, quantum dot based composites solar cells have emerged as promising candidates to increase the conversion efficiency and durability of operation. In this communication, the effect of the dot size distribution on the photoemission line shape of a quantum dot based composite solar cell is addressed. Three different types of size distribution (homogeneous, Gaussian, and power law) are considered and the photoemission spectrum is calculated as the convolution of the emission from a single quantum dot and the distribution function of their sizes within the composite. Despite of the qualitative agreement with experimental findings there are quantitative differences due to the omission of near field effects when dots are heavily packed and resonance phenomena due to Plasmonic resonances.

Keywords: *nanotechnology, quantum dots, photovoltaic cells, optical response, dot size distribution function.*

Introduction

The increasing energy demands of modern societies and the negative effects of fossil fuels on the environment despite of their concentrated output define two of the main challenges to be addressed by materials science and environmental engineering in order to secure a long term sustainable development. The production of electricity faces also another problem, the increasing urbanization, which creates an imbalance between highly, and populated urban areas and sparse populated rural ones demands from electric grids a smart distribution and control of the loads. Moreover, the cabling in rural areas makes the process of maintenance and distribution somehow inefficient. A better and optimal use of the solar energy might be the possible solution to such challenges. Different approaches have been adopted to enhance the conversion efficiency of the photovoltaic cells (PVC). Multi-junction cells take advantage of different wavelengths of the solar spectrum, however, most of them use toxic chemicals as components. A potential improvement is coming from nano-structured materials, for instance, quantum dots. Quantum dot based solar cells also take advantage of different sections of the solar spectrum. It is conditioned by the sensitivity of quantum transitions and photo-generation on the radii of quantum dots. Therefore, controlling the distribution of quantum dot sizes it is possible to access a wide range of the solar spectrum and to improve the photo-conversion process.

This communication is aimed at exploring the impact of the distribution of quantum dot sizes on the optical response of quantum dot based solar cells. Three types of dot sizes distributions are considered: A homogeneous, a Gaussian with a characteristic size and spread, and a power law with no characteristic size. It is hypothesized that the dot size distribution is controlling the efficiency of the photocells along with the intrinsic physical quantities.

Model and Results

A composite matrix containing quantum dots of spherical shape is considered. The distribution of radii of these quantum dots (QDs) is assumed to be one of the following options: An homogeneous

distribution, with QDs with radii ranging from a minimum to a maximum value with equal probabilities, a Gaussian distribution of radii with a characteristic value and some standard deviation due to fluctuations during the growth process, and finally a power law distribution with no characteristic size and decreasing for large values.

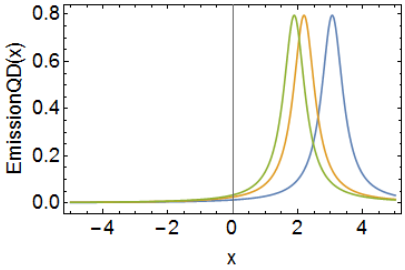

Fig 1: Emission spectrum of a QD for three different radii. The variable x, is the energy difference between levels.

Solving the Schrodinger equation for a single QD in spherical coordinates with the help of Wolfram Mathematica, the energy of the transition can be computed as a function of the QD radius. Assuming a Lorentzian shape for the emission spectrum and substituting the obtained excitation energies, the curves are presented in Fig 1 (blue line corresponds to the smallest radius, while the green one the largest radius). The emission from the whole composite matrix was represented as the convolution of the Lorentzian emission curve for a single QD times the probability distribution function (PDF) of the QD radii. Results are presented in Fig. 2.

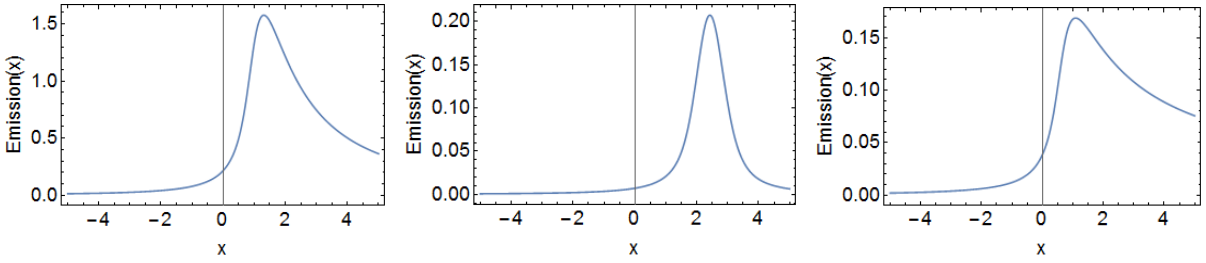

Fig 2: Emission spectrum of the QD obtained for three different distributions of radii: (Left) homogeneous, (Middle) Gaussian, and (Right) Power Law

It is worth to notice some limitations of this study: QDs geometries may differ from the spherical one. They may potentially be ellipsoids and needles; the emission spectrum from a collection of QDs should consider the effects of self-interference from different QDs emitting simultaneously as well as the tunneling between QDs is neglected.

Conclusions

There is valuable information about the distribution of radii in the QDs-based PVC within the emission line-shape. The line-shape control might constitute a method for quality control when QDs-based PVC are grown. Homogeneous distribution of radii tends to produce high emission power. Therefore, the PVC should work at better efficiencies. Real QDs-based PVC are closer to a combination of Gaussians with characteristic radii allowing the dots to emit in different but not infinite range of the electromagnetic spectrum.

Acknowledgments

Authors want to thank St. Thomas University facilities for completing this work during the SRI 2016, as well as the STEM-TRAC grant PO3C110190.

Conflicts of Interest

The authors declare no conflict of interest.

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Modeling the oscillating Belousov – Zhabotinsky chemical reaction

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Abstract: In this communication, the kinetics of the well-known Belousov-Zhabotinsky oscillating reaction is studied. The choice of this particular reaction came from its similitude with circadian rhythms and the molecular clocks involved in the human response to microgravity conditions and the presence of electro-magnetic fields. In the present communication, the chemical kinetics is modeled as a system on nonlinear differential equations following the scheme NKF. Critical points and conditions of stability are obtained, as well as the conditions leading to an oscillatory behavior. Since the study is limited to the kinetics only, convection effects were neglected cause the influence of the magnetic field on the reaction could not be addressed. Even though a system of partial differential equations will include such effect, in this communication, a possible explanation for the Magnetic Field Effect (MFE) is advanced which is in qualitative agreement with experiments performed by another research team from the School of STEM at St. Thomas University.

Keywords: *Belousov-Zhabotinsky, chemical reaction, magnetic field, circadian rhythms, dynamical systems, NKF scheme, chemical kinetics.*

Introduction

Many phenomena in Nature display periodic patterns. Chemical oscillating reactions are involved in biochemical processes, neurological responses, and circadian rhythms [1,2]. One of the conditions that might compromise the life of future astronauts venturing into long term space travels (for example to Mars) is the disruption of circadian rhythms due to outer space electromagnetic fields. Since most of the rhythms are controlled by the central nervous system, and the later operates on neurons, the use of a more simple system to study the expected changes will be desired. In this end, the Belousov-Zhabotinsky (BZ) reaction has emerged as one of the simplest dynamical models capable to address this problem. It is a non-linear oscillatory reaction that involves the production of molecular bromine from bromate and bromide ions in the presence of malonic acid. A realistic and a simple way of studying it would be using the same mathematical model of the Oregonator, created by Richard Field and Richard M. Noyes at the University of Oregon [1].

According to [3], the mechanisms of a magnetic field effect (MFE) on chemical reactions are classified into: (1) Radical pair mechanism, (2) Lorenz force, and (3) Magnetic force, proportional to the product of the magnetic susceptibility gradient of solutes and the square of magnetic flux density. Such a problem is important when addressing long-term space-travel physiology, in particular, the effect of magnetic fields on internal clocks of future astronauts. In this communication, the time evolution of the oscillating BZ chemical reaction is modeled and possible effects of magnetic field are addressed.

Model and Results

This communication is aimed at explaining the changes observed during the experiments performed at the chemistry laboratories in St. Thomas university school of STEM. The set up consists of two Helmholtz coils separated at a distance d, and generating a magnetic field distribution in between. At the middle point between the two coils, a petri dish with the chemical reagents is located such that the BZ reaction can be recorded in both conditions, in absence and presence of magnetic field.

The magnetic field distribution between the two coils was computed in [4] and is given by:

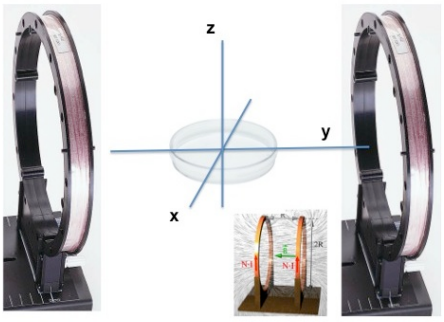

Fig. 1: Experimental setup to study the influence of the magnetic field on the BZ reaction.

$$B_r(r,y) = \frac{\mu_0 I}{2\pi}\frac{y}{r\sqrt{(r+a)^2+y^2}}\left[\frac{a^2+r^2+y^2}{(a-r)^2+y^2}E(k)-K(k)\right]$$

$$B_y(r,y) = \frac{\mu_0 I}{2\pi}\frac{y}{r\sqrt{(r+a)^2+y^2}}\left[\frac{a^2-r^2-y^2}{(a-r)^2+y^2}E(k)-K(k)\right]$$

where $k^2 = \frac{4ar}{(a+r)^2+y^2}$ and E(k) and K(k) are the elliptical integrals of first and second type respectively.

The kinetics of the BZ along with the fluid dynamics is considered as a reaction – diffusion problem. The kinetics follows the scheme FKN modified into the Oregonator model [1], which considers the chemical species X = $HBrO_2$, Y = Br^-, and Z = M_{ox}=Ferroin/Ferritin. The dynamical system in dimensionless form and following the quasi-static approximation (slow variation of Y) can be cast into:

$$\varepsilon\frac{dx}{d\tau} = x(1-x)-\frac{(x-q)}{(x+q)}f_0 z = f(x,z)$$

$$\frac{dz}{d\tau} = x - z = g(x,z)$$

where f_0 is the stoichiometric factor, q is a constant including several rate constants. The value of ε is small.

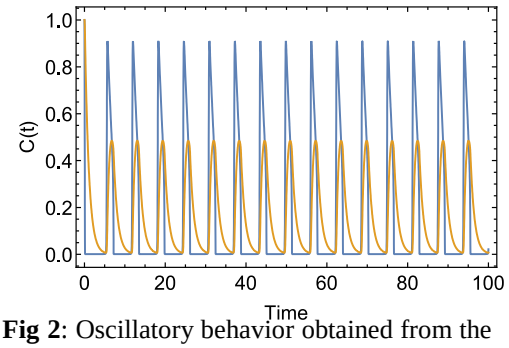

Fig 2: Oscillatory behavior obtained from the Oregonator model of the BZ reaction.

The condition for an oscillatory behavior (center) is equivalent to the requirement of Trace A = 0, while the Det A ≠ 0 (Hopf bifurcation), where A is the Jacobian of the above system.

In order to address the effect of the magnetic force, we will focus on the Ferroin/Ferritin ion. Hence the equation for z must be complemented with the Euler equation:

$$\frac{\partial\vec{v}}{\partial\tau}+(\vec{v}\cdot\vec{\nabla})\vec{v} = \frac{1}{\rho}\vec{\nabla}\left(\frac{B^2}{8\pi}\right)+\frac{(\vec{B}\cdot\vec{\nabla})\vec{B}}{4\pi\rho}$$

The term with B^2 conrresponds to the magnetic pressure and based on the spatial effects observed it does not seem responsible for their inner working. On the other hand, the last term is related with the tension along the magnetic lines, and it seems to be the source of the observed patterns [3]. Further modeling is going on in order to verify this hypothesis.

Conclusions

The Oregonator model is capable to reproduce the oscillatory behavior observed in experiments. The values of the parameter f_0 are critical to the stability of the system. Wave-trains, spirals and other two dimensional patterns require the inclusion of a diffusion term, transforming the Oregonator into a reaction-diffusion system. Magnetic field effects could be accounted by including an advection term, and it is part of an ongoing effort.

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 2016, as well as the STEM-TRAC grant PO3C110190.

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Braess Paradox in Electrical Networks – When more might mean less

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Abstract: Electrical grids are part of the network of connections maintaining a city alive nowadays. Most of these connections are wired in parallel in order to guarantee a sustainable flow of electricity plus being robust enough against failures. The Braess Paradox states that in a congested network, it may happen that adding a new path between destinations can increase the level of congestion. In this communication the Braess Paradox is investigated for several network configurations. Special interest is dedicated to the Wheatstone bridge and to those networks containing such configurations as part of their structural elements. The flow across the network as well as the overall resistance are computed and expressed in terms of network characteristics.

Keywords: *Braess Paradox, Wheatstone bridge, electrical networks, Smart Grid, adjacency matrix, geodesic path, clustering coefficient.*

Introduction

Electrical grids are part of the network of connections maintaining a city alive nowadays. Many times we see a limited amount of lines and poles, as well as, supportive local electrical transformer points (LETP). Most of these connections are wired in parallel in order to guarantee a sustainable flow of electricity plus being robust enough against failures. Why are we not making the system redundant and increasing the number of grid points and cabling? How the performance of a power grid network can be assessed from its connectivity pattern? In answering the first question, we hypothesized that despite of the economic cost of such approach there is a counter intuitive fact known as the Braess Paradox, which states that in a congested network, it may happen that adding a new path between destinations can increase the level of congestion [1 – 4]. This fact is extremely important when you are designing Smart Grids and Cities. The electrical grid can be mapped into a graph or network, where the hubs are vertices and power lines are edges. The idea is to optimize the topology of the network and make it a smart grid. In transportation networks, the phenomenon results from the decisions of network participants who selfishly seek to optimize their own performance metrics. In an electric power distribution network, an analogous increase in congestion can arise as a consequence of Kirchhoff's laws. To address the second question, we also hypothesized that power grid performance might be assessed through a combination of indices characterizing the networks and enabling quantify the easiness of connecting two distant points by walking the shortest path.

In this communication, the Braess Paradox is investigated for several network configurations. Special interest is dedicated to the Wheatstone bridge and to those networks containing such configurations as part of their structural elements. The flow across the network as well as the overall resistance are computed and expressed in terms of network characteristics.

Model and Results

Braess Paradox might be understood by appealing to the concept of signed graphs, in which positive edges (+) represent arcs along which the current flow encounters the least resistance, while negative edges (-) represent arcs with high resistance load. The collection of positive edges forms the shortest path in the model graph. Even though the results shown below are done for 10 different vertices with a varying amount of edges, it might be extended to the analysis of realistic electrical grids. The above algorithm was implemented in Wolfram Language and Mathematica and results are presented below.

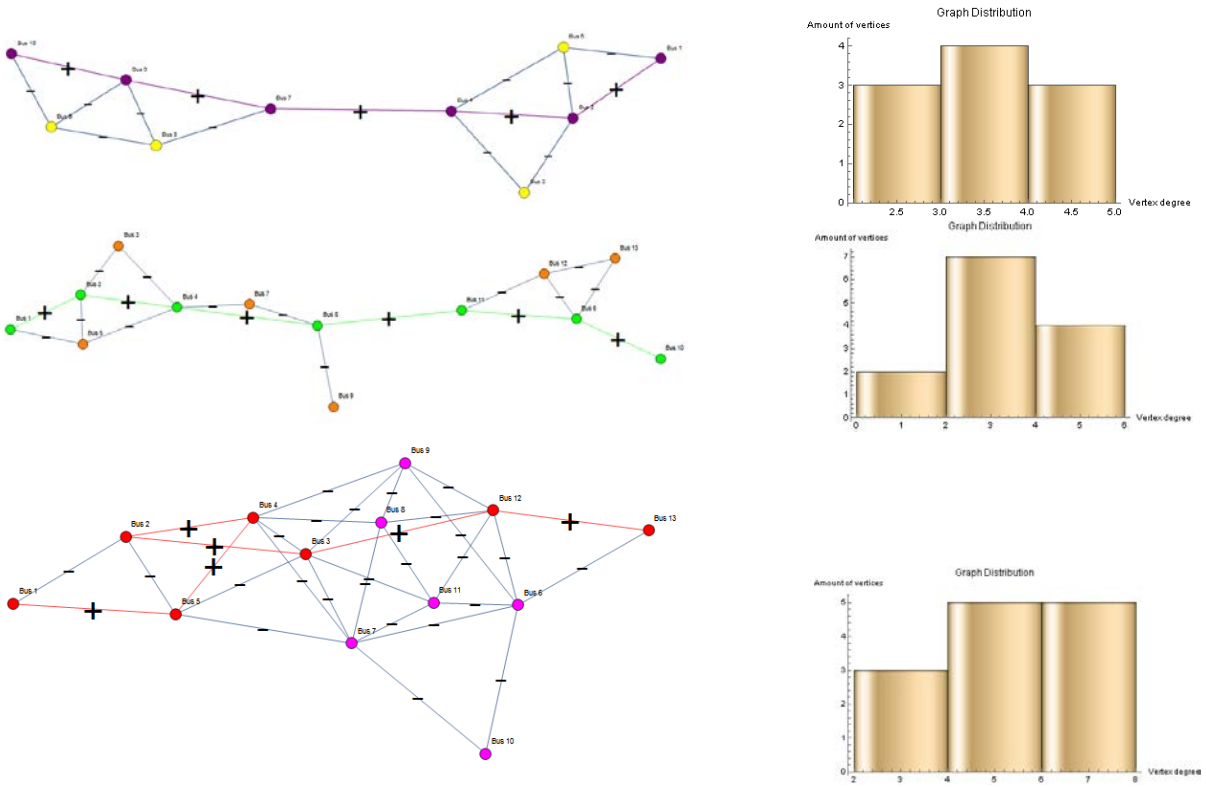

Fig 1: (upper panel) The reduced network graph (10 vertices) where the highlighted lines represent the shortest and convenient path from one node to any other that traverses the least amount of resistances present. These are labeled with "+" while all others are indicated with a "-". (middle panel) Upon creating the 13 - bus system with the added edges to the reduced network, we can see that based on the distribution of "+" & "-" lines in both network, the reduced network is balanced, but the 13 - bus network is unbalanced. Now, by comparing the values of the total resistance traversed between both networks, we see that the reduced network' s path was more efficient in avoiding the larger resistances. (lower panel) After creating a 13-bus network with 31 connections. Actually, the more edges you add to the network, the more unbalanced the system becomes.

Conclusions

Braess Paradox, a concept originally involved with traffic networks and its counterintuitive approach of adding an additional road to alleviate congestion, can be extended to its implications with electrical grids. Specifically, the consequences of erroneously increasing the number of grid points and cablings for better transmission of electrical power in a network may actually decrease the networks level of performance and lead to detrimental loses in electrical power flow and ultimately cause power outages across the grid.

Acknowledgements

Authors want to thank St. Thomas University facilities for completing this work during the SRI 2016, as well as the STEM-TRAC grant PO3C110190.

Conflicts of Interest

The authors declare no conflict of interest.

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Assessment of the impact of micro-meteorological conditions on plants growth

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Abstract: Agrometeorology is an interdisciplinary science aimed at finding and understanding the impact of changing weather conditions on plant's growth and their abilities to adapt to changing conditions. In this communication, the effect of outdoor temperature and humidity on plant's performance (Okinawa Spinach) is studied. To the extent of the period of study (two months during summer 2016) the following results were obtained: Plants were more sensitive to diurnal values of temperature and humidity, the stability of the Okinawa spinach grown in campus is in agreement with the documented facts about the preference of this plant for climatic regions similar to the existing in South Florida, and the type of soil used in the pods slightly affected the measured values.

Keywords: *Agrometeorology, temperature, humidity, chlorophyll level, soil moisture, plant physiology, plant adaptation.*

Introduction

Global climate changes and the acceleration of urbanization all over world constitute serious problems for the health of soils, the microbiome inhabiting in them, and ultimately to the agriculture. Intensive agricultural practices have increased the use of industrial fertilizers, which in many cases remediate temporarily and affect in the long term the soil biochemistry.

Agriculture is one of the most important practices carried by humans since early moments of civilization [1,2]. Agricultural productivity depends on both the quality of soils and weather conditions around plantations. The study of the conditions that might contribute to the soil health, crop yields, as well as the conservation of the microbiome are of tremendous relevance nowadays. In this end, the type of soil where crops and plants grow, the level of humidity, the typical temperatures, and the level of insolation are the physical characteristics that determine the survival and productivity of plants and soils. The yield of the plant is closely related with the efficiency of the photosynthesis. Therefore, the amount of chlorophyll is **a proxy** or indicator of the plant performance. In order to guarantee a sustainable agricultural development a full understanding of the inter-relationships between the biogeochemistry of soils, weather conditions at the micro-scales, and plant physiology and adaptation to changing climates is needed.

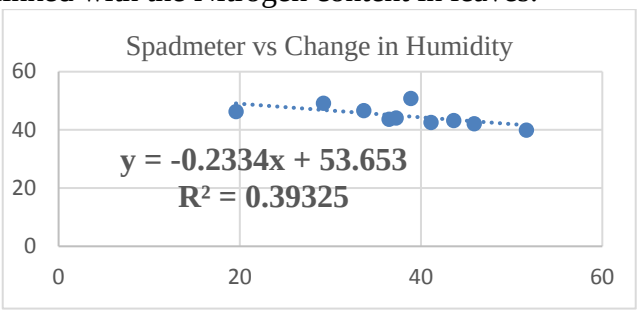
In this communication, the assessment of the impact of the outdoor temperature and humidity around the organic garden is performed, with emphasis on Okinawa Spinach. An evaluation of the micrometeorological conditions using mobile sensors from Pasco and how they compare with meso-scales is done, using the automated weather station operated with Earth-Networks (Weatherbug). Such studies are aimed at evaluating the impact of micrometeorological conditions of the effectiveness of artisanal soil in growing Japanese lettuces.

Experiments and Results

Okinawa Spinach is a dense, low growing plant to 70 cm high. Thriving in warm, wet conditions Okinawa Spinach does best in subtropical and tropical areas; it is sensitive to frost. It grows best in full sun to partial shade. It needs ample water; rich, fertile well-drained soil that is kept mulched and prefers a pH of between 6.1 and 6.5.

Fig1: Experimental set-up with 16 pots with Okinawa Spinach plants arranged under different conditions of insulation and with different soils.

A group of 16 pots with Okinawa spinach (*Gynura crepioides)* are placed outdoor in the botanical garden under different conditions of illumination and soil moisture. Meteorological conditions from the AWS – Weatherbug were recorded on a daily basis with 5 minutes resolution and extreme values included. These readings are representative of the overall weather conditions up to a radius of 5 miles around St. Thomas campus. On the other hand, mobile weather sensors from PASCO permitted the recording of weather conditions around the plants. This way, a comparison between the mesoscale and microscale behaviors can be performed. Additionally, a mobile soil moisture sensor from PASCO is recording the variation of soil moisture of five randomly selected plants. Mobile chlorophyll sensor (spad-meter SPA 502 Pro from Spectrum Technologies Inc.) is used to assess the chlorophyll levels in plants selected for this pilot study [3]. Plants are located in pots with different soils and different conditions of illumination. A higher spad value indicates higher chlorophyll content and then a healthier plant. It is linked with the Nitrogen content in leaves.



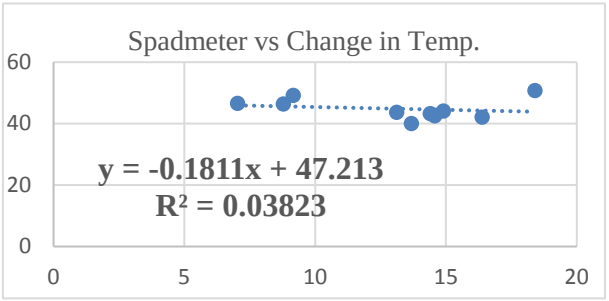


Fig 2: Changes in the measured SPAD values as functions of diurnal ranges of the temperature (left panel) and humidity (right panel) for the pot corresponding to case (d) shown above. As might be noticed, the Okinawa spinach is very tolerant to the diurnal changes in temperature, while it is slightly affected by diurnal changes in humidity.

Conclusions

To the extent of the period of time when measurements were performed is concluded that: No substantial changes are observed with the variations in diurnal values of the temperature; plants are more sensitive to diurnal variations in humidity of the atmosphere; the stability of the Okinawa spinach grown in campus is in agreement with the documented facts about the preference of this plant for climatic regions similar to the existing in South Florida; and the type of soil used in the pods slightly affected the measured values.

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Conflicts of Interest

The authors declare no conflict of interest.

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How might far different network topologies impact the development of epilepsy? – A modeling approach.

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Abstract: Interest in neurological disorders has grown exponentially over the last decade with rapidly developing technologies and more refined diagnostics. Epilepsy is the neurological disorders with well-localized sources of seizures. The understanding of conditions that lead to different types of epileptic seizures of different types, as well as the extent of the damage caused by these seizures is limited. Insight into these issues is especially critical in surgical procedures in cases of epilepsy that are currently non-treatable with medication. In this communication, two models of neuron dynamics (the Kuramoto model and the FitzHugh-Nagumo model) were analyzed. While the FitzHugh-Nagumo network model addresses an ensemble of neurons interacting each other and identifies their synchronic behavior, the Kuramoto model is used to investigate the synchrony between different cortical areas that belong to different brain zones from where EEGs are measured. In both cases, the influence of the connectivity matrix on the dynamical response is studied. Conditions favorable for epileptic seizure were assessed in terms of topological measures of the network. Centrality and clustering values were observed to be the most significant.

Keywords: *Neuronal networks, Fitzhugh Nagumo model, Kuramoto model, network topology, centrality, adjacency matrix, functional and anatomical networks.*

Introduction

Epilepsy is the 4th most common neurological problem in the USA, followed by migraine, 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. 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 [1]. Epilepsy is a medical condition characterized by seizures or disruptions of the electrical communication between neurons. Some epileptic seizures can be controlled with medications while others require surgical interventions. In these cases, surgeons must decide how much of the brain to remove or disconnect. Since our understanding of the inner workings of the brain is still at its infancy, there are many cases in which surgical procedures do not resolve episodic seizures.

This communication is aimed at assessing the relevance of the topology of the neuronal networks and how it impacts the synchronization between many neurons. Based on accumulated experience Refs. [2 – 5], it is hypothesized that some specific changes in neuronal networks are conducive to the appearance of seizures, for example, a lack of synchrony, with an escalating noise spread over extensive areas of the brain, can result in a frustrated dynamic state of neuron bundles. The goal is to translate results into the clinic to improve decision-making and accuracy during surgical procedures.

Model and Results

The brain is considered as one of the most challenging complex systems to be understood. Thus, models presented below are in agreement with methodologies used in complex systems. For instance, we are interested in the interplay between anatomical and functional complex networks. Two models of interest are solved for the sake of simplicity for model-designed networks: the Fitzhugh-Nagumo model which accounts for the dynamics of connected neurons, and the Kuramoto model, which

accounts for synchronization between different patches of cortical areas where neuronal networks are sparse (see Table 1).

Fitzhugh – Nagumo model (microscopic picture)

$$\frac{dv_i}{dt} = v_i - \frac{v_i^3}{3} - w_i + I_{inter} + I_{ext}$$

$$\varepsilon \frac{dw_i}{dt} = v_i + a - bw_i$$

$$I_{inter} = \sum_{j=1}^{N} G_{ij} a_{ij} (I_j - I_i)$$

Kuramoto model (mesoscopic picture)

$$\frac{d\theta_i}{dt} = \omega_i + \sum_{j=1}^{N} \sigma_{ij} a_{ij} \sin(\theta_j - \theta_i)$$

Table 1: Equations defining both models to be explored in this communication. In the case of the Fitzhugh Nagumo model, v represents the action potential of the neuron in the node (i), while w is the complement function. In the case of the Kuramoto model, θ is the phase of the (i) oscillator.

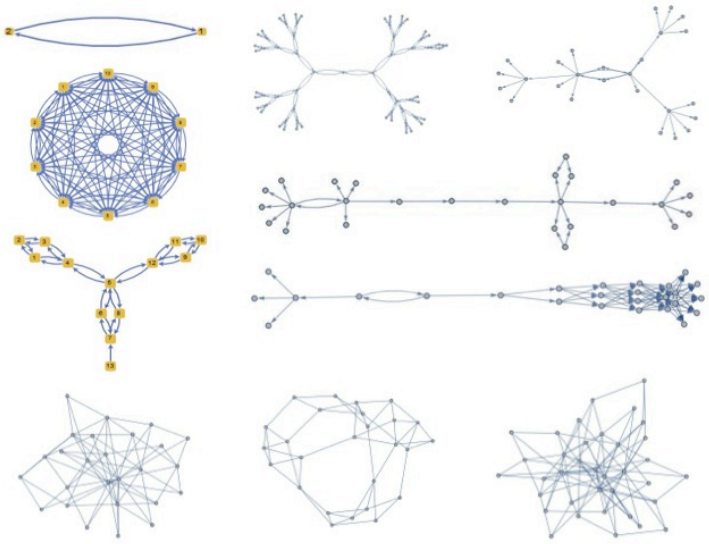

Fig. 1: Network configurations used in this communication. They are representative of local clusters of neurons. The maximum number of nodes was 128, and nodes were connected either through regular graphs or scale-invariant, or random graph.

Examples of neuronal models analyzed within this communication are summarized in Fig. 1. For each of the networks, the adjacency matrix **A** = || a_{ij} || is computed and the weights for connections were randomly generated. Weights were contained in the matrices **G** and **σ** for each of the models. In each case, topological measure as the vertex distribution, centrality, clustering coefficient, network's shortest distance, and synchronization parameters were computed.

Conclusions

Network topology influences the ability of bundle of nodes to reach the state of synchronization. A fair indicator is the clustering coefficient. The state of synchronization may suffer from a phenomenon similar to the Braess paradox observed in road networks. Networks of neurons with bridges are important because they might turn off the overall connectivity between different areas of the brain and therefore influence the appearance of seizures. A comparison with real epileptic brain networks obtained from EEG inverse signal processing, is planned for the future.

Conflicts of Interest

The authors declare no conflict of interest.

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Initial Description of the Local Ionospheric Response to Geomagnetic Storms

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Abstract: Geomagnetic storms are planet-wide disturbances of the Earth's magnetic field, closely related with solar activity events. In this work, we describe the response of our local ionosphere (as per foF2-critical frequency, TEC-total electron content) to geomagnetic activity (ap index) for year 2015. We found that for Equinox and Summer, the ionospheric parameters suffer a depletion from the quiet reference, indicating a more active recombination process due to the presence of fresh molecular mass. For winter conditions the ionospheric parameters increase over the quiet mean, corresponding with a prevalence of atomic elements, resulting in a less predominant recombination process. These results agree with previously published studies of mid to mid-low ionosphere.

Keywords: *ionosphere; ionospheric storm; geomagnetic storm; solar activity*

Author Contributions

Solar activity is the main source for disturbances of the geomagnetic field, which results in perturbations of the ionosphere, a layer of the earth's atmosphere with a high concentration of ions and free electrons. These disturbances are known to disturb technological system (*i.e.*, navigation and communication systems, power lines, generators, and transformers, satellites, etc.), in various degrees, depending on the intensity of the ionospheric storm, geomagnetic location of the system, and technological awareness.

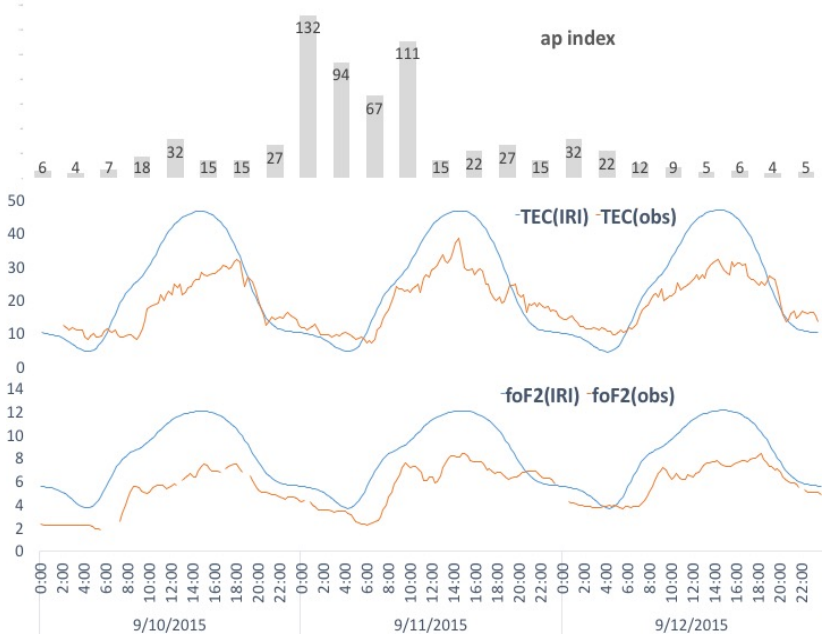

Figure 1: Equinox event showing a depletion of TEC and foF2 values (negative phase). Quiet values in blue, disturbed in brown.

In this work, we study the response of the ionosphere to disturbed events for south Florida, year 2015. The quiet ionospheric values were generated from the International Reference Ionosphere (IRI), while the foF2 disturbed values are from the sounder at the Eglin Air Force Base (EAFB), and the TEC disturbed values and geomagnetic ap-index from the National Geophysical Datacenter. Six events with ap $\geq$ 100 (units of 2 nT) were identified. Due to space restrictions, only two examples are offered, exposing the opposite seasonal ionospheric responses. For

example, Figure 1 shows the level of geomagnetic activity (ap-index, upper panel), quiet (blue) and disturbed (brown) TEC (expressed in 10^{16} e^-/m^2, mid panel), and foF2 (expressed in MHz, lower panel) for the period of September 10-12, 2015 (Equinox). Notice that the time scale at the bottom of the figure is common for all panels. In this figure it is easily observed that both parameters, TEC and foF2, show a consistent trend to lower values respect to the quiet conditions, a so-called negative phase. All Equinox/Summer events display the same behavior, in full agreement with previous results (*i.e.*, Araujo-Pradere *et al.*, 2006, 2002a) for equinox and summer conditions, with the exception of the abnormal ionospheric behavior during the past solar minimum (Araujo-Pradere, *et al.*, 2011), but including the normal variability (Araujo-Pradere, *et al.*, 2004).

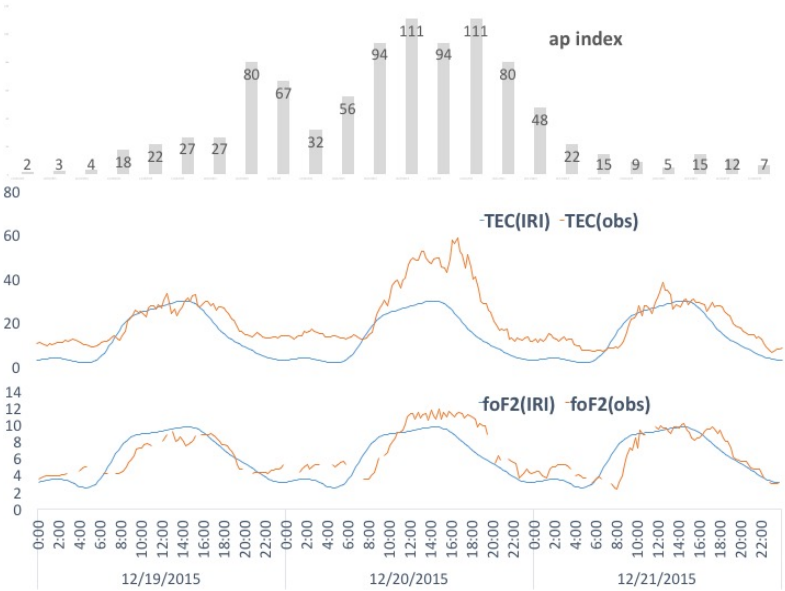

Figure 2: Winter event showing an increment of TEC and foF2 values (positive phase). Quiet values in blue, disturbed in brown.

A different picture is shown in Figure 2. Here, TEC and foF2 disturbed values are higher than the quiet reference, commonly identified as a "positive phase". This seasonal difference is explained by the fundamental summer-to-winter circulation, which transports the molecular rich gas to mid and low latitudes in the summer hemisphere over a day or two following the storm. In the winter hemisphere, poleward winds restrict the equatorward movement of the composition bulge. Consequently, the altered environment in summer depletes the F region midlatitude ionosphere to produce a negative phase, while in winter midlatitude a decrease in molecular species, associated with downwelling, persists and produces the positive storm (Araujo-Pradere *et al.*, 2002b).

Conflicts of Interest

The authors declare no conflict of interest.

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Belousov–Zhabotinsky Reaction: Effects of Magnetic Field Variations.

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Abstract: The Belousov-Zhabotinsky (BZ) reaction is a classical oscillatory reaction that is the subject of many studies. One of its applications is to model other oscillatory processes, such as circadian rhythm. More often than not, experiments done on people are impractical, thus requiring simulations or modeling to enlighten the world on the unknown. A study of the effects of magnetic fields on the BZ reaction is presented. Magnetic fields create a huge impact on our lives, so much so that without them we may not function properly. It is not practical to expose people to diverse magnetic environments, such as outer-space, since an assay that assesses this requires multiple readings as well as the ability to allow another person to do it. Therefore, the Belousov-Zhabotinsky reaction, a Helmholtz coil, petri dishes, and a timer were used to provide clues as to how magnetic fields affect oscillating reactions. Our results indicate that the BZ reaction is a good model to replicate oscillatory biological reactions. Furthermore, the experimental conclusion is that reactions exposed to strong magnetic fields will oscillate slower than those exposed to weak magnetic fields.

Keywords: *oscillatory reactions, Belousov-Zhabotinsky reaction, magnetic field effect.*

Introduction

Since the 1950's the Belousov–Zhabotinsky reaction, otherwise known as BZ, has been employed as a model to study biological related oscillating reactions.[1] One factor that plays a part in possible changes in biological reactions, such as birds migrating or when a person falls asleep, involves the magnetic field created by the earth's core. Inversely, the lack of a magnetic field also causes differences in the biological clock. Places without magnetic fields or a large difference in the strengths of magnetic fields include outer space and other planets in the galaxy. Due to the fact that testing on people or even animals is deemed unethical or not practical, the Belousov-Zhabotinsky reaction provides the perfect solution. With this oscillating reaction and a Helmholtz coil, different magnetic fields can be tested on this particular solution thereby allowing a simulation of a living being traveling in space.

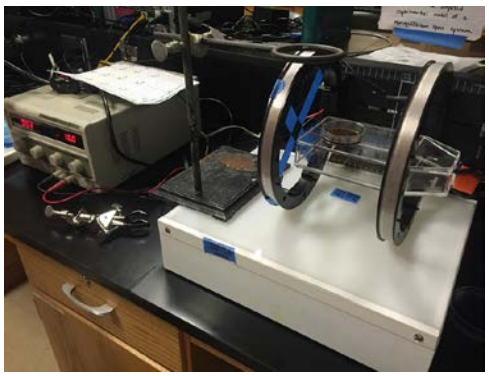
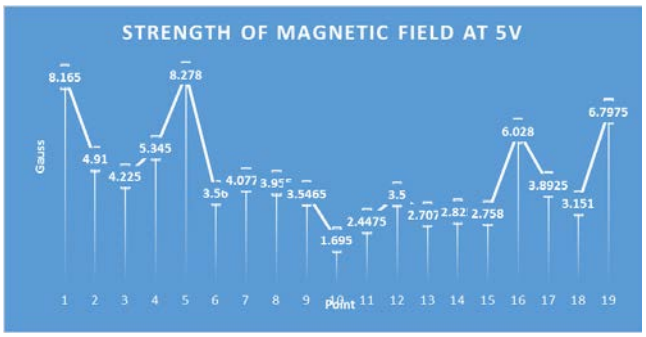


Fig. 1: DC power supply which is connected to the Helmholtz coil. A petri dish is placed on top of a suspended flask to ensure that the solution is directly in the most uniform area.

Methods and Results

The environment to which this experiment takes place in must have minimal interference. Once familiar with the BZ reaction, the solution can be portioned in order to minimize waste. The apparatus should then contain a Helmholtz coil attached to a DC power supply. Quickly after the solution is mixed, it is placed in to a small petri dish and suspended directly in the middle of the Helmholtz coil in order to insure magnetic field uniformity within the mixture. From this point all chemical wave fronts are recorded for the duration of the reaction.



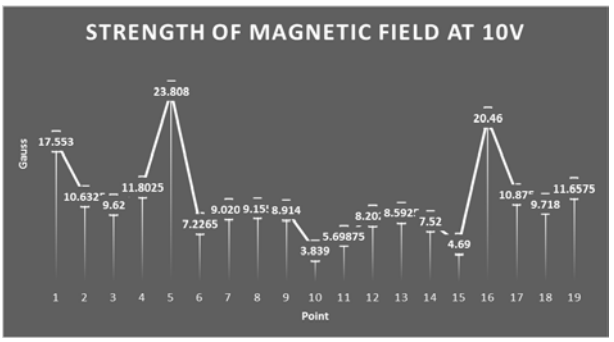


Fig. 2: The graphs are representations of the information obtained from using a sensor connected to an interface as an average. The measurements were taken along the horizontal axis.

Although the Helmholtz coil creates an almost uniform magnetic field, the horizontal axis was measured in order to find the most consistent area. This allowed the proper positioning of the petri dish and ultimately ensuring that the solution had little to no interference. This was accomplished by using graphing paper suspended within the Helmholtz coil and a sensor connected to an interface which measured the magnetic field in Gauss.

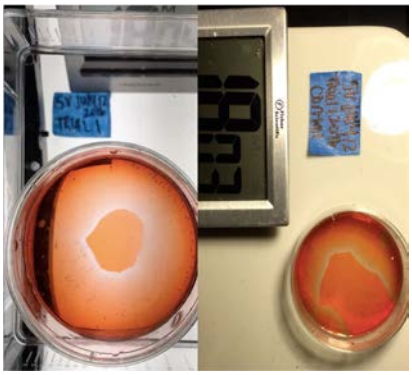


Fig. 3: ***From left to right: Side by side comparisons during a 5 volt trial where the left is the assay and the right is the control. The picture on the left shows an acceleration of the oscillating process of the BZ reaction***.

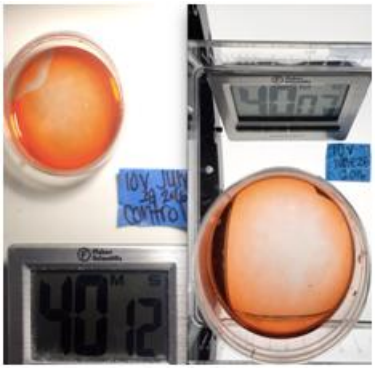


Fig. 4: ***From left to right: Side by side comparisons during a 10 volt trial where the left is the assay and the right is the control. The picture on the left shows a deceleration of the oscillating process of the BZ reaction***.

Conclusions

Considering that the magnetic field on Earth ranges roughly from 0.25 to 0.65 Gauss, the data from this experiment suggests that reactions exposed to high magnetic fields will oscillate slower than those exposed to lower magnetic fields. The varying magnetic fields affect the iron diffusion in the chemical wave. The research done here with the Belousov-Zhabotinsky reaction is vital to understanding how changes in magnetic fields effect the oscillating reactions within living organism, which could be extrapolated to the potential colonization of other planets.

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 2016, as well as the STEM-TRAC grant PO3C110190.

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Solvent Variations of the Briggs-Rauscher Reaction

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Abstract: The Briggs-Rauscher (BR) oscillatory reaction is one of the more interesting and colorful oscillatory reactions. It has surpassed the demonstration realm, as it has found use as a method to assess antioxidant capacity. However, this application as an antioxidant assay is limited to water-soluble samples. In the constant search for different, novel applications, we report the effects of various sample solvents on the behavior of the BR reaction. Our investigation looked at how changes in the solvent used to dissolve samples altered the time intervals of BR reaction's oscillations. The solvents used were ethanol, isopropanol, 1-propanol, acetone, and acetonitrile. Addition of ethanol had no effect on the BR oscillations. Isopropanol, 1-propanol, and acetone shorten the oscillation time. A test using acetonitrile discarded solvent polarity effects. Our results suggest that solvents that accelerate the enol pathway rate affect the oscillations of the BR reaction. Finally, samples can be safely dissolved in ethanol and used in the BR reaction.

Keywords: *oscillatory reaction, Briggs-Rauscher reaction, solvent variation, and enol pathway.*

Introduction

The Briggs-Rauscher (BR) reaction is an oscillating reaction that changes between two cycles back and forth until it reaches equilibrium. The two cycles the reaction oscillates between correspond to a radical state and a non-radical state. The main reaction is:

$$IO_3^- + 2\ H_2O_2 + CH_2(CO_2H)_2 + H^+ \rightarrow ICH(CO_2H)_2 + 2\ O_2 + 3\ H_2O \quad \text{(Eq 1)}$$

This main reaction can be broken down into two component reactions. The first component reaction;

$$IO_3^- + 2\ H_2O_2 + H^+ \rightarrow HOI + 2\ O_2 + 2\ H_2O \quad \text{(Eq 2)}$$

can occur via a radical or a non-radical process. When $[I^-]$ is low, the radical process dominates; when $[I^-]$ is high, the non-radical process dominates. The BR reaction is mostly used as demonstration.[1] Recently, Cervellati reported its use as a method to assess antioxidant capacity.[2] Application as an antioxidant assay is limited to water-soluble samples. In this short communication we report the effects of various sample solvents on the behavior of the BR reaction. Our results describe how changes in the solvent used to dissolve samples altered the time intervals of BR reaction's oscillations.

Methods and Results

A typical preparation of the Briggs Rauscher reaction was utilized.[3] When all stock solutions were prepared the solvents were tested as follows. Take 5mL of the sodium iodate solution, 5mL of starch solution, and 10mL hydrogen peroxide. Once a stir bar has been placed in a 100mL beaker, start to mix the sodium iodate solution and starch solution in the beaker over a stirring plate. Then add the peroxide; the solution turns amber yellow then dark blue. Start the timer when the first dark blue color appears until the next dark blue appears. This is the oscillation time (usually 13-18 seconds). This is also the control time for each trial. Repeat the step above and when the second deep blue color appears, add 1mL of solvent solution. Measuring the time from the second blue to the third blue appearance determines any solvent effects.

The solvents investigated were water, ethanol, 1-propanol, isopropanol, acetone, and acetonitrile. Addition of water and ethanol had no effect on the oscillation time (Figure 1, left). Acetone, 1-propanol, and isopropanol shortened the oscillation time (Figure 1, right). Acetonitrile had no effect on the BR oscillation time.

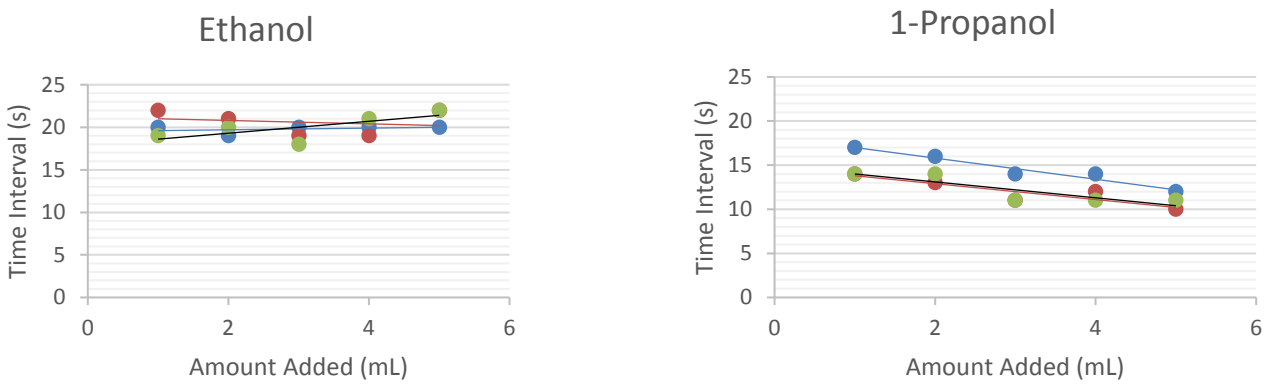

Fig. 1: (Left) Effect of ethanol on the oscillation time of the BR reaction. (Right) Effect of 1-propanol on the oscillation time of the BR reaction.

Results can be understood analyzing the second component reaction:

$$HOI + CH_2(CO_2H)_2 \rightarrow ICH(CO_2H)_2 + H_2O \qquad \text{(Eq 3)}$$

The dramatic change from amber to dark blue occurs as the iodide ion slowly is being produced and is less than the HOI, and the excess HOI reacted with hydrogen peroxide allows for a conversion to the iodide ion, creating the dark blue change. This is where the enol reaction mechanism takes place.

$$I^- + HOI + H^+ \rightarrow I_2 + H_2O \qquad \text{(Eq 4)}$$

$$I_2 + CH_2(CO_2H)_2 \rightarrow ICH(CO_2H)_2 + I^- + H^+ \qquad \text{(Eq 5)}$$

Leopold *et al*. suggested an intramolecular mechanism, featuring a six-member cyclic transition state, is postulated to account for the results on the enolization of malonic acid (Eq 5).[4] We hypothesize that sample solvents that disrupt the postulated mechanism will affect the BR oscillations. In the case of 1-propanol and isopropanol, longer and branched alcohols disrupt the proposed mechanism. Two suggested disruption pathways: De-stabilization of proposed six-member ring, and alcohol reaction with iodide. Acetone can readily undergo enol reaction mechanism pathway, which has the effect of adding more malonic acid. Finally, polarity can be discarded as a factor since acetonitrile did not disrupt the BR oscillations.

Conclusions

Addition of ethanol had no effect on the BR oscillations. Acetone, isopropanol, and 1-propanol shorten the oscillation time. A test using acetonitrile discarded solvent polarity effects. Our results suggest that solvents that disrupt the enol pathway affect the oscillations of the BR reaction. Finally, samples can be safely dissolved in ethanol and used in the BR reaction.

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 2016, as well as the STEM-TRAC grant PO3C110190.

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Analysis of Oyster Plant (*Tradescantia spathacea*) Extracts via Maceration, Soxhlet Extraction, Thin Layer Chromatography and Cytotoxicity Assays

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Abstract: The Oyster plant (*Tradescantia spathacea*) is a fleshy or succulent perennial garden herb. It is utilized for ornamental purposes in many tropical and subtropical climates. Medicinally, the plant is used for colds, sore throat, whooping cough, nasal bleeding, and also as an anti-inflammatory. Oyster Plants were grown and harvested from the organic garden at St. Thomas University. The different parts of each plant – leaves, stems, roots and flowers – were separated, cleaned, and dried at 40°C. Specimens were then grinded and prepared as extracts using maceration and Soxhlet extraction. All the extracts were rotevapored and analyzed by thin layer chromatography (TLC) with different mixtures of polar and nonpolar solvents. The spots were developed and visualized with iodine and UV light. Root and leaf fractions contained the majority of organic compounds. The present work reports the best solvent for extraction and the most effective conditions for TLC separation. Preliminary experiments testing ethanol-containing extracts for anticancer properties are also discussed.

Keywords: *medicinal plant, extraction, separation, chromatography, cancer, cytotoxicity*

Introduction

Natural products from plants play crucial roles in human life and are critical to the field of medicine. Plant components have been shown to be valuable sources for anticancer drug discovery.[1] Numerous studies have demonstrated that extracts from herbal medicines or mixtures have anticancer potential *in vitro* or *in vivo*.[2,3] Recent data suggests *Tradescantia spathacea* inhibits growth of a breast cancer cell line and affects Wnt/β-catenin signaling.[4] In addition, phenolic and flavonoid contents present in *T. spathacea* were recently reported to have antioxidant activities.[5] The aim of our work is to isolate potential new anticancer compounds from *T. spathacea* extracts through bioassay guided fractionation.

Fig 1. *Tradescantia spathacea*, an invasive plant species in Florida, grown in an organic garden at St. Thomas University.

Materials and Methods

All plants were grown under similar conditions. Solvents mixtures used for extraction were: ethanol/hexane 3:1; ethanol/hexane 1:1; ethanol and hexane alone. The dry material was extracted in one day using a Soxhlet extractor with ethanol and dichloromethane. Extracts were analyzed through thin layer chromatography (TLC).

MCF7 breast cancer cells (American Type Culture Collection) were cultured for 24 hours on 96-well plates in Eagle's Complete Media: Eagle's Minimum Essential Medium, 10% fetal bovine serum, 1X insulin-transferrin-selenium, and 1X penicillin/streptomycin. Leaf extracts prepared with ethanol/hexane 3:1, ethanol/hexane 1:1, and ethanol alone were solubilized in DMSO and added to the cells 24 hours later. Three extract concentrations were screened: 200, 100 and 20 µg/mL. After 24 hours, the culture media was replaced with RPMI1640 (without phenol red), 10% fetal bovine serum. A MTT cell viability assay was performed. Data from extract-treated cells were compared to that obtained from untreated controls.

Results

Preliminary data indicated that dichloromethane is a better extracting solvent. Compounds were separated with CH_2Cl_2/Isopropanol 4:6 and with petroleum ether/acetone/ cyclohexane 5:3:5. Numerous compounds of different polarities were found in roots and leaves (Fig.2). Preliminary data from MTT assays indicated a trend of greater cytotoxicity in 200µg/mL ethanol/hexane 3:1 leaf extracts compared to untreated controls.

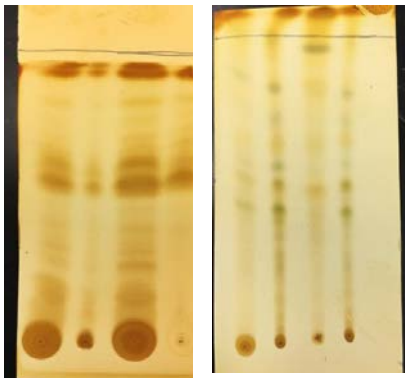

Fig.2: TLC of roots / TLC of leaves

Discussion

Column chromatography was done to separate the main components with promising results. More components were found in root and leaf extracts than stem and flower extracts. The infrared spectra of solids isolated from leaf extracts suggested the presence of phenolic and other polar compounds. Cytotoxicity assays are being replicated, and higher extract concentrations are being tested. Once the data is assessed, extracts will be tested in cell-invasion assays to study metastatic processes, and crude extracts with anticancer properties will be chemically fractionated to isolate active compounds.

Conflicts of Interest: Authors have no conflicts of interest.

Acknowledgments: We thank St. Thomas University's Summer Research Institute as well as STEM-TRAC grant PO3C110190. In addition, we thank Marrisa Lee, Leana Ramos, Milagros Mulero, Tashani Brown, Carlos Planchart and Luis Cendan for technical assistance.

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Studying the role of DLGAP1 transcripts in autism using human neural progenitor stem cells

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Abstract: This communication provides a brief review of autism spectrum disorder (ASD), including psychological perspectives and biological insights from whole brain, as well as cellular and genomic studies. Our progress towards conducting a functional study on the role of DLGAP1 RNA transcripts in human glutamatergic neurons derived from ASD and control, non-autistic, induced pluripotent stem cells is also described.

Key words: *autism (ASD), induced pluripotent stem cell (ipsc), neural progenitor cell (npc), DLGAP1*

Introduction

Autism Spectrum Disorder (ASD) is a neurological condition characterized by two core features: (1) impairments in social interaction and communication and (2) the presence of restricted interests and/or repetitive behaviors. Spectrum refers to ASD's numerous clinical presentations, including variations in intelligence, learning disabilities, compulsive behavior, and speech deficits [1]. Studies in the USA, Canada, South America, Asia, and Europe identified ASD in 1-2% of their populations [2]. ASD is 4.5 times more common among males than females [3].

Psychology research proposes that behavior commonly associated with ASD results from alterations in several cognitive processes, including social cognition and global processing [4]. The Social Cognition Deficit theory describes aspects of ASD as altered processing of human stimuli, such as other people's emotions, in contrast to processing of non-human stimuli, such as objects. The Weak Central Coherence theory argues the general population is primed for central coherence, integrating details in the context of a global, meaningful whole. In contrast, ASD individuals orient their cognitive processing in a more detail-focused, decontextualized, local manner. Lastly, the

Complexity Theory suggests individuals with ASD experience cognitive overload when processing tasks of certain complexity.

From a biological perspective, several models have emerged describing the neuropathology of

autism [5]. Studies on brain volume and gray/
white matter volume have led to a theory that the brain of autistic individuals undergoes accelerated growth during early childhood followed by a deceleration in growth later in life. MRI studies on functional connectivity support the premise of global hyperconnectivity in the brains of ASD individuals. Indeed, altered neurotransmission, caused by an imbalance of excitatory and inhibitory signaling during development, is hypothesized to be an underlying cause of autism.

In the brain, excitatory and inhibitory signaling are primarily mediated by two neurotransmitters: glutamate and GABA. Postnatally, during excitatory signaling, glutamate transmission results in depolarization and excitation of the post-synaptic neuron. Postnatally, GABA is inhibitory. Its post-synaptic transmission results in hyperpolarization, so a stronger signal is required for future neural excitation. This inhibitory role for GABA is a shift from its prenatal function as an excitatory transmitter. Since neurite outgrowth is dependent on neuronal activity, deficits in prenatal excitatory GABA signaling in an individual with ASD could result in an initial period of reduced neurite outgrowth. A postnatal period of excitatory glutamate signaling could then lead to an increased number of “local” circuits, which are hyperactive, due to deficient postnatal GABA inhibitory signaling. These reinforced local circuits would later prevent the elimination of superfluous synapses during synaptic pruning [6].

On a genetic level, over 700 loci are implicated in autism, highlighting ASD’s complexity and heterogeneity [7]. Based on these findings, approximately 25% of current autism cases have an identifiable genetic cause. Disruptions in several genes involved in synaptic development and function, as well as cortical neuron identity, have been associated with autism [5,6].

A functional study of DLGAP1

Recently, whole transcriptome sequencing was performed on cultured cortical neurons, derived from the induced pluripotent stem cells (ipscs) of individuals with autism and control, non-autistic individuals [6,8]. Three Discs large homolog-associated protein 1 (DLGAP1) transcripts were differentially expressed. DLGAP1 is a scaffolding protein, expressed in a structure termed the post-synaptic density, in glutamatergic synapses [9]. A 1.7 fold increase in DLGAP1, a 1.93 fold decrease in DLGAP1 antisense 1, and a 1.51 decrease in DLGAP1 antisense 2 were observed in ASD neurons compared to controls [6].

The aim of our research is to examine the role of DLGAP1 in autism biology. We will study how reducing DLGAP1 antisense 1 and 2 transcripts affects ASD and control, non-autistic glutamatergic neurons. While the functions of these transcripts are unknown, one hypothesis is that these non-coding RNAs negatively regulate DLGAP1 protein-coding mRNA expression. Based on the transcriptome data, we hypothesize that reducing DLGAP1 antisense transcripts in control neurons will cause them to develop more like autistic neurons. Conversely, decreasing DLGAP1 protein coding mRNA in ASD neurons will ameliorate ASD phenotypes.

Currently, we are culturing ASD and control human neural stem/progenitor cells differentiated from ipscs, reprogrammed from skin biopsies. A protocol, which mimics the steps of neurogenesis during cortical development, was used to terminally differentiate the cells into glutamatergic neurons [6]. Visible differences were observed in the morphology of ASD neurons compared to control neurons. While we are still characterizing these differences, our findings support a previous report showing significant changes in axon and dendrite length, branch count, and process count between ASD and control cultures [6].

To reduce DLGAP1 antisense 1 and 2 levels in ASD and control neurons, we designed six different short hairpin RNAs (shRNAs). shRNAs reduce protein expression levels by targeting complementary RNA for degradation. Each shRNA was molecularly cloned into a lentiviral vector, and the insertion was verified

by DNA sequencing. Lentiviruses carrying the shRNAs and a control shRNA, with no known RNA target, were produced and titered. Procedures were established to amplify DLGAP1 antisense 1 and 2 cDNA in preparation for quantifying shRNA-mediated transcript reduction utilizing real time reverse transcriptase PCR.

Conclusion: Future experiments will transduce the shRNA lentiviruses into ASD and control cortical stem/progenitor cells. The cellular morphology, differentiation, maturation, and electrophysiology, as well as synaptic structure and function, of glutamatergic neurons will be examined and quantified. This data will provide valuable insight to the role of DLGAP1 antisense 1 and 2 in glutamatergic neurons during normal development as well as in cases of ASD.

Conflicts of Interest: Authors have no conflicts of interest. IRB approvals were attained from the Univ. of Miami and St. Thomas Univ. **Acknowledgments:** This work was funded by STEM-TRAC grant PO3C110190. We thank Deliabell Hernandez, Vadym Trokhymchuk, Mang Cing (St. Thomas Univ.); Arielis Ortiz, Claudia Martinez-Crespo, Melissa Suarez (Miami Dade College); Kinsley Belle and Francelethia Johnson (Univ. of Miami) for technical assistance.

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Contrasting Effects of Vermiculture-based Fertilizers on Growth of *Brassica oleracea* var. *sabellica*

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Abstract: Organic fertilizers not only maintain soil fertility but enhance the biological activity of the soil, which improve the efficiency of nutrient use by the plants. Vermicompost, a nutrient-rich organic fertilizer made by composting earthworms can be used in the preparation of aqueous extracts known to increase crop yield and plant health. Vermicompost was produced through cultivation of red wriggler worms (*Eisenia fetida*), a common composting species and used in the preparation of aqueous fertilizing mixtures. Kale (*Brassica oleracea* var. *sabellica*), a leafy vegetable known for its high nutritional properties, was grown in an organic garden at St. Thomas University. Plants were fertilized with one of three vermicompost-based solutions containing different combinations of organic additives, such as fish emulsion, corn syrup and/or a seaweed blend. Mixtures were prepared weekly and applied, in 4 gal. doses to the plants in each study group twice a week over the course of 10 weeks. Upon harvest, the height, wet weight, and dry weight of the plants were measured. ANOVA and Tukey test analysis showed a significant positive effect on all vermicompost-treated plants compared to the control. Kale plants receiving the mixture T1 (a combination of vermicompost and fish emulsion) resulted in significantly greater height, and dry weight in comparison to the other treatments.

Keywords: *Eisenia fetida; kale; vermicompost; worm tea*

Introduction: For many societies around the world, environmental and financial conditions often present barriers to agricultural stability, making the development of affordable and reasonably manageable agricultural techniques for such groups a complex process. Vermicompost, a waste by-product resulting from the breakdown of organic material by earthworms, is among the most promising of soil enrichment materials. Analyses have shown it to be rich in various nutrients such as potassium, nitrate, and calcium [1], as well as several phytohormones [2]. Earthworms are generalist organisms which serve as primary decomposers alongside fungi and soil bacteria, and their feeding behavior assists in the improvement of soil quality through their tunneling (which helps maintain an aerated environment conducive to microbial and fungal growth) and the breakdown of organic matter into vermicompost [3]. *Eisenia fetida*, commonly known as the red wriggler worm, is a popular composting species. The cultivation of this species is a relatively inexpensive process, and can be conducted under a generous range of temperatures and conditions. As a result, a scientific interest in the agricultural potential of vermiculture has developed, particularly regarding the use of solutions derived from vermicompost, often referred to as "worm teas."

Materials and Methods: Worms were housed in a Worm Factory 360® vermicomposting unit. They were cultivated using a substrate composed of peat moss and shredded newspaper, and covered with regularly dampened newspaper. Chopped iceberg lettuce was provided on a weekly basis. Kale was planted in raised mounds in four independent beds containing a soil mixture of 1 part organic soil: 1 part sand. Seeds were planted one week prior to treatments.

All worm tea solutions contained 454 grams vermicompost and 3 tbsp fish emulsion in 4 gallons of chlorine-free water. In addition, Treatment 1 contained 3 tbsp unsulfured black-strap molasses, Treatment 2 received 3 tbsp dark corn syrup, and Treatment 3 contained 3 tbsp fish emulsion and 400 mL seaweed solution. In preparation of each fertilizer solution, compost was weighed and wrapped in mesh fabric bags which were tied with zip ties. Each fertilizer treatment was churned using aeration pumps, and

stored in plastic 5 gallon buckets. Treatments were applied twice per week. Kale plants were removed 9 weeks after initiation of treatments, washed in water and their length was measured. They were then placed in an incubator to dry at 70 °C for four days. Thereafter dry weight was recorded. ANOVA and Tukey analyses were performed on kale height data.

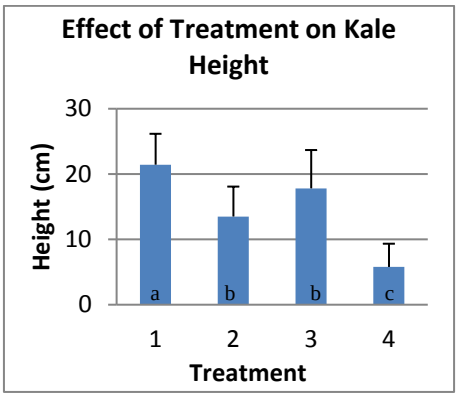

Fig. 1. Height of kale plants.

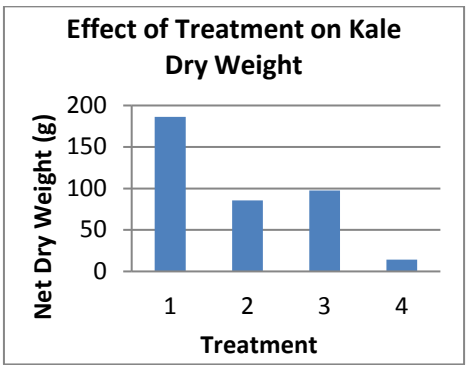

Fig. 2: Dry weight of kale plants.

Results and Discussion: Kale plants receiving worm tea solutions displayed significantly greater height increase over the control group (Fig. 1), pointing to the beneficial effects of using vermicompost-liquid fertilizers supplemented with additives. Treatment T1 resulted in a significantly greater height increase over T2 or T3 (which produced results comparable to one another). The average dry weights for all fertilizer treatments showed a notable increase over the control group, particularly in the case of treatment T1 (Fig.2). Treatment 1 contains unsulfured black-strap molasses as an additive. Molasses serves as a good source of carbohydrates, and is also high in calcium, magnesium, iron, and potassium, as well as in B-complex vitamins [4]. It is commonly incorporated as fertilizer in organic gardens because its high sugar content promotes the growth of beneficial soil microbes [5].

Conclusions: Treatment T1, which contained the unsulfured black-strap molasses additive, resulted in the greatest height growth, as well as a greater average weight increase among all vermicompost-based treatments.

Conflicts of Interest: The authors declare no conflict of interest.

Acknowledgements: Authors thank St. Thomas University, the Department of Education STEM-TRAC grant PO3C110190, and the USDA-HSI FCCAgE Agricultural Education grant.

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Mighty Fruits: Antioxidant Performance of Various Fruits

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Abstract: Antioxidants help fight free-radicals, which are produced by stress and later can induce many health problems. L-Ascorbic acid (Vitamin C) is a great antioxidant, and it can be found in a vast variety of fruits. Additionally, vitamin C is marketed as an over-the-counter remedy for the common cold. This motivates our desire to understand vitamin C's antioxidant properties. This investigation presents the antioxidant capacity of various fruits that are known to be sources of vitamin C, and their comparison to pure vitamin C using the Briggs-Rauscher (BR) oscillatory reaction. The antioxidant species scavenge free radicals formed in the BR reaction, lengthening the time intervals of the reaction's oscillations; the higher the antioxidant capacity, the longer the oscillation delays. The samples that were tested were: L-ascorbic acid, Sunny-D®, red grape juice, white grape juice, pineapple juice with pulp, pineapple juice without pulp, mango juice, and kiwi juice. Pure vitamin C could only be tested at low concentrations, as high concentrations completely interrupt the BR reaction. Kiwi exhibited the best antioxidant capacity of the tested samples, followed by mango juice. The antioxidant performance of orange juice resembled that of vitamin C the most. This result suggests that vitamin C is the main antioxidant present in orange juice. The other fruits exhibited antioxidant performances different to pure vitamin C. We ascribe these observations to the presence of other molecules, such as flavonoids and tannins, which also show antioxidant capacity.

Keywords: *antioxidants, oscillatory reaction, Briggs-Rauscher reaction, and vitamin C.*

Introduction

Vitamin C (L-Ascorbic acid) is a great antioxidant present in many fruits. Consuming vitamin C helps provide the needed antioxidant intake while also helping decrease the production of free radicals. Antioxidants are very important when it comes to free radicals. Antioxidants help control the production of free radicals. Free Radicals are blamed as the cause of many diseases, such as heart disease, cancer, and diabetes. Lacking antioxidants in the body can become very dangerous because the production of free radicals will increase and the chances of developing these diseases will also increase. Antioxidants can be found in many fruits such as the ones that were tested. Antioxidants in a daily diet may delay and even prevent cell damage that is caused by free radicals.

The Briggs-Rauscher (BR) reaction is an oscillating reaction that changes between two cycles back and forth until it reaches equilibrium. The two cycles the reaction oscillates between correspond to a radical state and a non-radical state. The BR reaction is mostly used as demonstration.[1] Recently, Cervellati reported its use as a method to assess antioxidant capacity.[2] In this method, the presence of an antioxidant increases the oscillation time in the BR reaction. In this short communication we report the antioxidant performance of pure vitamin C by using the BR oscillating reaction. Additionally, we determine the antioxidant performance of various fruit juices while also comparing the results to a standard (vitamin C).

Methods and Results

A typical preparation of the Briggs Rauscher reaction was utilized.[3] When all stock solutions were prepared the solvents were tested as follows. Take 5mL of the sodium iodate solution, 5mL of starch solution, and 10mL 3% hydrogen peroxide. Once a stir bar has been placed in a 100mL beaker, start to mix the sodium iodate solution and starch solution in the beaker over a stirring plate. Then add the peroxide; the solution turns amber yellow then dark blue. Start the timer when the first dark blue color appears until the next dark blue appears. This is the oscillation time (usually 13-18 seconds).

This is also the control time for each trial. Repeat the step above and when the second deep blue color appears, add 1mL of antioxidant solution. Measuring the time from the second blue to the third blue appearance determines the antioxidant performance.

Concentrations above 2% of vitamin C completely disrupted the BR reaction. The reason for this observation is that high vitamin C concentrations alter the BR mechanism, reacting with iodine. The average slope of the orange juice was the most comparable to that of L-ascorbic acid, suggesting that the main antioxidant species in orange juice is vitamin C (Figure 1). Sunny-D® seems to have a higher slope than regular orange juice. Sunny-D® contains thiamin hydrochloride (vitamin B), as well as other fruits. These factors contribute to why Sunny-D® has higher strength in antioxidant than orange juice. Red grape juice had a higher level of antioxidant strength than white grape juice (Figure 2). Red grapes contain flavonoids which give them a higher antioxidant level. Juices with pulp also contain flavonoids, which is why pineapple juice with pulp had higher antioxidant potency than pineapple juice with no pulp. Mango juice and Kiwi juice are the fruits that have the highest slope average. The reason being is both of these fruits contain beta carotene which causes them to have higher antioxidant strength.

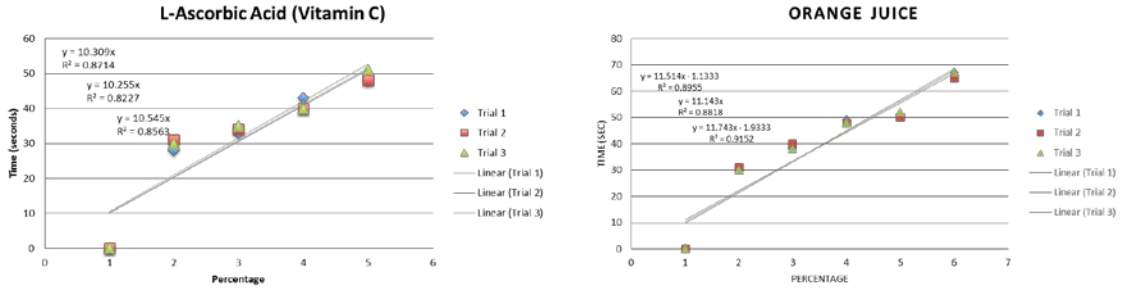

Fig. 1: (Left) Antioxidant performance of pure vitamin C. (Right) Antioxidant performance of orange juice.

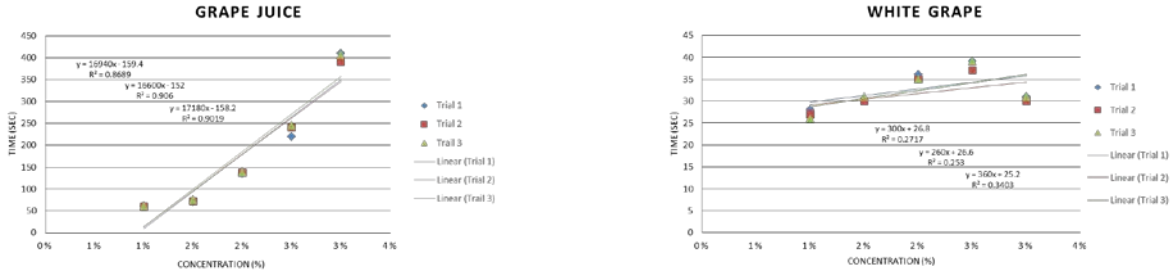

Fig. 2: (Left) Antioxidant performance of red grapes. (Right) Antioxidant performance of white grapes.

Conclusions

The Briggs-Rauscher oscillating reaction is effective assessing antioxidant performance. When the concentration of L-ascorbic acid is above 2%, it will completely stop the reaction. Kiwi had the highest antioxidant strength of the fruits, but it could be due to the various vitamins Kiwi contains of ethanol had no effect on the BR oscillations. In the future, we will test other fruits that are said to have a high level of antioxidant performance, and possibly isolate the active components of the fruits.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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Structure-Activity Relationships (SARs) of Antioxidant Molecules

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Abstract: The public's demand for antioxidant supplements has increased recently. As more of these supplements hit the market, an understanding of what makes a molecule a good antioxidant is paramount. The aim of this research project is to evaluate the antioxidant capacity of several phenols and related derivatives using the Briggs-Rauscher (BR) oscillatory reaction. The antioxidant species scavenge free radicals formed in the BR reaction, lengthening the time intervals of the reaction's oscillations; the higher the antioxidant capacity, the longer the oscillation delays. In phenol structures, the amount of hydroxyl (OH) groups affects the antioxidant capacity. By adding a hydroxyl group in a specific ring position, such as *ortho* or *para*, an increase of antioxidant capacity was observed. Previous research supports that *ortho* substitution of an electron donor increases the stability of the phenol radicals. Our results showed that *meta* substituted phenols were the most active antioxidants. We rationalize this observation by considering the low pH (~2) of the Briggs-Rauscher reaction. It was also noticed that a monophenol showed less antioxidant capacity than a polyphenol structure. Finally, we observed that the number of OH substituent does affect the antioxidant capacity.

Keywords: *antioxidants, oscillatory reaction, Briggs-Rauscher reaction, structure-activity relationship, and polyphenols.*

Introduction

Free radicals (FR) and reactive oxygen species (ROS) been suggested as potentially being important causative agents of aging and several human diseases such as cancer, inflammatory and degenerative diseases, emphysema, central nervous system injury, and autoimmune disease. The use of antioxidants for the prevention of damage caused by free radicals thereby assumes great importance for health and traditional medicine. Currently, there are many products on the market which claim to contain antioxidants.

The Briggs-Rauscher (BR) reaction is an oscillating reaction that changes between two cycles back and forth until it reaches equilibrium. The two cycles the reaction oscillates between correspond to a radical state and a non-radical state. The BR reaction is mostly used as demonstration.[1] Recently, Cervellati reported its use as a method to assess antioxidant capacity.[2] In this method, the presence of an antioxidant increases the oscillation time in the BR reaction. In this short communication we evaluate the antioxidant capacity of several phenols by using the Briggs-Rauscher reaction. We also determine structure-activity relationships within the tested phenol derivatives. A structure activity relationship shows how the feature of a chemical structure relates to the biological activity related with that specific chemical.

Methods and Results

A typical preparation of the Briggs Rauscher reaction was utilized.[3] When all stock solutions were prepared the solvents were tested as follows. Take 5mL of the sodium iodate solution, 5mL of starch solution, and 10mL 3% hydrogen peroxide. Once a stir bar has been placed in a 100mL beaker, start to mix the sodium iodate solution and starch solution in the beaker over a stirring plate. Then add the peroxide; the solution turns amber yellow then dark blue. Start the timer when the first dark blue color appears until the next dark blue appears. This is the oscillation time (usually 13-18 seconds). This is also the control time for each trial. Repeat the step above and when the second deep blue color

appears, add 1mL of antioxidant solution. Measuring the time from the second blue to the third blue appearance determines the antioxidant performance.

Throughout our experiment there were some phenols that were tested that did not have any antioxidant activity. Cinnamic acid, p-coumaric acid, and p-hydroxybenzoic acid, had minimal affect; however, the oscillation delay was not consistent throughout the trial. SARs were present in each phenol structure.[4] The phenol with the hydroxyl substituent in the meta position showed the greatest antioxidant activity (Figure 1). Among the phenol derivatives, pyrogallol, and resorcinol were the most active antioxidants. Since resorcinol has the hydroxyl group in the meta position, it exhibited the most antioxidant activity. In the phenol acids, the most active antioxidants were caffeic acid and gallic acid. Gallic acid contains 3 OH groups with a carboxylic acid attached directly to the ring. However, caffeic acid showed to be more active than gallic acid even though it has two OH groups attached to the ring. The studied polyphenol molecules contained aromatic and phenolic rings that possess some patterns mentioned earlier, such as multiple OH substituents in specific positions. The most active that was tested in this experiment was quercetin, which exhibited these specific patterns. Finally, some of the samples lost antioxidant activity with exposure to air and light.

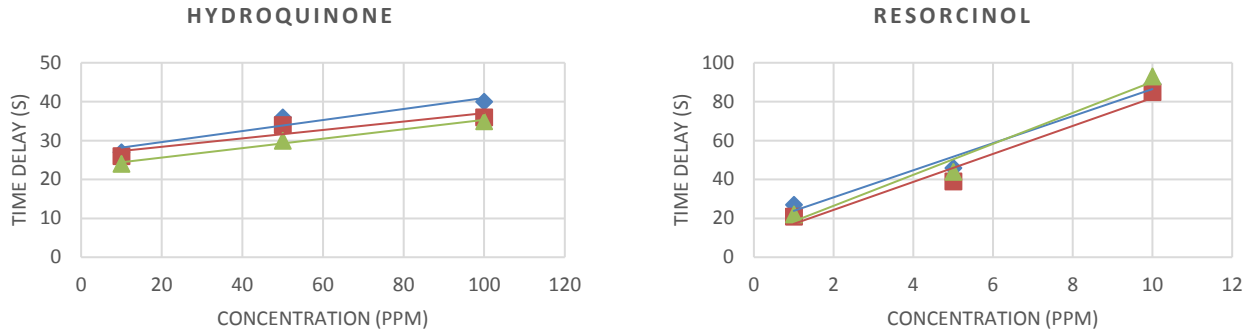
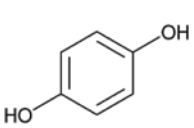
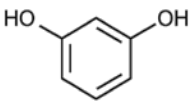


Fig. 1: (Left) Antioxidant performance of hydroquinone. (Right) Antioxidant performance of resorcinol. The steeper slope indicates more antioxidant activity.





Conclusions

The Briggs-Rauscher oscillating reaction is effective assessing antioxidant performance. We consistently observed that OH substituents affect antioxidant activity. Hydroxyls located in the meta position exhibited the greatest antioxidant activity. In the future, we aim to develop a more encompassing SAR for antioxidant molecules.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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Powerful plants: Antioxidant capacity of selected plants

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Abstract: Different cultures from around the world have used plants from their natural surroundings to treat different ailments. The action mechanisms of these natural remedies are diverse, yet many studies suggest their antioxidant properties bring about their effectiveness. This project presents the determination of the antioxidant capacity of selected plants, and comparing those results to a Trolox standard. The Briggs-Rauscher (BR) oscillating reaction was used to determine the antioxidant capacity of the samples. The antioxidant species scavenge free radicals formed in the BR reaction, lengthening the time intervals of the reaction's oscillations; the higher the antioxidant capacity, the longer the oscillation delays. The samples consisted of aqueous and ethanolic extracts from the leaves of *Annona muricata, Moringa oleifera, Petiveria alliacea, Hamelia patens,* and *Gynura bicolor*. To analyze the results we used the Relative Antioxidant Performance (RAP), where the slopes of the samples were compared to the Trolox standard. Since most of these leaves are traditionally used in teas, we hypothesized that the aqueous extracts would exhibit the highest antioxidant capacity. Except for the aqueous extracts of *Moringa oleifera* and *Petiveria alliacea*, our hypothesis was proven correct, with *Hamelia patens* showing the highest RAP. These results were attributed to the solubility in water of the active antioxidant molecules versus their solubility in ethanol. These observations suggest that antioxidant properties are present, and could be a plausible pathway to their therapeutic properties. Furthermore, these extracts are complex mixtures of natural ingredients; therefore, we should not dismiss any potential synergistic effects between different ingredients.

Keywords: *antioxidants, oscillatory reaction, Briggs-Rauscher reaction, natural products, Annona muricata, Moringa oleifera, Petiveria alliacea, Hamelia patens, and Gynura bicolor.*

Introduction

Many plants that are used as natural remedies possess antioxidant properties. These plants contain phytochemicals, which are non-nutritive plant chemicals that have protective or disease preventive properties. Most phytochemicals have antioxidant activity and are suspected to reduce the risk of developing certain types of cancer and other diseases related to reactive oxygen species (ROS). ROS have been suggested as causative agents of aging and several human diseases such as cancer, inflammatory and degenerative diseases, emphysema, and autoimmune disease. The use of antioxidants for the prevention of damage caused by ROS thereby assumes great importance for health and traditional medicine.

The Briggs-Rauscher (BR) reaction is an oscillating reaction that changes between two cycles back and forth until it reaches equilibrium. The two cycles the reaction oscillates between correspond to a radical state and a non-radical state. The BR reaction is mostly used as demonstration.[1] Recently, Cervellati reported its use as a method to assess antioxidant capacity.[2] In this method, the presence of an antioxidant increases the oscillation time in the BR reaction. In this short communication we test the antioxidant capacity of aqueous and ethanolic extracts from the leaves of *Annona muricata, Moringa oleifera, Petiveria alliacea, Hamelia patens,* and *Gynura bicolor*. Finally, we determined their Relative Antioxidant Performance (RAP) using Trolox as a standard.

Methods and Results

A typical preparation of the Briggs Rauscher reaction was utilized.[3] When all stock solutions were prepared the samples were tested as follows. Take 5mL of the sodium iodate solution, 5mL of

starch solution, and 10mL hydrogen peroxide. Once a stir bar has been placed in a 100mL beaker, start to mix the sodium iodate solution and starch solution in the beaker over a stirring plate. Then add the peroxide; the solution turns amber yellow then dark blue. Start the timer when the first dark blue color appears until the next dark blue appears. This is the oscillation time (usually 13-18 seconds). This is also the control time for each trial. Repeat the step above and when the second deep blue color appears, add 1mL of sample solution. Measuring the time from the second blue to the third blue appearance determines any solvent effects. All samples were dissolved in water or ethanol. Therefore, we had an aqueous and an ethanolic solution for each sample. Using ethanol does not affect the BR oscillations.[4]

Excluding *Moringa oleifera* and *Petiveria alliacea*, all of the plants' antioxidant potency was exhibited in the aqueous solution. *Hamelia patens* was shown to have the highest RAP value, translating into containing the highest levels of antioxidant capacity compared to the other plants. The aqueous solutions of *Hamelia patens* and *Gynura bicolor* showed a marked difference in antioxidant capacity when compared to their ethanolic extracts. This observation suggests a high water solubility of its antioxidant molecules. *Annona muricata* showed about the same antioxidant capacity for both solvents. Both *Annona muricata* and *Petiveria alliacea* are currently studied by other groups for potential cancer treatment alternatives. From our study we can suggest that antioxidants are part of their promising effectiveness, but antioxidants are not the only pathway. We cannot discard the presence of some phytochemicals in both water and ethanol extracts, while some are shown to be exclusive to one or the other. Flavonoids are shown to be present in both extracts while tannins are present solely in ethanol extracts. Rutin is an example of tannin present in *Moringa oleifera*, making the ethanolic extract stronger in antioxidant activity.

The relative antioxidant performance (RAP) of the different plants was determined using Trolox as a standard (table below). We measure the time the BR oscillation was delayed as a function of increasing concentration; the higher the concentration, the longer the delay. A best-line fit produced a slope for each of the samples.

Extract	RAP *Annona muricata*	RAP *Moringa oleifera*	RAP *Petiveria alliacea*	RAP *Hamelia patens*	RAP *Gynura bicolor*
aqueous	0.00381	0.00238	0.00152	0.0450	0.00647
ethanolic	0.00348	0.00378	0.00378	0.0218	0.000533

Conclusions

In general, aqueous solutions exhibited more antioxidant capacity than ethanolic solutions. The water solubility of the antioxidant species affects the antioxidant capacity. In the future we would like to test aqueous and ethanolic extracts for anticancerous activities, and produce more concentrated extracts through a freeze-dry process.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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Antioxidant capacity of common dietary supplements

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Abstract: Today's health-conscious society consumes a wide variety of dietary supplements in order to improve quality of life. An increasing number of these supplements are marketed as antioxidants. Therefore, it is of great importance to understand the performance of these supplements as antioxidants. This investigation presents the antioxidant capacity of several common dietary supplements using the Briggs-Rauscher (BR) oscillatory reaction. The antioxidant species scavenge free radicals formed in the BR reaction, lengthening the time intervals of the reaction's oscillations; the higher the antioxidant capacity, the longer the oscillation delays. The samples experimented, Beta carotene, Lutein, Quercetin, Folic Acid, and L-Glutathione, all exhibit antioxidant activity. Trolox, a water-soluble form of vitamin E, was established as the standard to assess each supplement's antioxidant capacity. It was noted that the time delay within the BR reaction oscillations was significantly affected with increasing concentrations of each substance. Also, sodium iodate proved to be better than potassium iodate in the BR reaction, as precipitation was not a factor that altered results in the BR reaction. In addition the antioxidant capacity was quantified by the calculation of the Relative Antioxidant Performance (RAP), which measures the sample slope over the standard slope or the slope of Trolox. Finally, we observed that exposure to light can affect the antioxidant capacity.

Keywords: *antioxidants, oscillatory reaction, Briggs-Rauscher reaction, Trolox, and supplements.*

Introduction

An imbalance between free radicals (oxidants) and antioxidants, can potentially lead to disease such as heart attack, Alzheimer's disease, and tumors. Free radicals can lead to a variety of problems. In particular, they react with – and damage – lipids, proteins and nucleic acids, including DNA. Our marketplace is flooded with supplements marketed as antioxidants, but their purported benefits are the subject of ongoing investigations.

The Briggs-Rauscher (BR) reaction is an oscillating reaction that changes between two cycles back and forth until it reaches equilibrium. The two cycles the reaction oscillates between correspond to a radical state and a non-radical state. The BR reaction is mostly used as demonstration.[1] Recently, Cervellati reported its use as a method to assess antioxidant capacity.[2] In this method, the presence of an antioxidant increases the oscillation time in the BR reaction. In this short communication we test if the Briggs-Rauscher oscillating reaction can determine antioxidant performance. In order to do this, we first established the use of Trolox as a standard for antioxidant capacity within the Briggs-Rauscher reaction. We measured the antioxidant capacity of L-glutathione, folic acid, β-carotene, and lutein, and finally determined the Relative Antioxidant Performance (RAP) of the L-glutathione, folic acid, β-carotene, and lutein using Trolox as a standard.

Methods and Results

A typical preparation of the Briggs Rauscher reaction was utilized.[3] When all stock solutions were prepared the solvents were tested as follows. Take 5mL of the sodium iodate solution, 5mL of starch solution, and 10mL hydrogen peroxide. Once a stir bar has been placed in a 100mL beaker, start to mix the sodium iodate solution and starch solution in the beaker over a stirring plate. Then add the peroxide; the solution turns amber yellow then dark blue. Start the timer when the first dark blue color appears until the next dark blue appears. This is the oscillation time (usually 13-18 seconds).

This is also the control time for each trial. Repeat the step above and when the second deep blue color appears, add 1mL of sample solution. Measuring the time from the second blue to the third blue appearance determines any solvent effects. All samples were dissolved in water or water/ethanol mixtures. Addition of ethanol does not affect the BR oscillations.[4]

Among the several testing methods available to determine the relative antioxidant activity of pure compounds, plant extracts, drinks, etc., the Trolox Equivalent Antioxidant Capacity (TEAC) assay is the most commonly used. We were able to establish Trolox as a standard for the BR reaction method (Fig 1). It is noted that for the substances L-Glutathione, Folic Acid and Quercetin the concentration greatly affects the level of antioxidant effect. Utilizing Lutein that had been stored in amber flasks which protected it from direct light and limiting air exposure provided more consistent results.

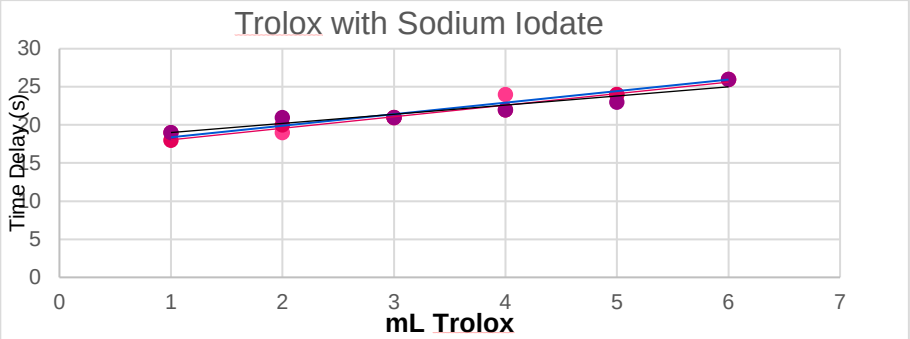

Fig. 1: Effect of Trolox on the oscillation time of the BR reaction. A saturated aqueous solution of Trolox was used.

$$RAP = \frac{\text{slope of sample}}{\text{slope of standard}}$$

The relative antioxidant performance (RAP) of the different supplements was determined using Trolox as a standard (table below). We measure the time the BR oscillation was delayed as a function of increasing concentration; the higher the concentration, the longer the delay. A best-line fit produced a slope for each of the samples. Our observations show that quercetin has the best antioxidant performance of the tested supplements.

RAP Lutein	RAP β-carotene	RAP L-glutathione	RAP Folic acid	RAP Quercetin
0.41	0.4	0.0009	0.61	2.31

Conclusions

The Briggs-Rauscher oscillating reaction is effective detecting antioxidant presence. The time delay which represents the antioxidant strength significantly increases with increased concentrations. Determining the Relative Antioxidant Performance (RAP) of the various supplements demonstrated highest performance in Quercetin. Performing the Briggs Rauscher reaction with sodium iodate provided more consistent results. Trolox can be used as a standard within the Briggs Rauscher Oscillatory Reaction, but ethanol is a better solvent for it than water.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

Authors want to thank St. Thomas University SRI 2016, and STEM-TRAC grant P03C110190.

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Antioxidant capacity of selected teas and cocoa

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Abstract: Diverse teas are consumed around the world for their calming, soothing effects. Many people attribute curative properties to tea. The same can be said for cocoa, and its processed form chocolate. Furthermore, these attributed health-giving properties are suggested to come from their antioxidant properties. This study presents the determination of the antioxidant capacity of selected teas (*Camellia sinensis*) and cocoa (*Theobroma cacao*), and comparing those results to a caffeine standard. The Briggs-Rauscher (BR) oscillating reaction was used to determine the antioxidant capacity of the samples. The antioxidant species scavenge free radicals formed in the BR reaction, lengthening the time intervals of the reaction's oscillations; the higher the antioxidant capacity, the longer the oscillation delays. The samples consisted of aqueous preparations of Green tea, Black tea, Cocoa (pure powder), and Dark chocolate. To analyze the results we used the Relative Antioxidant Performance (RAP), where the slopes of the samples were compared to the caffeine standard. We hypothesized that the aqueous preparations of the samples would exhibit antioxidant capacity. Our hypothesis was proven correct, with green tea showing consistently higher RAP than decaffeinated green tea, and dark chocolate exhibiting slightly more antioxidant capacity than pure cocoa powder. Black tea proved to be less antioxidant than green tea. These observations suggest that antioxidant properties are present, and could be a plausible pathway to their attributed health-giving properties. Finally, these preparations are complex mixtures of natural ingredients; therefore, we should not dismiss any potential synergistic effects between different ingredients.

Keywords: *antioxidants, oscillatory reaction, Briggs-Rauscher reaction, tea, and cocoa.*

Introduction

Antioxidants are speculated to positively affect the health of humans in regards to certain reactions that take place within the body. Tea (*Camellia sinensis*), such as Green Tea and Black Tea, is speculated to have antioxidants present, which help remedy certain ailments. Certain health benefits have been attributed to consuming cocoa (*Theobroma cacao*) and dark chocolate due to its purported antioxidant presence. Antioxidants help control the production of free radicals. Free Radicals are blamed as the cause of many diseases, such as heart disease, cancer, and diabetes. Lacking antioxidants in the body can become very dangerous because the production of free radicals will increase and the chances of developing these diseases will also increase.

The Briggs-Rauscher (BR) reaction is an oscillating reaction that changes between two cycles back and forth until it reaches equilibrium. The two cycles the reaction oscillates between correspond to a radical state and a non-radical state. The BR reaction is mostly used as demonstration.[1] Recently, Cervellati reported its use as a method to assess antioxidant capacity.[2] In this method, the presence of an antioxidant increases the oscillation time in the BR reaction. In this short communication we test if the Briggs-Rauscher oscillating reaction can detect presence of antioxidants. Furthermore, we determine the Relative Antioxidant Performance (RAP) of chocolate and tea.

Methods and Results

A typical preparation of the Briggs Rauscher reaction was utilized.[3] When all stock solutions were prepared the solvents were tested as follows. Take 5mL of the sodium iodate solution, 5mL of starch solution, and 10mL 3% hydrogen peroxide. Once a stir bar has been placed in a 100mL beaker, start to mix the sodium iodate solution and starch solution in the beaker over a stirring plate. Then add the peroxide; the solution turns amber yellow then dark blue. Start the timer when the first dark blue color appears until the next dark blue appears. This is the oscillation time (usually 13-18 seconds).

This is also the control time for each trial. Repeat the step above and when the second deep blue color appears, add 1mL of tea or chocolate solution. Measuring the time from the second blue to the third blue appearance determines the antioxidant performance.

The different dilutions of the antioxidant containing samples show different levels of antioxidant performance. Certain brands of Black teas and green teas also exhibited remarkable antioxidant presence. Green tea consistently showed higher antioxidant capacity than black tea. We ascribe this observation to the higher degree of oxidation of black tea (less antioxidants present). Dark chocolate showed better antioxidant capacity than cocoa. This is plausible due to the processing and addition of ingredients to make chocolate.

The relative antioxidant performance (RAP) of tea was determined using caffeine as a standard. Our observations also suggest that caffeine is not the only antioxidant species in green tea, as the RAP for green tea is greater than 1. The observation of a higher RAP for decaffeinated black tea versus regular black tea is somewhat confusing. We attribute this result to using two different brands of tea.

$$\mathrm{RAP} = \frac{\text{slope of sample}}{\text{slope of standard}}$$

	Slopes of Caffeine	Slope of Black Tea	Slope of Green Tea	Slope of Decaf Tea
Trial 1	7	4.5	13	12.4
Trial 2	6	4.5	14.5	12.4
Trial 3		4.1	15.5	12.3
Average	6.5	4.4	14.3	12.4

RAP of Black Tea	RAP of Decaf	RAP of Green Tea
.67	1.90	2.2

Conclusions

The Briggs-Rauscher oscillating reaction is effective assessing antioxidant performance. Chocolate, cocoa, and tea affect the reaction, confirming their antioxidant activity. Determining the Relative Antioxidant Performance (RAP) of the green tea using caffeine as a standard showed the green tea has consistently better antioxidant performance than decaffeinated black tea and regular black tea. Using the RAP of cocoa in comparison to caffeine as the standard, the dark chocolate showed to have a better antioxidant performance than the cocoa. Our future aims are to perform additional runs with new samples in order to corroborate the decaffeinated tea data, and isolate and test the other active ingredients in tea and cocoa.

Conflicts of Interest

The authors declare no conflict of interest.

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Determination of the antioxidant capacity of coffee

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Abstract: Coffee (*Coffea arabica*) is one of the most consumed drinks in our society. It is grown in many regions around the world, developing different flavors and aromas. Its active ingredient, caffeine, is sought after for its stimulating properties, and purported therapeutic effects. This study presents the determination of the antioxidant capacity of coffee, and the assessment of those results using a caffeine standard. The Briggs-Rauscher (BR) oscillating reaction was used to determine the antioxidant capacity of the different coffee samples. The antioxidant species scavenge free radicals formed in the BR reaction, lengthening the time intervals of the reaction's oscillations; the higher the antioxidant capacity, the longer the oscillation delays. The samples consisted of caffeine (5%), and freshly brewed samples of espresso coffee (1%), decaf (1%), Costa Rican coffee (5%), Cuban Split Pea Blend (1%) and Jamaican Blue Mountain Coffee (5%). All samples show antioxidant capacity. To analyze the results we used the Relative Antioxidant Performance (RAP), where the slopes of the samples were compared to the caffeine standard. Jamaican Blue Mountain Coffee exhibited the highest RAP at the 5% dilution; Cuban blend was the highest RAP at the 1% dilution. To further examine the Cuban blend, we tested roasted split peas (10%), and they showed no antioxidant capacity. These observations suggest that antioxidant properties are present in coffee, and could be an explanation to its attributed health-giving properties. Finally, brewed coffee is a complex mixture of natural ingredients; therefore, we should not dismiss any potential synergistic effects between different ingredients.

Keywords: *antioxidants, oscillatory reaction, Briggs-Rauscher reaction, coffee, and caffeine.*

Introduction

Free radicals (FR) and reactive oxygen species (ROS) been suggested as potentially being important causative agents of aging and several human diseases such as cancer, inflammatory and degenerative diseases, emphysema, central nervous system injury, and autoimmune disease. The use of antioxidants for the prevention of damage caused by free radicals thereby assumes great importance for health and traditional medicine. Currently, there are many products on the market which claim to contain antioxidants. One of those is coffee, widely consumed around the world. Its active ingredient, caffeine, is sought after for its stimulating properties, and purported therapeutic effects.

The Briggs-Rauscher (BR) reaction is an oscillating reaction that changes between two cycles back and forth until it reaches equilibrium. The two cycles the reaction oscillates between correspond to a radical state and a non-radical state. The BR reaction is mostly used as demonstration.[1] Recently, Cervellati reported its use as a method to assess antioxidant capacity.[2] In this method, the presence of an antioxidant increases the oscillation time in the BR reaction. In this short communication we test if the Briggs-Rauscher oscillating reaction can determine antioxidant performance. Furthermore, we determine how the various coffee blends, Costa Rican Coffee, Jamaican Blue Mountain, Cuban Split Pea Blend Coffee, and decaffeinated Coffee, affect the BR reaction. Finally, we determine the Relative Antioxidant Performance (RAP) of coffee using caffeine as standard.

Methods and Results

A typical preparation of the Briggs Rauscher reaction was utilized.[3] When all stock solutions were prepared the solvents were tested as follows. Take 5mL of the sodium iodate solution, 5mL of starch solution, and 10mL 3% hydrogen peroxide. Once a stir bar has been placed in a 100mL beaker, start to mix the sodium iodate solution and starch solution in the beaker over a stirring plate. Then add the peroxide; the solution turns amber yellow then dark blue. Start the timer when the first dark blue color appears until the next dark blue appears. This is the oscillation time (usually 13-18 seconds).

This is also the control time for each trial. Repeat the step above and when the second deep blue color appears, add 1mL of coffee solution. Measuring the time from the second blue to the third blue appearance determines the antioxidant performance. All the coffee samples were dark roast, and were brewed using the espresso method.

The different dilutions of the antioxidant containing samples show different levels of antioxidant performance. The Jamaican Blue Mountain Coffee showed the best antioxidant performance at a 5% scale. The Cuban Split Pea Blend coffee showed an antioxidant performance at a 1% scale. Independently we roasted and grounded split peas to discern any antioxidant contributions. The split peas had little effect on the antioxidant performance of the Cuban-Split Pea blended coffee.

The relative antioxidant performance (RAP) of coffee was determined using caffeine as a standard. We measure the time the BR oscillation was delayed as a function of increasing concentration; the higher the concentration, the longer the delay. A best-line fit produced a slope for caffeine and for each of the samples. Our observations suggest that caffeine is probably the main antioxidant species in coffee, but not the only one present.

$$\mathrm{RAP} = \frac{\text{slope of sample}}{\text{slope of standard}}$$

	Slope of Caffeine	Slope of Jamaican Blue Mountain	Slope of Costa Rican	Slope of coffee	Slope of decaf	Slope of Cuban Split Pea	Slope of Split Peas
Trail 1	0.6	6.3	1.3	3.2	0.8	6.74	0.08
Trail 2	0.7	7	4.6	2.1	2.8	6.14	0.22
Trail 3	0.65	7	3.1	4.3	1.4	7.04	0.18
Average	0.65	6.766667	3	3.2	1.666667	6.64	0.16

		Jamaican Blue Mountain	Costa Rican	Coffee	Decaf	Cuban Split Pea	Split Pea
RAP		10.41	4.62	4.92	2.56	10.22	0.25

Conclusions

The Briggs-Rauscher oscillating reaction is effective assessing antioxidant performance. The varieties of coffee tested affect the reaction, confirming their antioxidant activity. Caffeine could be used as a standard to determine the Relative Antioxidant Performance (RAP). Our future aims are to the other ingredients in each coffee to see if they have an impact on the antioxidant performance.

Conflicts of Interest

The authors declare no conflict of interest.

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RELATIONSHIP BETWEEN AGE AND TWO SPECIES OF PROTOZOA IN CATTLE IN THE ECUADORIAN AMAZON

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Abstract:

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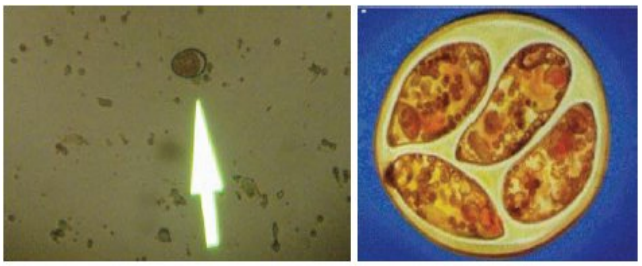

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RELATIONSHIP BETWEEN AGE AND TWO SPECIES OF PROTOZOA IN CATTLE IN THE ECUADORIAN AMAZON

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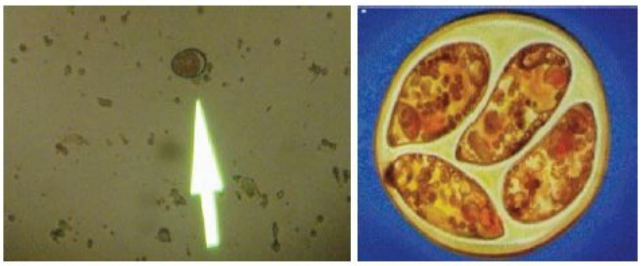

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MANUSCRIPT

A PROTOTYPE WEB APPLICATION PACKAGE FOR BASIC DNA AND PROTEIN ANALYSIS USING R LANGUAGE

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ABSTRACT

Analysis of DNA and protein has become a very important aspect in the field of research, especially for Bioinformatics. This is important as the basic analysis of these protein and DNA can lead to further advanced analysis of the sequence, which may lead to new discoveries. Basic analysis of sequences is done in the industry, research as well as education. R language is a statistical program that is used in the analysis of DNA and protein sequences, through the application of packages in the Comprehensive R Archive Network. This analysis package helps to analyze sequences, but in a command prompt analysis. However, the process is slow as the researcher has to enter several lines of codes to obtain the result for the analysis. The research is to develop a prototype web application package with an interactive new interface for the DNA and protein analysis. The prototype is fully coded in R with options to download the results as well as providing information about the codes being used for the analysis and the package reference. This application is made to assist in the sequence analysis of DNA and protein without having to write the codes.

KEYWORDS: R, Bioinformatics, sequence analysis, web application, statistics

INTRODUCTION

Bioinformatics is a hybrid field consisting of different field such as biology, statistics, chemistry and genetics with the addition of information technology in the analysis and the interpretation of biological data. Some of the fields in Bioinformatics includes sequence and structure analysis of sequences of DNA and protein. Manipulation of sequences can be done via sequence analysis in Bioinformatics which includes statistical outputs as a theoretical value and result. R language is a GNU language(Kim, 2007) that emphasizes in statistical analysis with simple data analysis and data visualization. The packages in R are in alphanumeric form that helps programmers to script codes using the packages (Jinlong, 2011) There are packages in the R archives which contains codes for statistical analysis, including sequence manipulation. R language is an open source language, thus it is freely distributed among people in the Internet. R is also integrative as it allows the language to be implemented with other languages such as C++ (Dirk and Romain, 2011) and Java. In the field of Bioinformatics, R language is used in the analysis of sequences to produce statistical data using analysis packages which is accessed using a R terminal that requires writing long lines of codes and sometimes in a strange arrangement. These R codes can be used in the analysis of DNA sequences ("What is DNA", 2014) such as length, GC count (Zheng and Wu, 2010, Oliver and Marin, 1996, Henke et al, 1997 , base count (Lobry and Lobry, 1999) as well as reverse and complement of the sequences. It can also be used in the protein analysis in the study of evolutionary analysis (Mehmet *et al*, 2006), length determination (Kingshuk and Ken, 2009, Luciano and Samuel, 2005), the isoelectric point of the protein (Kawashima *et al*, 1999, Widmann *et al*, 2010), translation from DNA to protein, amino acid statistics (Kawashima *et al*, 1999) as well as the Dot Plot analysis of both sequences (Gibbs and Mcintyre, 1970). The R codes can be used to generate graphical plots such as box-plots, histograms and charts for better analysis of data (Tina, 2014)

PROBLEM STATEMENT

Previous analysis of DNA and protein sequences using R has been done using the command line analysis, which is redundant and time consuming. Besides that, the R language is deemed difficult due to the strange code structure, making it difficult for users to learn.

METHODOLOGY

Agile Unified Process

Agile Unified Process has four stages. The Inception stage where initial analysis for R and Bioinformatics is being done, Elaboration is done to see the compatibility of the R language to the current analysis packages in the repository. Construction is where analysis packages is combined with the documentation package under one large web application, using a special web application package as a framework. Then, the prototype is uploaded to the online server and deployed for testing.

Packages used for the analysis:

a) Shiny : web application package for the R codes. Combination of the analysis package as well as the documentation package is combined within the framework.
b) knitR : Dynamic report generating package using R language. The package acts as a secondary R terminal to include the input, codes and output into a report
c) seqinR: analysis package created for sequence extraction and analysis from the databases or from random input from the user.
d) rBase : the base package in R itself which is installed with the R language. Provides the structure and syntax for the R codes

RESULTS

Several comparison analysis has been conducted in terms of the methods used for the DNA and protein sequencing and the prototype produces an accurate analysis of the data, compared to the current online web application tool as well as the command line analysis. The prototype also has a good response from the users in terms of knowledge input and provides a good documentation which includes the input, codes for the analysis and the output as well. Based on the user acceptance survey and prototype evaluation survey, the prototype web application attains a good response in terms of user interface, system analysis as well as the result production. In terms of Bioinformatics, the users agree that statistical analysis is a very important aspect in the sequence analysis in bioinformatics. They also agree that providing the codes for the analysis in the interface as well as the documentation provides new knowledge in terms of analysis method and the codes being used for the analysis. The new documentation format is also preferred by the respondents as it shows the input, codes and the output of the analysis in the same report.

The deployed application is also tested with a random number of sequences for Protein and DNA analysis. The prototype web application is compared with another online web application tool as well as the R command line. Comparison are made for all the analysis present in the prototype web application, numeric and graphical. The prototype web application has shown to produce a good and accurate analysis of the sequences, similar to the R command line analysis as well as the online web application. Certain analysis such as the Dot Plot analysis and the Amino Acid Statistics which produces a graphical output is produced similarly in the R command line, but not in the online web application tool.

DISCUSSION

The prototype passes the user acceptance test in terms of user interface, system analysis as well as result production. This is because compared to the command line analysis which is R language's main access, the users don't have to write long lines of codes to access the analysis packages. Only a click of a button and the results will be shown in the interface as well as the report. There are two spaces for the input, which allows the user to do a rough comparison of the two sequences as well in the report produced. Reports are separated between the numerical analysis and the graphical analysis Figure 2 to prevent clutter in the report production as well as helping the users to understand the results better. Implementation of R language codes in the help section provides the users with a new knowledge because the prototype not only provides a simple explanation about the analysis method being used on the

sequences but also the codes that is being used for the manipulation of the sequences. This provides a new knowledge in users as they could perform the sequence analysis, learn about the methods being used in the sequence analysis as well as the codes that is being used for the analysis.

The prototype is also easy to use as the users only has to enter the input once to obtain all the outputs of the analysis such as in Figure 3. This method prevents the redundancy of users to enter the same sequence every time they want to perform sequence analysis. This also reduces the overall time taken for sequence analysis to be done, as the users do not have to take their time in writing long lines of codes redundantly to obtain the results of the sequence analysis. Presence of graphical outputs provides an easier way to interpret the sequence analysis results compared to the textual output such as the dot plot analysis and the amino acid statistics function in the prototype web application. The presence of the user interface such as in Figure 1 and Figure 4 helps in providing a better access to the sequence analysis because the packages in R are commonly accessed in command line prompt. The user interface helps to access the analysis and documentation packages in R without the users having to write the codes to access the packages and still obtain the results as accurate as the command line analysis. The user interface provides a good medium to link the users with the R sequence analysis packages without having to access the command line prompt.

CONCLUSION

The prototype web application for the basic DNA and protein analysis using R language provides a simple basic analysis of sequences with the addition of information about the methods of the analysis as well as the codes that is being used for the analysis as well. The prototype has several tabs for the information and analysis of the sequences. Users shows preferences for the prototype in terms of user interface, system analysis as well as result production. Accuracy of the prototype can also be proved by the accurate result comparisons with the command line and the online web application tool. Redundancy and time can be reduced as the users do not have to write long lines of codes for the analysis.

ACKNOWLEDGEMENT

Firstly, I would like to thank my student Sivakumar chandran and Prof Dato Gapar for helping this work in a great manner.

CONFLICT OF INTEREST

There is no conflict of interest

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FIGURES

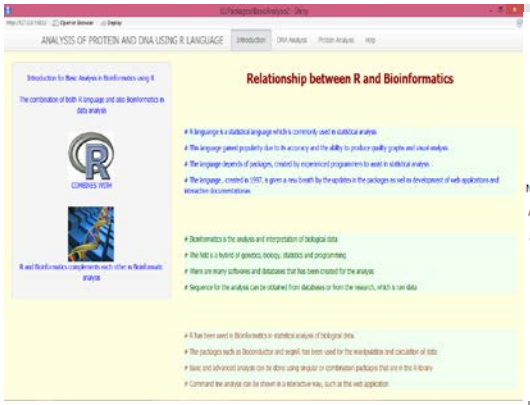

FIGURE 1: INTRODUCTION INTERFACE

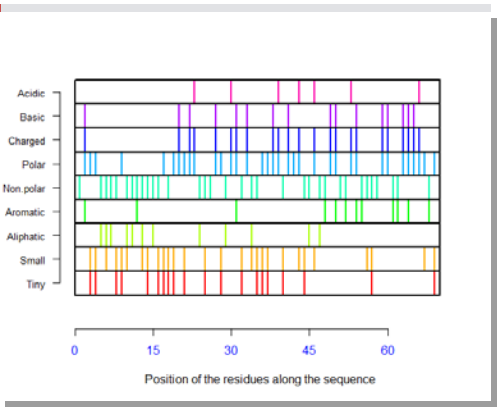

FIGURE 2 : AMINO ACID STATISTICS

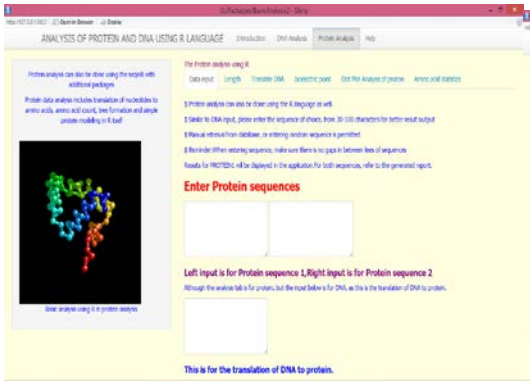

FIGURE 3: DATA INPUT INTERFACE

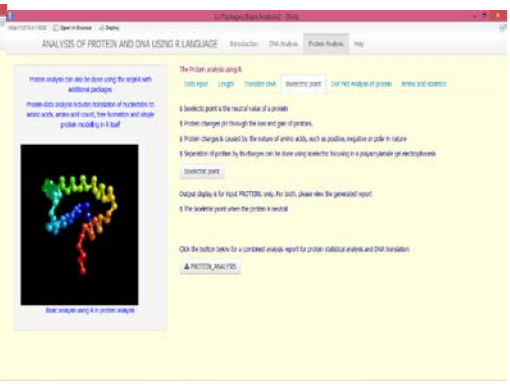

FIGURE 4 : ANALYSIS METHOD

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Artificial Neural Network Schedulers for Food Webs

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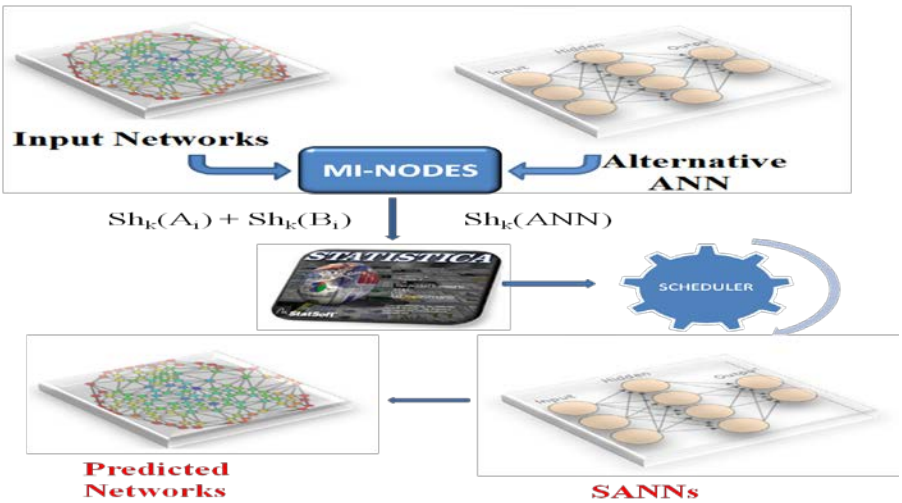
[3] IKERBASQUE, Basque Foundation for Science, 48011, Bilbao, Biscay, Spain.

Abstract: *In this work, we introduce by the first time a new type of algorithm aimed to predict the more promising topology of one ANN to be trained in order to model a given dataset of complex system. 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. Using information parameters as inputs, we developed one scheduler for 338050 outputs of 10 different ANNs for the respective 33805 pair of nodes in 73 Biological Networks. The overall accuracy of the SANN-HPC schedulers found was of >72% for Biological Networks; in training and validation series.*

Keywords: *Artificial Neural Networks, High Performance Computing, Biological Networks*

Graphical Abstract:



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GCUB 2017, Campaign to Launch the Meeting of The Coimbra Group of Brazilian Universities in University of Alfenas

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This is the first announcement of the campaing to launch the GCUB 2017 Meeting of The Coimbra Group of Brazilian Universities (GCUB). GCUB was formally established as an association of university leaders on November 27, 2008, during the academic ceremony at the Great Hall of the Rectory of the University of Coimbra. The GCUB consists of national universities - federal, state, religious and community. Some of the most important Brazilian universities today make up the group of 72 institutions associated with 51 federal universities, 15 state universities and 6 Community and confessional universities. The current president of GCUB is Prof. Paulo Márcio de Faria e Silva, Rector of Federal University of Alfenas, Brasil.

The GCUB welcomes more than one million students enrolled in undergraduate; Furthermore, it holds more than 90% of sense-strict Graduate programs and research groups consolidated in the country. It is the only organization of its kind that brings together, in a predominantly academic forum, a representative set of knowledge institutions in Brazil can claim, legitimately, the historical-institutional status University.

Despite its short existence, the GCUB is responsible for programs for internationalization of Brazilian universities with countries like Mexico, France, Portugal, Switzerland, Canada, Colombia, Peru, among others. The GCUB has partners, sponsors and collaborators, among

them: Brazilian government agencies such as:

- The Ministry of Education and Culture (MEC).
- Higher Education Personnel Improvement Coordination (CAPES).
- National Council for Scientific and Technological Development (CNPq).
- The Ministry of Foreign Affairs (MRE); International organizations - Organization of American States (OAS), IESALC / UNESCO.

As well as, university networks like the *National Association of Universities and Instituciones de Educación Superior* (ANUIES), *Agence Universitaire de la Francophonie* (AUF), *Colombian Association of Universities* (ASCUN), *Coimbra Group* (CG), Univerdades of Unión de America Latina y el Caribe (UDUAL) and *National Rectores Asamblea* (ANR).

This year, as part of a more general strategy for the internationalization of the GCUB meeting, the MOL2NET Conference Series on Multidisciplinary Sciences will act as the online host conference of the GCUB 2016. MOL2NET is an International Conference Series to Foster Interdisciplinary Collaborations in Sciences with emphasis on Experimental Chemistry (all branches), Materials Science, Nanotechnology, Life Sciences, Medicine, and Healthcare, along with Data Analysis, Computer Sciences, Bioinformatics, Systems Biology, and Complex Networks Sciences.

The conference series per se, is the result of the synergy between IKERBASQUE, Basque Foundation for Sciences, the Departmentof Organic ChemistryII,Faculty of Science and Technology, University of Basque CountryUPV/EHU, and the Faculty of Informatics, University of Coruña (UDC). MOL2NET-01, the first edition of this conference series, was held in Dec 2015 (http://sciforum.net/conference/mol2net-1).

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.

NOTES: The conference is Totally Online; no physical presence is needed 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). Please, see the following instructions: (1) Read call for papers [Link], (2) Read instructions to authors and download template .doc file [Link], (3) Submit short communications (2-3 pages), reviews, papers, or videos: [Link].

Consequently, this section serve as both: (1) publication media for communications, posters, or plenary conferences (videos) of GCUB 2016 and also (2) online discussion media to post comments, questions, and answers about GCUB 2016.

The authors of GCUB meeting should send their papers using the same central online system and selecting section G for their papers, posters, or videos, please, click here to submit: MOL2NET-GCUB Submission.

GCUB 2017 Meeting Chairman

Prof. Dr. Ihosvany Camps

Universidade Federal de Alfenas, Unifal-MG, Alfenas, MG, Brasil.

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Prof. Paulo Márcio de Faria e Silva,

Rector of Universidade Federal de Alfenas. Unifal-MG, Alfenas, MG. Brasil

SCIFORUM

SRI08

SRI-08: The 8th Annual Undergraduate Summer Research Symposium of Saint Thomas University.

David Quezada, Email: dquesada@stu.edu
Saint Thomas University (STU), Carnival Building, Room 115, Miami, FL, USA.

Humberto González-Díaz, Email: humberto.gonzalezdiaz@ehu.eus
(1) Department of Organic Chemistry II, University of Basque Country (UPV/EHU), Biscay, Spain.
(2) IKERBASQUE, Basque Foundation for Science, Bilbao, Biscay, Basque Country, Spain.

Abstract: Dear colleagues we welcome you to the symposium of the Summer Research Institute (SRI) with Head Quarters (HQ) at Saint Thomas University (STU), and supported by Miami Dade College (MDC), Miami, Downtown, FL, USA . This is face-to-face (in person) workshop associated to and hosted online by MOL2NET-2, International Conference of Multidisciplinary Sciences, 2016, MDPI, Sciforum, Basel, Switzerland, with HQs, University of The Basque Country (UPV/EHU), and supported by IKERBASQUE, Basque Foundation for Science, Basque Country, Bilbao, Spain.

Welcome Message

Eight years have passed since the First Annual Undergraduate Summer Research Symposium. Over this period of time, the School of Science, Technology, and Engineering Management of Saint Thomas University (STU) in partnership with Miami Dade College (MDC) and with the continuous support of the faculty and staff has provided an excellent internship program to our students and those coming from our partner's institutions. From the start the Carnival Cruise Lines Science and Technology Building focused on the hands-on research experience. This places our students in an excellent position to gain entrance into graduate, or professional Schools, or to directly enter the workforce in South Florida. Most students in other institutions do not have this research opportunity until they reach graduate school.

The faculty and staff of the School of Science are committed to providing a quality education in the sciences and offer the unique opportunity to talented undergraduate students, to experience hands-on research in ten research laboratories alongside their professors. In addition, the Summer Research Institute (SRI) has enhanced the instrumentation capacity of our institution. Such improvements have allowed deepening our research projects as well as to establish new alliances in research and

development. Results from our projects already circulate in local, national and international conferences, augmenting this way the visibility of the institution and the pride that students might have for their faculty and work accomplished. The eight edition of the SRI offered a continuous lecture series "Moving into the Future" on a weekly basis. Ten speakers from University of Miami, Florida International University, and local Technological companies came to St. Thomas to share their wisdom as an in kind contribution.

This year, the memories of the Annual Symposium will be published online in the open source forum MOL2NET of 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, inter-disciplinarity, teamwork and networking. I hope you will enjoy the program and the presentations.

Special thanks to our sponsors, Miami Dade College, STEM-TRAC grant, St. Thomas School of Science, Technology, and Engineering Management, Yager Foundation, Monet Coiffure, In Fashion Forever, and Physics & Mathematics Solutions, who provided funding for major and minor activities associated with the 2016 edition. Follow the link to download and/or read the full program [SRI08 Program]

Program Overview

8:00 – 9:00 Registration and Poster Setup. Judges meet in room CCL 111
9:00 – 9:05 Invocation by Rev. Alfred Cioffi
9:05 – 9:20 Opening Comments by Dr. Adrienne Vynne, Dean of School of STEM
9:20 – 9:35 Opening Comments by Dr. Irma Becerra, Provost of St. Thomas University
9:35 – 9:45 Session Introduction by Dr. David Quesada, Coordinator SRI 2016
9:45 – 10:00 Oral Presentation 1
10:00 – 10:15 Oral Presentation 2
10:15 – 10:30 Oral Presentation 3
10:30 – 10:55 Break
11:00 – 11:15 Oral Presentation 4
11:15 – 11:30 Oral Presentation 5
11:30 – 11:45 Oral Presentation 6
12:00 – 13:00 Lunch Break courtesy of the School of STEM
13:00 – 15:00 Poster Session
15:00 – 15:30 Judges meet in CCL 111 to choose the awards
15:30 – 15:45 Announcement of Awards

Submission Notes

This workshop is planned to be held on Sept, 2016. However, 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. After abstract approval you need to send your communication. Firstly, you need to submit only the title, authors, and short abstract. Secondly, you could submit your full work in .doc and .pdf format after receiving the approval email from the chairperson. Please, download and use the following .doc file as [SRI08 2016 Official Template] to submit your work. For more details contact the Chairman of the workshop.

Be aware:

on step 1, you should select MOL2NET 2016, International Conference on Multidisciplinary Sciences, 2nd edition (Conference),

on step 2, you should select SRI08: Saint Thomas University Research Experience for Undergraduates, Miami, 201**6.**

Author Contributions

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Symposium Committee: follow the link to see the full symposium committee, please take into consideration that the committee list may change upon final completion. http://sciforum.net/conference/mol2net-02/sri-08

SciForum
MOL2NET

MOL2NET Introductory talk by Ph.D. Yong Liu,
Audio: Chinese Mandarin (官话)

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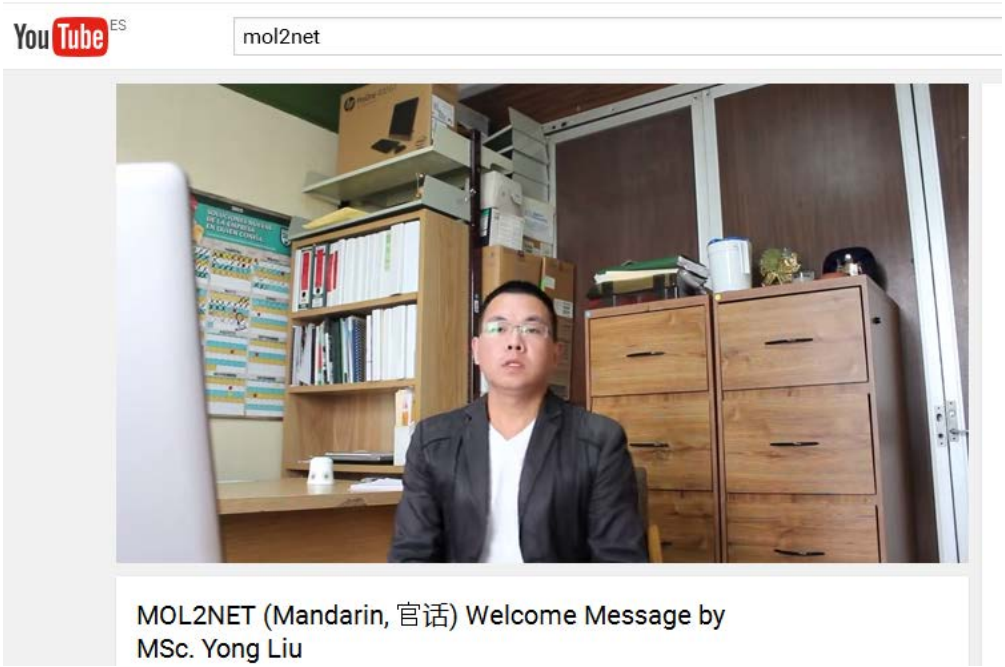
(b) Key Laboratory of Subtropical Agro-ecological Engineering, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, Hunan, P. R. China
(c) Computer Science Faculty, University of A Coruna, Spain.



Abstract: 大家好，我叫刘勇，是 H. Gonzalez-Diaz 教授的一名在读博士研究生。受 Humberto Gonzalez-Diaz 教授之托，在此为他将于 2015 年 11 月 15-30 号在科学论坛上组织一个关于 Molecular to Social Network（Mol2Net）的国际会议作中文的宣传。Mol2Net 会议是一个结合分子科学，生物医学，数据研究，社会网络建立科学的国际会议。Mol2Net 会议涉及到实验科学方面，包括化学，药物学，癌症蛋白组学，神经科学，纳米科学，流行病学，分子生物学，生物医学等。计算科学包括理论计算学，社会科学，计算化学，生物信息学，社会网络分析，大数据预测分析等等，或是与之相关的研究领域。同时，这次议会将接收的会议论文发表在 MDPI 的杂志期刊上。我们诚挚地欢迎您的加入。更多信息请关注：http://sciforum.net/conference/mol2net-02, Published on Youtube in Jul 13, **2015**

Youtube link: https://youtu.be/E6GJVmE0RTs

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Dimers Derived From Densely Substituted Unnatural Prolines As Precursors Of γ-Peptides And Their Use In Organocatalysis

Maddalen Agirre, Mª de Gracia Retamosa, Andrea Ruiz-Olalla and Fernando P. Cossío*

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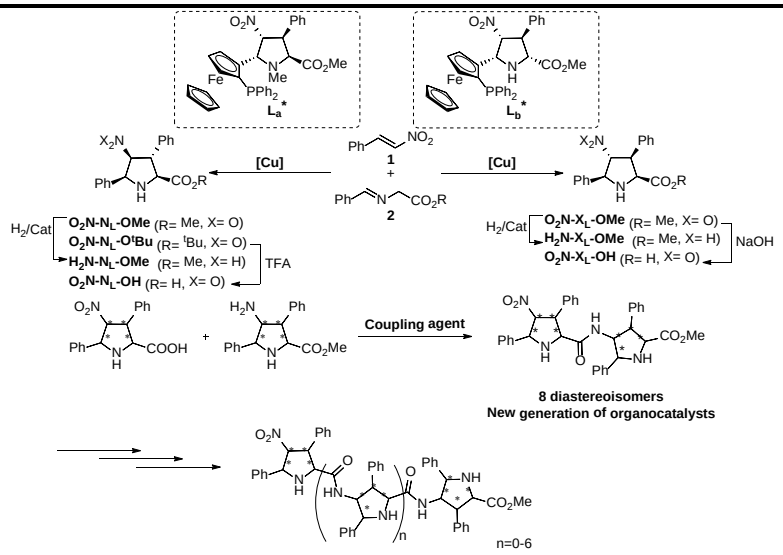
The synthesis of novel hybrid ferrocenylpyrrolidine ligands $\mathbf{L_a}^*$ and $\mathbf{L_b}^*$ via [3+2] cycloadditions has been described by our group.[1] Both ligands in turn provided densely substituted unnatural L- and D-proline derivatives in a stereodivergent manner. The powerful feature of having nitro and ester groups gives the opportunity to orthogonally synthesize different γ-proline oligopeptides with different substitution patterns and chiral centers. Supported by the efficiency of Proline-based organocatalysts in numerous chemical transformations, our densely substituted pyrrolidine derivatives have been used in aldol reactions with good results.[1,2] In this communication, we present our results on the structure/activity relationship of the new generation of oligomeric catalysts. The main conclusion is that in the case of the γ-dipeptides, the stereochemistry of the aldol adducts depends on both monomeric units in a nearly additive manner.



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Food sources and emerging methods to obtain ellagic acid

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Abstract: Polyphenolic compounds present in food have been widely investigated because of their health benefits as antioxidants, in particular their role in the prevention or treatment of chronic diseases. The current bibliographic investigation presents a collection of the main sources of ellagic acid, as well as structural and safety features related to its applications on dietary. The quantitative information has been compiled in databases of food composition directed to different professionals such as dieticians, innovative food-producing industries, agronomists, botanists, etc. General issues of the importance of structure activity relationship and cheminformatics studies related to Food Science were described. New biotechnological methods applied to obtain ellagic acid from agro-industrial waste, were also analyzed. Different aspects of efficacy and safety of ellagic acid as diet-derived antioxidant are presented and discussed.

Keywords: food science; food composition database; cheminformatics; ellagic acid; polyphenol

Mol2Net YouTube channel*: http://bit.do/mol2net-tube*
YouTube link: *please, paste here the link to your personal YouTube video, if any.*

1. Introduction

During the last decades, the interest in ellagic acid (EA) and other polyphenolic phytochemicals has increased. Some of the stakeholders are nutritionists, pharmaceutical industry, agriculture/food, and consumers [1]. This interest is mainly due to the discovery of its in vitro antioxidant effects, as well as its alleged role in the prevention and treatment of various chronic diseases [2-7].

Chronic diseases are still one of the main death causes in developed countries [8,9]. It has been shown that diets rich in fruits and vegetables are associated with lower mortality rates associated with some of these diseases such as cardiovascular disease and some types of cancer [10,11]. For their antioxidant activity, polyphenols on diet may contribute to this relationship as they are present in fruits, vegetables, grains, seeds and some drinks such as wine, tea, beer, etc. [1].

For these reasons many investigations regarding EA have been focused on characterize its bioactivity. Their multiple activities have been determined (Figure 1), among which are: antiviral, anti-mutagenic and anti-cancer effects [12-17]. EA also exerts potent preventive and therapeutic effects against several types of cancers, including colon cancer, breast cancer, prostate cancer, skin cancer, esophageal cancer and osteogenic sarcoma [18,19]. The anticarcinogenic properties of EA have drawn increasing attention globally [9]. EA has been considered an antioxidant [16,20,21]. The antioxidant activity is one of the proposed activities related to the anti-cancer effects, along the anti-inflammatory and anti-proliferative effects [22,23].

Currently, Food Sciences focus their efforts on the characterization of polyphenols and EA. The content in food and other matrices is essential to assess its potential benefits in terms of biological activity. This knowledge has to be matched with the design of new carrier matrices of these phytochemicals such as fortified foods, functional foods, nutraceuticals and dietary supplements [24]. It has been observed that in some cases, when the amounts of some bioactives were manipulated in the production of dietary supplements, the results were not as expected [3].

The aim of the present study is focused on the main dietary sources of EA that have been compiled so far in food composition databases (FCDB). These food sources were analysed from the perspective of the health benefits and the potential of agricultural production. Development and use of modern biotech and cheminformatic methodologies for characterization of biological structure-activity relationship are also addressed.

2. Results and Discussion

EA is a naturally occurring phenolic constituent, that is contained in ellagitannins, in grapes, nuts, strawberries, black currents, raspberries, green tea, pomegranates and nuts [9]. Ellagitannins are a complex class of polyfenols characterized for one or more hexahydroxydiphenoyl (HHDP) moieties esterified to a sugar, usually glucose.

Hong-Mei et al., 2014 compiled the EA content in different foods and plants, described by several authors [25-32]. Observed for this data that the content of this polyphenol varies widely, from values of 2-160 mg/100g dry weight (Figure 1). This variability in the content has been observed for all the polyphenols [33]. The phenolic composition of fruits varies greatly among cultivars [33]. They have been described multiple factors affecting the polyphenol content in food [34]. Maas, J.L. et al. (1991), studied the strawberry cultivars for EA content, realizing that this lack may indicate that selection for EA content can be highly specific for tissue type; e.g., for high fruit content but low leaf content [35,36].

Phenol-Explorer Database contains detailed data on the specific amounts and content in foods of the major classes of polyphenols. Version 3.6 contains information of nine subclasses of flavonoids, stilbenes, lignans and other polyphenols within which are two subclasses of coumarins and five subclasses of phenolic acids where EA is located. It also contains information about 155 food processing and its influence on the

content of 161 polyphenols covering 35 processes and also includes RFC.

The database of Phenol-Explorer compiled the content in EA of food. An example of that, organized by families and species, is shown in Table 1. This database still does not include the content of this polyphenol determined in mango (*Mangifera indica*), guava (*Psidium guajava*), peper (*Capsicum anunn*), peach (*Pras persica*), gooseberry (*Ribes grosularia*), chamomile (*Camelia sinensis*), camu-camu (*Myrciaria dubia*), radish (*Raphanus sativus*), beetroot (*Beta vulgaris*)[37].

The main sources for obtaining EA are condensable tannins, ellagitannins and complexes [38]. The traditional method of production has been through processes involving highly polluting substances such as acids and alkalis with high costs of heat energy. Today is a challenge to generate new knowledge about the processes of biodegradation of ellagitannins and thus help to eliminate the limiting factor in the development of biological technologies. The latter focus their research on the importance of determining for example the type of enzymes that are involved in this process. Parallel is also a challenge from obtaining industrial raw materials, agricultural products and crop residues like pomegranate, muscadine grape cultivars, berries family, etc.

Can be generalized that the process of obtaining EA is supported on two sides: the extraction and biodegradation of tannins (Figure 2). They have been used as conventional methods; other unconventional methods as solvent extraction [39-42], assisted with microwave and ionic liquids, ultrasound and supercritical fluids with cosolvents, can also be used [43-45].

Currently there are controversial aspects leading to deepen the characterization and benefit/safety of polyphenols. A debated aspect is related to their advantage when they as antioxidants, as current studies are inconclusive and sometimes contradictory [3]. The possible genotoxic activity of EA has been evaluated *in silico* using the TOPSMODE approach proposed by Estrada [46]. In this study Yordi et al. (2015) considered the EA as part of an external data set of phenolic acid with prooxidant reports. As part of this study, this compound presented a probability of being active of 0.932 [47].

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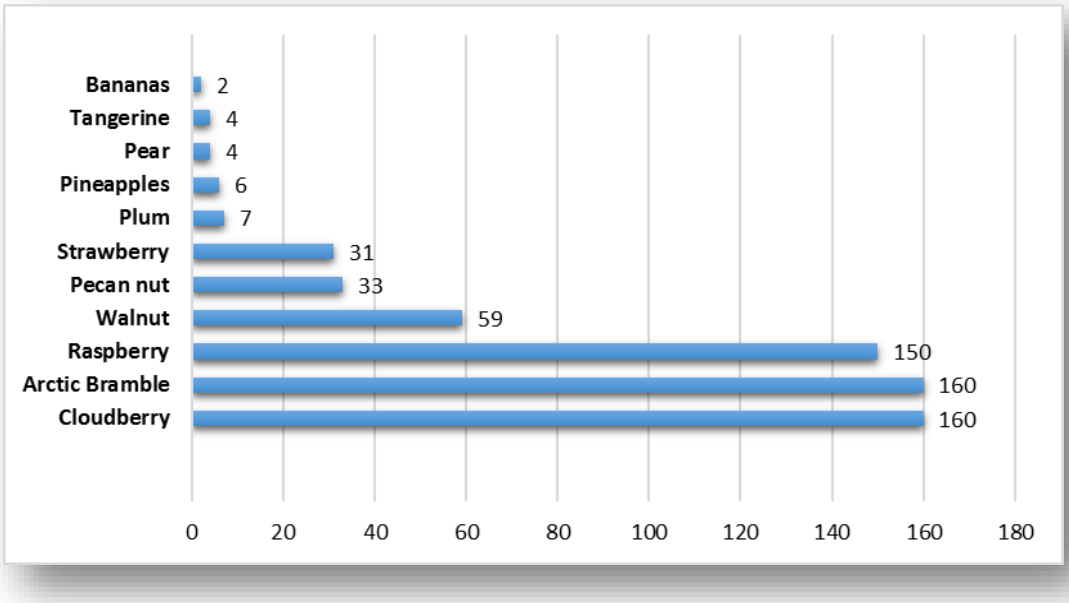

Figure 1. EA minimal values in mg/100g dry weight of plants and fruits [37].

Table 1. Ellagic acid (determined by chromatography) containing plant species includes in Phenol-Explorer database. Adapted from [37].

Phenol Explorer Database		
Family ***specie*** -vernacular name	**Food classification**	**EA mean content (mg/100 g)**
Fagaceae		
Castanea P. Mill - Chestnut	Seeds - Nuts	735.44
Juglandaceae		
Juglans ailanthifolia Carr. -***Japanese Walnut***	Seeds - Nuts	15.67
J. regia L. - Walnut	Seeds - Nuts	28.50
J. regia L. - dehulled Walnut	Seeds - Nuts	5.90
J. regia L. - Walnut	Alcoholic beverages (Liquor)	1.22
Quercus sp. - Oak	Alcoholic beverages (Brandy)	1.13
Quercus sp. - Oak	Alcoholic beverages (Rum)	0.21
Quercus sp. - Oak	Alcoholic beverages (Scotch whisky, Rum)	0.82
Lythraceae		
Punica granatum L.-Pomegranate	Fruit (from juice concentrate)	17.28 9.13*
Punica granatum L.-Pomegranate	Fruit (pure juice)	2.06 3.97 *
Rosaceae		
Fragaria spp - Strawberry	Fruit (raw)	1.24
Fragaria spp - Strawberry	Fruit (raw)	2.85*
Rubus sp. - Blackberry	Fruit	43.67
R. chamaemorus L. - Cloudberry	Fruit	15.30
R. idaeus L. - Red raspberry	Fruit (jam)	1.14
R. idaeus L. - Red raspberry	Fruit (jam)	0.08**
R. idaeus L. - Red raspberry	Fruit (jam)	0.13***
R. idaeus L. - Red raspberry	Fruit (jam)	1.00****
R. idaeus L. - Red raspberry	Fruit (pure juice)	0.84
R. idaeus L. - Red raspberry	Fruit (raw)	2.12
R. idaeus L. - Red raspberry	Fruit (raw)	0.20**
R. idaeus L. - Red raspberry	Fruit (raw)	0.36***
R. idaeus L. - Red raspberry	Fruit (raw)	2.27**
Vitaceae		
Vitis rotundifolia Michx -Muscadine grape (Black)	Non-alcoholic beverages	0.90
Vitis rotundifolia Michx. - Muscadine grape (Green)	Non-alcoholic beverages	0.93

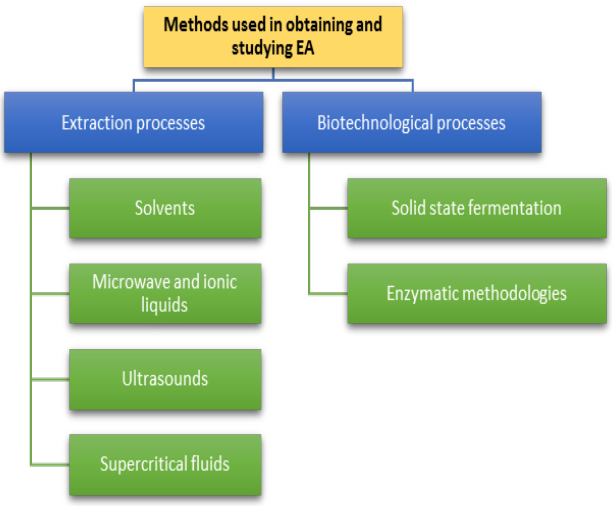

Figure 2. Methods used in obtaining and studying EA [37].

3. Materials and Methods

The main dietary sources of EA and the use of modern biotech and cheminformatic methodologies were analysed from scientific literature from the perspective of the health benefits and the potential of agricultural production (Figure 3).

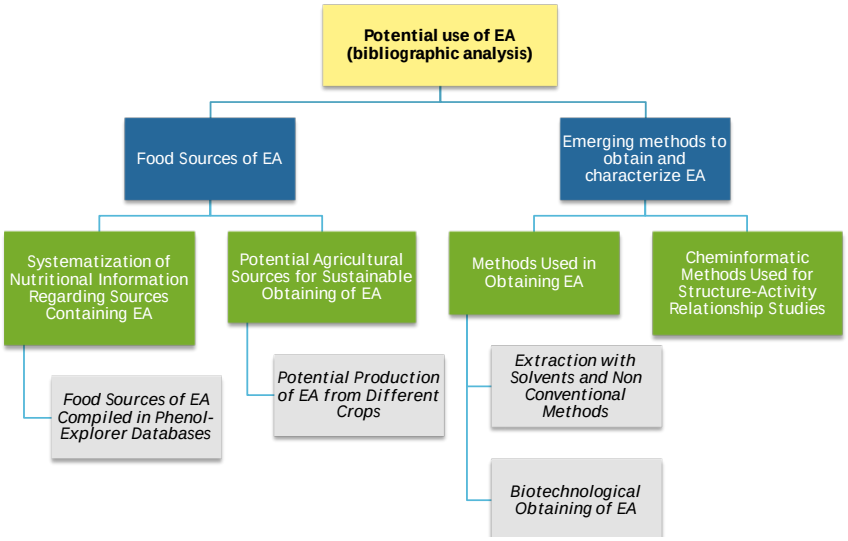

Figure 3. Scheme of the applied methodology

4. Conclusions

- New chemical technologies for EA obtaining and industrial scale are conditioned to the development of new methods and biotechnology.
- The generation of sustainable technological alternatives is necessary.
- The database information collected here can help in a future sustainable agricultural development of potentially rich EA crops, controlling genetic, biotic and abiotic factors that influence their content.
- Many fruits have higher concentrations of antioxidants found in the bark, seeds and pulp residues, which are by products of industrialization thereof. These elements show the wide possibilities that arise in the future for commercial production of EA.
- Epidemiological studies that linked higher intake of polyphenols and EA are required.
- To meet these challenges, the use of bioinformatics and in silico studies is truly important. In vivo and in vitro studies are starting points and sources of valuable information to consider the introduction of in silico methods, such as QSAR, in Food Sciences.

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Author Contributions

All the authors contributed equally for the execution of the work and the writing of the manuscript

Conflicts of Interest

The authors declare no conflict of interest.

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A QSAR Study towards Predicting the Adsorption of Environmental Pollutants by Multi-Walled Carbon Nanotubes

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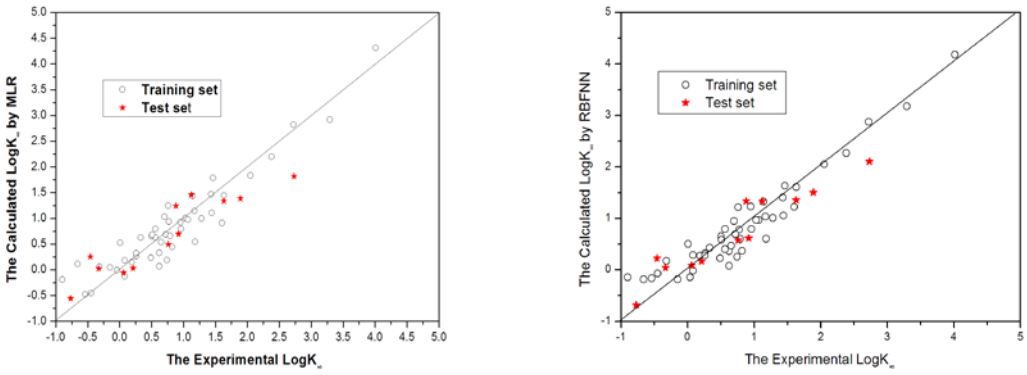
Abstract: Nanotechnology has led to the development of new materials with unique properties and a wide variety of applications. Meanwhile, it has raised great concerns regarding their properties and potential adverse effects to humans and the environment. In this work, a Quantitative Structure-Activity Relationship (QSAR) modeling study was carried out for predicting the adsorption property of a set of 59 environmental pollutant aromatic compounds into multi-walled carbon nanotubes. We report a systematic evaluation of multiple linear regression (MLR) and artificial neural network (ANN) methods along with a variety of structure representations and feature selection algorithms. Judging from the attained statistical results, our derived QSAR models have an acceptable overall accuracy and robustness, as well as good predictivity on external data. This QSAR study suggested also that the adsorption ability of these compounds is mainly explained by size, charge and hydrophobicity factors. Moreover, it showed to be a simple, precise and credible tool forward-predicting the adsorption of aromatic compounds by multi-walled carbon nanotubes.

Keywords: Environmental Pollutants, Multi-walled Carbon Nanotubes, Quantitative Structure-Activity Relationships (QSAR); Multiple Linear Regression (MLR); Artificial Neural Networks (ANN)

Graphical Abstract:



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2D-QSAR modeling was performed on the adsorption property of a set of 59 environmental pollutions aromatic compounds into multi-walled carbon nanotubes by MLR and RBFNN.

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Introduction:

QSAR studies forward-modeling nanomaterials are still in an early stage. Many research efforts now aim at a better understanding of the properties and behavior of nanomaterials (including nanoparticles). As a desire for contributing to the use of the QSAR methods to tackle nanomaterials, we will present the development of 2D-QSAR models based on a series of aromatic compounds known to adsorb on MWCNTs.

Materials and Methods:

A data set of 59 aromatic compounds with the adsorption parameter K_∞ was used as dependent variable after log-transforming ($\log K_\infty$) to be of practical use in the following QSAR modeling. All molecular structures were drawn and energetically optimized by molecular mechanics MM^+ and the semi-empirical PM3 and MOPAC, then were brought into the CODESSA program to calculate five kinds of descriptors. The MLR and ANN were then used to build and validated the models.

Results and Discussion:

The best-fit linear regression model found using four descriptors is given below along with its MLR statistical parameters.

$$\mathrm{Log}K_\infty = 5.002\times10^{-3}GI + 5.262E_{\mathrm{min}}^{\mathrm{C\text{-}H}} - 4.084RNN - 5.456Q_{\mathrm{max}} - 32.596$$

N = 47, R^2 = 0.886, RMS = 0.3374, F = 351.71, ρ = 11.75

In this equation, GI stands for the gravitation index (all bonds), $E_{\mathrm{min}}^{\mathrm{C\text{-}H}}$ for the minimum exchange energy for a C-H bond, RNN represents the relative number of N atoms, and Q_{max} is the maximum partial charge. For the discussion of the descriptors, one can conclude that small, less charged compounds are expected to be adsorbed more favorable by these nanomaterials. Prediction results were obtained using the external test set (N = 12). In this case, the obtained statistical parameters were the following: Q^2 = 0.894, RMS = 0.3654, F = 75.01. The training quality and the predicting ability of the RBFNN model can be judged from the obtained statistical results, *i.e.* for the training set (N = 47): R^2 = 0.906, RMS = 0.2903, F = 403.84; and for the external test set (N = 12): Q^2 = 0.894, RMS = 0.3654, F=75.01.

A Williams plot, *i.e.* a plot of the standardized cross-validated residuals *vs.* leverage values (or hat values, $h = 3m/n$, for this study, m = 4, n = 47) can be used for an immediate and simple graphical detection of both the response outliers and structurally influential chemicals in the model.

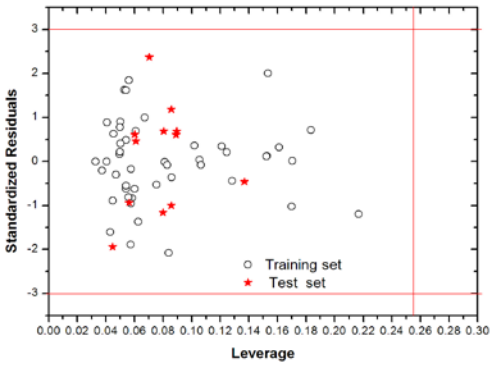

Fig. 1. The William plot for the training and test sets.

Conclusions:

QSAR models (MLR analysis and RBFNN) have been developed to predict the adsorption of aromatic compounds by multi-walled carbon nanotubes. The acceptable statistical results of both these models proved their robustness as well as their predictive power. In addition, the developed MLR model provided some useful insights regarding which descriptors are most related to the adsorption ability of the analyzed compounds, suggesting that to be mainly governed by size, hydrophobic and charge factors.

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Antimicrobial peptides of *Lactobacillus plantarum* UTNCys3.4 strain isolated from native fruits of Ecuadorian Amazonia inhibit the growth of foodborne pathogens

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Abstract: Lactic acid bacteria are known as the most versatile microorganisms used as probiotic or functional foods. In the recent years, lactobacillus species were exploited for the selection of strains with antimicrobial molecules to be used in bio-preservation of food products. In this study, we isolated and identify *Lactobacillus plantarum* UTNCys3.4 strain from *Solanum stramonifolium* fruits of Ecuadorian Amazon rainforest and evaluated for the presence of bacteriocin-like compounds. However, using agar-well diffusion bioassay, the crude supernatant fluid (CFS) derived Cys3.4 strain showing elevated antimicrobial activity against *E. coli* ATCC 25922, *Salmonella enterica subsp. enterica* ATCC 51741, *E. coli* O157:H7 UTNEc1, *Enterococcus aeruginosa* UTNEn1, *Salmonella* Typhi UTNSm2 and *Shigella* UTNShg1 was further characterized for the presence of bacteriocin-like molecules. The antimicrobial activity of CFS was stable after heating at the ranking 60 to 121°C for 30 min, was activated by exposure at acidic pH of 2.0 to 6.0 and decline at 10.0, suggesting that the inhibitory activity is acid dependent. Its active principle was proteinaceous in nature since the bacteriocin was inactivated by proteolytic enzymes, while resistance to lysozyme indicated their non-lipid and carbohydrate moiety form. These released compounds were detected after 3 hours of bacterial growth indicating that the peptides were primary metabolites which might act as a barrier against pathogens at the early stage of growth.

Keywords: lactic acid bacteria, antimicrobial peptides, bacteriocin-like inhibitory substances, foodborne pathogens, biopreservation

1. Introduction

The presence of spoilage microorganisms in foods is a serious problem worldwide, thus, identifying new alternatives for preservation has converted in an attractive approach to be investigated. Lactic acid bacteria (LAB) are known as inhibiting invading Gram-negative bacteria due to the presence of several active substances such as short-chain fatty acids or hydrogen peroxide,

proteins such bacteriocins or bacteriocin-like inhibitory substances (Yang et al., 2012). As the inhibitory capacity is specie-dependent, nowadays, many investigations are focused on identification of novel strains with a larger spectrum to inhibit pathogenic bacteria of food (da Silva et al., 2014). In Ecuador, due to defective storage condition, poor manufacturing practices, most artisanal typical dishes (i.e. mote, chicha, chocho) contains a significant number of pathogenic and spoilage microorganism, therefore the risk of developing diseases is elevated; thus, the authorities are implementing new strategies to reduce the contamination by pathogenic microorganism. Accordingly, the research was centered on identification of natural ingredients to be used in preservation. Previously, in our laboratory, several LAB have been characterized (Benavidez et al., 2016). Among them we selected *Lactobacillus plantarum* Cys3.4 of Amazon microbiota to be analyzed for the presence of bacteriocin-like inhibitory substances (BLIS).

2. Results and Discussion

Tropical fruits of Amazon might be a greater source of benefic microorganisms providing a newly source of functional compounds to be for further use in industry.

Spectrum of antimicrobial activity

Antimicrobial activity against pathogens is one of the important properties of a probiotic LAB. The LAB isolate was identified based on API50CHL carbohydrate profile and 16S rRNA sequencing as *L. plantarum* Cys3.4 with 99% identity, and was was deposited at GenBank with accession number KY110685. The results from agar-well assay measurements showed that CFS of Cys3.4 has a broad spectrum of inhibitory activity, with the greatest activity registered towards *E. coli* ATCC25922 (20.66 ± 0.94mm) and *Shigella sonnei* ATCC25931 (19.33 ± 0.47mm) followed by, *E. coli* UTNEc1 (15.33 ± 0.47mm), *Salmonella* UTNSm2 (14.67 ± 0.94mm), *Shigella* UTNSgh1 (13.67 ± 0.94mm) and *Enterobacter* UTNEn1 (12.67 ± 0.94mm). In order to investigate on the nature of inhibitory molecules, CFS was submitted to different treatments. However, the level of activity varied from 6400 AU/ml to 3200 AU/ml upon removal of organic acids (TFS) and respectively, hydrogen peroxidase (NCFS). No activity was detected upon the treatment with proteolytic enzymes, indicating that the active compounds are proteinaceous in the nature. Moreover, the, the activity remain stable (6400 AU/ml) after treatment with non-proteolytic enzymes suggesting the non-lipid and carbohydrate moiety content of released molecules. The results showed that BLIS of Cys3.4 remained stable after heat at all temperatures tested (6400 AU/ml) with a slightly decrease upon autoclaving (3200 AU/ml). Maximum level of activity was recorded in acidic condition (pH, 2.0) of 12800 AU/ml, while a slightly decrease was registered with the increasing of pH, from 6400 AU/ml at pH 4.0 to 800 AU/ml at the pH 10.0. These results suggested that the activity is pH-dependent and their accumulation produced during fermentation process can be adverse for the microorganisms sharing the same microenvironment. Activity of Cys3.4 bacteriocin was initially detected in the early exponential phase of growth in MRS broth (3 hours) reaching the maximum concentrations at the stationary phase (**Figure 1**). An increasing production was registered at 24 and 30 hours of incubation suggesting that the bacteria Cys3.4 must be grown for at least 24 hours to get optimum activity towards pathogen. Similar results of *L. brevis* FPTLB3 showed that the maximum production was at the end of logarithmic phase of growth (Banerjee et al. 2013). In this study, if initial pH of bacterial culture was 6.0, at 24 hours of incubation, pH declined at about 4.0, which correlates with the optimum production of bacteriocin.

Effect of Cys3.4 BLIS on indicator cells

The viable cell counts of indicator cells was reduced about 2.5 fold from 6.2 log CFU/ml to 2.32 log CFU/ml after 3 hours of incubation with CFS of Cys3.4 suggesting the bacteriocidal manner to suppress the pathogen growth. (**Figure 2**). Addition of CFS in the late phase does not result in the same level of inhibition suggesting that the Cys3.4 bacteriocin activate in the early stage of target growth. Bacteriocin Cys3.4 was adsorbed 94% to both indicator cells. Adsorption was influenced by the incubation temperature as well as EDTA and SDS nor pH, Triton-X and NaCl (**Table 1**).

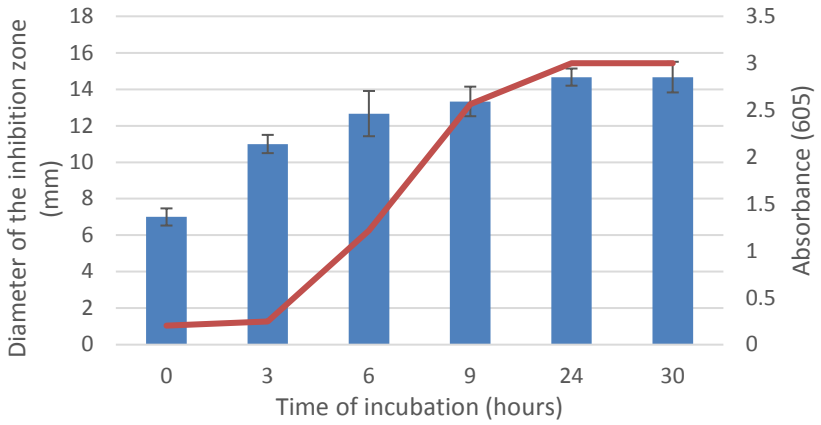

Figure 1. Production of bacteriocin Cys3.4 throughout time and inhibitory activity (mm) against *E. coli* ATCC25922.

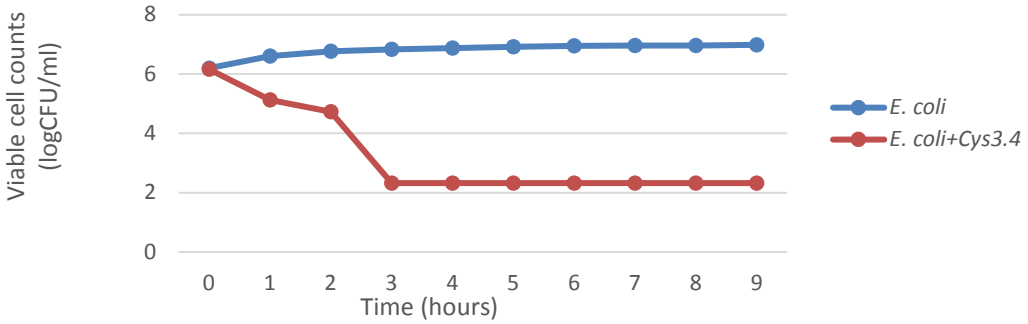

Figure 2. Effect of Cys3.4 on the viability of *E. coli*

Table 1. Effect of pH, temperature and chemicals on adsorption cellular

Adsorption cellular of Cys3.4 (%)		
Treatment	*E. coli* Ec1	*Salmonella* Sm2
pH		
2.0	90.38	87.87
4.0	94.47	95.3
6.0	96	95.9
Temperature (°C)		
4	74	69
15	98	87
30	95	100
37	91	91
45	94	95
Chemicals (1%), pH 6.5		
NaCl	92	88
Triton X-100	99	71
EDTA	25	41
SDS	55	52
CFS	94	94

3. Materials and Methods

Bacterial strain and culture media

LAB were isolated from native fruits of *Solanum stramonifolium* plant *(*Sucumbios Provence). The identification was performed using morphological, biochemical and genetic analysis. API50CHL strips (Biomerieux, Marcy l'Etoile France, cat # 50300) were used to identify the specie following manufacturer's instructions. Moreover, the strain specie was validated by 16S rRNA sequencing (Macrogen. Inc, Korea).

Antimicrobial activity bioassay

Antimicrobial activity was performed using the agar-well diffusion method (Benavidez et al., 2016). Indicator strains: *E. coli* ATCC25922, *Shigella sonnei* ATCC25931, *E. coli* O157:H7 UTNEc1, *Enterococcus aeruginosa* UTNEn1 (isolated from local fresh cheese), *Salmonella* Typhimurium UTNSm2, *Shigella* UTNShg1 (isolated from cooked chicken). The experiments were run in triplicate the mean values of the inhibition zones were estimated. As reference, *L. plantarum* ATCC8014 has been used.

Characterization of BLIS-producing Cys3.4

To rule out the possible inhibition activity of organic acids, the CFS was heated at 80°C for 10 minutes, the pH adjusted at 6.0 (TFS). In other batch, TFS was treated with 1 mg/ml catalase to eliminate the possible activity as results of hydrogen peroxide presence (NCFS). NCFS was treated with proteolytic enzymes at the final concentration of 1mg/ml: proteinase K, pepsin, catalase, lysozyme and lipase (Sigma-Aldrich Corporation, USA) incubated for 2 hours at 37°C and 5 min at 100°C to inactive the enzyme. In other experiment, aliquots of CFS were incubated for 30 minutes at 60, 80, 90, 100 and 121°C and different pH (2.0, 4.0, 6.0 and 10.0) followed by determination of bacteriocin titer expressed as arbitrary units per ml (AU / ml). One arbitrary unit was defined as the highest dilution showing about 2 mm of inhibition zone on the indicator lawn. Residual activity of each-treated supernatant was determined against *E. coli* ATCC25922.

Kinetics of bacteriocin production *in vitro*

One hundred milliliter of MRS broth was inoculated with 18 hours culture (2 %, v/v) of Cys3.4 and incubated at 37°C without agitation. Antimicrobial activity (mm) and optical density (605 nm) of the culture were measured at intervals of 3 hours for 30 hours.

Cell lysis and adsorption of Cys3.4 to target cell

Twenty ml of CFS (corresponding to 6400 AU/ml) was added to 100 ml culture of 3 h old culture (OD605 = 0.2) of indicator strains. Incubation was performed at 37°C for 9 hours and OD605 was measured every hour using spectrophotometer UV-VIS (Nova60, Millipore, Merck) followed by plate-agar method to determine the number of viable cells. As control untreated indicator strains cultures has been used. Adsorption of bacteriocin to indicator cells was performed following a method describe by Yang et al. 2012. The effect of pH, temperature and chemicals on adsorption were evaluated.

4. Conclusions

This study demonstrated the capacity of *L. plantarum* Cys3.4 strain to suppress several pathogenic bacteria, which are the main cause of food illness in population. The inhibitory activity *in vitro* was highly related with the presence of bacteriocin-like molecules and depends at least in part, by lowering pH and/ or the presence of organic acids. We shall further investigate the condition for the improvement of bacteriocin secretion along with the potential preservative *in vivo*.

Acknowledgments

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Author Contributions

KG, AB and CO performed the experiments. GNT design the experiments, interpret the data and wrote the manuscript.

Conflicts of Interest

"The authors declare no conflict of interest".

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SUIWML01: International Workshop on Machine Learning in Biomedicine, Soochow, China, 2016

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SUIWML01 Editorial

Dear all we are glad to welcome you to the SUIWML2016: Soochow University International Workshop Series on Machine Learning and its Applications in Biomedicine. This is one scientific conference series of the School of Computer Science and Technology of Soochow University, PCR, China. This is workshop also is a section of MOL2NET-2.

This workshop is focus on Machine Learning. Machine learning is the most growing branch of computer science, driven by the ongoing explosion in the availability of data. Machine learning evolved from artificial intelligence and deals with many different problems and aspects to solve various tasks, including knowledge discovery, data mining, decision support and etc. A grand challenge is to discover relevant structural patterns and/or temporal patterns in complex data, which are often hidden and not accessible to the human expert.

Biomedicine is a branch of medical science that applies biological and other natural-science principles to clinical practice. The branch especially applies to biology and physiology, which has been the dominant health system for more than a century. Nowadays, the dramatic growth of medical and biological data has created an unprecedented opportunity for machine learning in the pattern recognition and machine learning community. Many medical and biological problems involve challenging approaches to pattern discovery and learning.

This workshop aims at highlighting the on-going research both the advancement of machine learning technologies and the improvements of biomedicine, and trying to bringing together researchers from the related fields to foster discussion and elicit open problems on machine learning and its applications in biomedicine. The workshop will consist of invited talks, contributed presentations, and posters. We plan to include an opening tutorial and an overview of the state-of-the-art techniques. Invited talks will be given by leading experts from both machine learning and biomedicine. We hope this workshop will not only provide an opportunity for international researchers to exchange ideas and present the latest promising work, but also create a platform to discuss and identify important future topics and directions in related fields for further research and collaboration.

This workshop is planned to be held in middle of Nov, 2016. However, the submission **is open and the publication of communications will be ASAP after acceptance, all the year**. For more details, see Schedule & Program page and 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. After abstract approval you need to send your communication. Please, download and use the following template to write your communication SUIWML template

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Topics of particular interest include (but not limited to)

Machine Learning;

Supervised learning;

Unsupervised learning;

Reinforcement learning;

Transfer learning;

Manifold Learning;

Multi-task learning; and Active Learning.

Applications in Biomedicine;

Biomedical decision support systems;

Medical informatics;

Bioimage mining;

Medical (business/financial) intelligence;

Electronic medical record (EMR) data mining;

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Bioinformatics;

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An Efficient Residual Q-learning Algorithm based on Function Approximation

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Abstract:

A number of reinforcement learning algorithms have been developed that are guaranteed to converge to the optimal solution when used with lookup tables. However, these algorithms can easily become unstable when implemented directly with function approximation. We proposed an efficient Q-learning algorithm based on function approximation (FARQ), which not only can guarantee the convergence but also has a fast learning rate. The algorithm performs gradient decent on mean squared Bellman residual and adopts a new rule to update value function parameter vector. In addition, the algorithm introduces a new factor, named forgotten factor to accelerate the learning rate of the algorithm. Applying the proposed algorithm, Q-learning, and Actor-Critic algorithm to the traditional Grid World and the pole balancing problems, the experimental results show that FARQ algorithm has the faster convergence rate and better convergence performance.

Keywords:

Reinforcement Learning, Q-learning algorithm, Function Approximation, Gradient Descent, Bellman Residual

1 INTRODUCTION

Reinforcement Learning (RL) [1] is considered as a kind of Machine Learning method, which can be applied to handle problems where the process model is not available in advance. The agent explores an environment and through the use of a reward signal to maximize the expected cumulative rewards to achieve a certain goal [2]. The traditional RL algorithms have been guaranteed to converge to the optimal solution, which use the lookup tables to store the value functions for problems with discrete state and action spaces [3]. The lookup table is generally applied to the problems with small-scale continuous state space and action spaces, but when dealing with continuous actions, or action space with large discrete sets, the algorithms will suffer from slow learning rate or even cannot converge. This problem is named the curse of dimensionality [4], which leads to the increasing computational complexity exponentially with the number of dimensions. As a result, designing a more efficient algorithm to solve the problem of slow learning rate and unstable converge will be of great importance in RL.

RL algorithm with function approximation is a new research hotpot in Machine Learning. In the learning process of algorithms with function approximation, a set of parameters were adopted to describe the state-value function or action-value function [5-6]. The agent chooses the optimal action according to the approximation, and the learning experiences can be generalized from the subsets to the whole state space. In the 1990s, Tsitsiklis et al, applied function approximation to RL algorithms, and the stability and convergence of the algorithms were proved both theoretically and experimentally [6].

However, the Q-learning based on function approximation cannot converge, because the target policy and the behavior policy are inconsistent to make the different distribution of sample data [7]. Gordon attempted to improve the Q-learning algorithm, and the proposed algorithm can converge steadily, but the performance is not ideal [8]. Hasselt adopted two function approximations to eliminate the overestimations of action values, and proposed Double Q-learning [9]. Nowadays, Q-learning has been used to find solutions on many problems [10-12] and was an inspiration to similar algorithms, such as dual iterative Q-learning [13], Policy Gradient and Q-learning [14], and hybrid Q-learning [15]. These algorithms have mostly been proposed in order to speed up convergence rates compared to the original Q-learning algorithm.
In this paper, we proposed a Residual Q-learning algorithm with Function Approximation (FARQ) in order to speed up convergence rate and improve the stability of the original Q-learning algorithm. The algorithm adopts gradient-descent method to adjust the parameter vector and use function approximation method to update function parameter vector. It is worth noting that, the Q-learning algorithm with function approximation does not necessarily guarantee convergence. To avoid this problem, we consider Bellman residual as a good choice to guarantee convergence of algorithm, and the new algorithm introduces a new rule to update value function parameter vectors. Applying the proposed algorithm, Q-learning, and Actor-Critic algorithm to the traditional Grid World and the pole balancing problems, the experimental results show that FARQ algorithm has better convergence comparing with the original Q-learning algorithm and has faster learning rate comparing with the traditional algorithms based on lookup tables. In addition, FARQ has better robustness to the growth of the state space. This paper is organized as follows: Section 2 describes MDPs and the original Q-learning algorithm. The FARQ algorithm is presented in Section 3. Experimental results are analyzed and discussed in Section 4. In Section 5, we make a final conclusion and the future topics.

2 RELATED LITERATURE

2.1 Markov decision process

In RL, Markov decision process (MDP) can be used to model the problem. The learning task satisfying the Markov property can be modeled by a 4-tuples $M = (X, U, R, T)$, where

- X is a set of system states;
- U is a set of system actions;
- R is the reward function, where $R(x,u,x')$ is the reward obtained from the environment when ending up at state x' after executing action u at state x;
- T is the state transition function, where $T(x,u,x')$ is the probability of ending up at state x' after executing action u at state x.

The ultimate goal of RL is to learn a policy, which is a mapping from state-action pair to the probability of taking the action at the state. According to the output of the policy is an action or an action choice probability, the policy usually can be divided into deterministic policy and random policy, where the deterministic policy is represented as $\bar{h}: X \to U$, a map from a state to an action; and the random policy is represented as $h: X \times U \to [0,1]$, a map from a state-action pair to a probability. For example, $u = \bar{h}(x)$ is an action that chosen at state x and $P(u \mid x) = h(x,u)$ is a probability of choosing action u at state x. For convenience, we use h to represent a policy. We assume that time step is k, state is x_k, and policy is h. Agent chooses action u_k according to the current state and policy, and the state moves from x_k to x_{k+1}. In the learning process, algorithm repeats the above process, and agent can obtain the best policy through interacting with the environment to maximize the cumulative rewards.

In order to evaluate how good the policy is, the value function is proposed in RL. The value function is divided into the state value function $V^k(x)$ and the action value function $Q^k(x,u)$, where $V^k(x)$ represents the expected return at state x under a policy h and $Q^k(x,u)$ represents the expected return at the state action pair (x,u) under a policy h. $V^k(x)$ and $Q^k(x,u)$ are the unique solutions to their Bellman equation, which are denoted by:

$$V^*(x) = \max_{u \in U}\{R(x,u) + \gamma \sum_{x' \in X} T(x,u,x')V^*(x')\} \quad (1)$$

$$Q^h(x,u) = R(x,u) + \gamma \sum_{x' \in X} T(x,u,x') \sum_{u' \in U} h(x',u')Q^h(x',u') \quad (2)$$

Where $0 \le \gamma \le 1$ is the discount factor; the best policy h can maximize the cumulative rewards, and the correspond deformation of the optimal value function $V^*(x)$ and $Q^*(x,u)$ are shown in equation (3) and equation (4).

$$V^*(x) = \max_{u \in U}\{R(x,u) + \gamma \sum_{x' \in X} T(x,u,x')V^*(x')\} \quad (3)$$

$$Q^*(x,u) = R(x,u) + \gamma \sum_{x' \in X} T(x,u,x')\{\max_{u' \in U} Q^*(x',u')\} \quad (4)$$

It is worth noting that equation (3) and equation (4) are also called Bellman optimality equation.

2.2 Q-learning algorithm

Temporal difference learning (TD-Learning) is the central and novel ideas in RL, which was introduced by Sutton in 1988 [4]. Q-learning is a special form of TD learning, and it belongs to the off-policy methods, because the learned action-

value function of Q-learning can directly approximates the optimal action-value function, independent of the policy being followed.

The Q-learning algorithm is given as follows [1].

Algorithm 1 Q-learning algorithm

1: Initialize: $Q(x,u)$ arbitrarily
2: Repeat (for each episode):
3: Initialize x
4: Repeat (for each step of episode):
5: Choose u from x using policy derived from Q (e.g., $\varepsilon-greedy$)
6: Take action u ,observe R , $x^{'}$
7: $Q(x,u)=Q(x,u)+\alpha\left[R+\gamma\max_{u^{'}}Q(x^{'},u^{'})-Q(x,u)\right]$
8: $x\leftarrow x^{'}$
9: Until x is terminal

3 Q-LEARNING WITH FUNCTION APPROXIMATION

3.1 Approximation of Value Functions in FARQ Algorithm

The traditional algorithms in RL often use lookup tables to store the value function. The agent chooses the action according to the return of the state action pair. For an MDP with a continuous state space or continuous action space, the updating of action-value function is shown as equation (5).

$$Q(x,u)\overset{\alpha}{\leftarrow}R+\gamma\max_{(x^{'},u^{'})}Q(x^{'},u^{'}) \tag{5}$$

Where $x^{'}$ is the next state of x , R is the immediate reward. The value of $Q(x,u)$ is modified to be closer to the value of $R+\gamma Q(x^{'},u^{'})$ with a learning rate α . At the end of the learning process, the equation (5) can guarantee to converge to the optimal action-value function under the condition that the learning rate α gradually decreases to zero. The lookup table is a very simple and effective method for problems with small state spaces, but it is not appropriate for the application with large state spaces. As a result, the lookup table was approximated by the function approximation, that is, the action-value function $Q(x,u)$ can be represented as a parameterized functional form with parameter vector θ . The target output $R+\gamma Q(x^{'},u^{'})$ can be any approximation of $Q(x,u)$, and all the parameter vectors would be adjusted through gradient descent, so the actual output $Q(x,u)$ could be closer to the target output $R+\gamma Q(x^{'},u^{'})$. The gradient descent update for action-value prediction of Q-learning is shown as equation (6).

$$\Delta w=\alpha\left(R+\gamma\max_{(x^{'},u^{'})}Q(x^{'},u^{'})-Q(x,u)\right)\frac{\partial Q(x,u)}{\partial w} \tag{6}$$

A common approach is called batch updating [16], where every value function is updated only once by the sum of all increments. All the available experience is processed with the new value function to produce a new overall increment, and so on, until the value function converges. So the batch version of the equation (6) is shown as equation (7).

$$\Delta w_{d}=-\alpha\sum_{x}\left[R+\gamma\max_{(x^{'},u^{'})}Q(x^{'},u^{'})-Q(x,u)\right]\left[-\nabla_{w}Q\left(x,u\right)\right] \tag{7}$$

Where $R+\gamma\max_{(x^{'},u^{'})}Q(x^{'},u^{'})-Q(x,u)$ may be positive or negative . If it's positive, it means that the value of $R+\gamma\max_{(x^{'},u^{'})}Q(x^{'},u^{'})$ is greater than the value of $Q(x,u)$, the actual value approximates to the desired value in the direction of growth, and the algorithm learns along the positive gradient direction, so the algorithm eventually diverges. If it's negative, it means that the value of $R+\gamma\max_{(x^{'},u^{'})}Q(x^{'},u^{'})$ is less than the value of $Q(x,u)$, the actual value approximates to the desired value in the direction of decrease, and the algorithm learns along the negative gradient direction, so the algorithm eventually converges. From the above description, we can know that Q-learning with function approximation cannot guarantee to converge definitely.

In order to solve the above problem, we consider Bellman residual as a good choice to guarantee the convergence of the algorithm, and the new algorithm constructs a new rule to update parameter vector of the value function. The value of the action-value function is $Q(x,u)$ and the target output function for sample training is $R+\gamma\max_{(x^{'},u^{'})}Q(x^{'},u^{'})$, the Bellman residual is defined as the mean square error of the two values, which is shown as equation (8).

$$E = \frac{1}{n}\sum_{x}\left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u)\right]^2 \quad (8)$$

For the problem with continuous action space, only when the action-value function is optimal, Bellman residual is zero. By applying the gradient descent method to equation (8) can guarantee E to converge to a local minimum. After the state x moves to x' with an immediate reward R, the parameter update vector of Q-learning-Bellman is given by Equation (9).

$$\Delta w = -\alpha\left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u)\right]\left[\frac{\partial}{\partial w}\gamma \max_{(x',u')} Q(x',u') - \frac{\partial}{\partial w}Q(x,u)\right] \quad (9)$$

The difference between Equation (9) and equation (6) is that equation (9) can guarantee the convergence of Bellman residual, but equation (6) cannot. As a result, applying function approximation to Q-learning algorithm, equation (9) can guarantee the algorithm to converge to the optimal solution. The batch version of the equation (9) is shown as equation (10).

$$\Delta w_{rg} = -\alpha\left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u)\right]\left[\nabla_w \gamma \max_{(x',u')} Q(x',u') - \nabla_w Q(x,u)\right] \quad (10)$$

Where w, Δw_{rg}, the gradient of $Q(x,u)$ and $Q(x',u')$ are all vectors. When the step-size parameter α satisfies the stochastic approximation theory [1], Δw_{rg} can guarantee E to converge to a local minimum. However, comparing with Q-learning algorithm, Q-learning-Bellman sometimes converges slowly. Take the star problem in Figure 1 as an example. Initialize $w_5 = 0$ and $w_4 = 10$. In the learning process from state 4 to state 5, Q-learning only decreases the value of w_4, but Q-learning-Bellman increases the value of w_5 while decreasing the value of w_4. Therefore, Q-learning-Bellman algorithm will lead the agent to learning in two directions, and the learning rate will become slower.

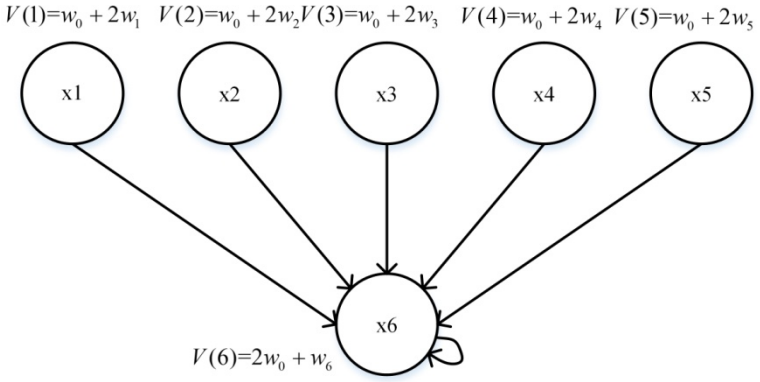

Figure 1: The star problem

Q-learning with function approximation has a faster learning rate but cannot guarantee the convergence and Q-learning with Bellman residual can guarantee the convergence but has a slower learning rate. Considering the two cases, we want to find an algorithm which not only can guarantee the convergence, but also has a faster learning rate. In figure 3, the dotted line represents the hyperplane that is perpendicular to the gradient, the above of dotted line represents the negative gradient direction which causes a decrease in E and the below of dotted line represents the positive gradient direction which causes an increase in E. The vector Δw_{rg} is for Q-learning-Bellman, and the vector Δw_d is for Q-learning. The update vector we need should be as close as possible to Δw_d and still remain to the up of the dotted line, which can guarantee the convergence

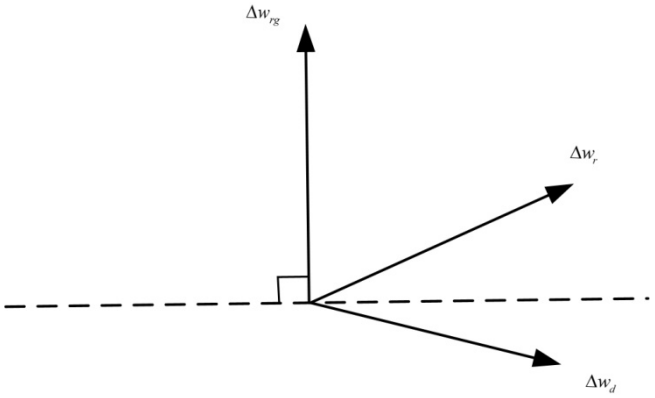

Figure 2: Action-value function parameter update vector for FARQ algorithm

of the algorithm and make the algorithm learn quickly, such as Δw_r in figure 3. Therefore, the above two value function parameter vectors are combined with parameter Φ (a constant between 0 and 1), and the new parameter update vector Δw_r is defined to be:

$$\Delta w_r = (1-\Phi)\Delta w_d + \Phi \Delta w_{rg} \tag{11}$$

From the equation (11), we can know that the new vector can ensure the convergence of the algorithm with an appropriate Φ, because Δw_r causes E to decrease. In addition, the algorithm might have a fast learning rate, because Δw_r lies as close as possible to Δw_d.

3.2 Selection of Φ in FARQ Algorithm

An important question is how to choose an appropriate Φ in FARQ algorithm. A Φ of 1 is guaranteed to converge, and a Φ of 0 might be expected to learn quickly. However, both of them are not the best choice, and it requires to analysis by trial and error.

When the two vectors Δw_r and Δw_{rg} are orthogonal, then the calculation of Φ would be:

$$\begin{aligned} &\Delta w_r \cdot \Delta w_{rg} = 0 \\ &((1-\Phi)\Delta w_d + \Phi \Delta w_{rg}) \cdot \Delta w_{rg} = 0 \\ &\Phi = \frac{\Delta w_d \cdot \Delta w_{rg}}{\Delta w_d \cdot \Delta w_{rg} - \Delta w_{rg} \cdot \Delta w_{rg}} \end{aligned} \tag{12}$$

If the equation (12) yields a Φ outside of the range $[0,1]$, then the angel between the vectors Δw_r and Δw_{rg} is acute. From figure 3 we can see that the vector Δw_r changes to the above of the hyperplane, which means that the algorithm has converged, so a Φ of 0 should be used for maximum learning rate in the next learning. When the denominator of Φ is 0, then the value of Φ is 0, this means that E is at a local minimum, or the vectors Δw_r and Δw_{rg} point in the same direction. In either case, the algorithm can guarantee to converge. If the equation (12) yields a Φ between the range $[0,1]$, then the value of Δw_{rg} is 0, which means that E has converged to a local minimum. Theoretically, E will decrease to zero as the number of iterations increasing, and the algorithm converges eventually. Therefore, any Φ above this value will ensure convergence. In summary, when Φ satisfies the range $[0,1]$, the FARS algorithm can guarantee to converge.

In order to make the angle between the two vectors Δw_r and Δw_{rg} is acute, adding any small, positive constant k to Φ. Therefore, FARS algorithm should firstly calculate the numerator and the denominator, secondly check whether the denominator is zero. If the denominator is zero, then Φ is 0. If it is not, the algorithm should evaluate equation (12), including the addition of a small constant k, then check whether the resulting Φ lies in the range $[0,1]$. The corresponding deformation of equation (11) is shown in equation (13).

$$\begin{aligned} \Delta w_r &= (1-\Phi)\Delta w_d + \Phi \Delta w_{rg} \\ &= -(1-\Phi)\alpha \sum_x \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[-\nabla_w Q(x,u) \right] \\ &-\Phi \alpha \sum_x \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[\nabla_w \gamma \max_{(x',u')} Q(x',u') - \nabla_w Q(x,u) \right] \\ &= -\alpha \sum_x \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[\Phi \nabla_w \gamma \max_{(x',u')} Q(x',u') - \nabla_w Q(x,u) \right] \end{aligned} \tag{13}$$

At the end of an episode, the vectors w_d and w_{rg} are updated vectors of the value function parameter in Q-learning and Q-learning-Bellman respectively. In order to satisfy the requirements of faster convergence, we introduce a new factor μ (a small, positive constant) in the updating of w_d and w_{rg}, which is named forgotten factor. We use w_d and w_{rg} to approximate Δw_d and Δw_{rg}, the vectors w_d and w_{rg} are updated according to equation (14) and equation (15).

$$w_d = (1-\mu) w_d - \mu \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[-\nabla_w Q(x,u) \right] \tag{14}$$

$$w_{rg} = (1-\mu) w_{rg} - \mu \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[\nabla_w \gamma \max_{(x',u')} Q(x',u') - \nabla_w Q(x,u) \right] \tag{15}$$

In addition, the marginally-stable Φ is calculated by equation (16).

$$\Phi = \frac{\sum_{w} w_d \cdot w_{rg}}{\sum_{w} \left(w_d - w_{rg}\right) \cdot w_{rg}} + \mu \tag{16}$$

3.3 FARQ ALGORITHMS

Residual Q-learning Algorithm with function approximation combines the traditional Q-learning algorithm and Q-learning-Bellman algorithm, which constructs a new method to update parameter vector of action-value function. By choosing an appropriate Φ and introducing a forgetting factor, the FARS algorithm has better performance and faster learning rate.

The complete algorithm is given as follows.

Algorithm 2 FARQ algorithm

Initialize: $w = 0$; $x \in X$; α ; Φ=0
Repeat
  For each $x \in X$
    $Q = Q(x,u)$
    $Q(x,u) = R + \gamma \max_{(x',u')} Q(x',u')$
    $w_d = (1-\mu) w_d - \mu \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[-\nabla_w Q(x,u) \right]$
    $w_{rg} = (1-\mu) w_{rg} - \mu \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[\nabla_w \gamma \max_{(x',u')} Q(x',u') - \nabla_w Q(x,u) \right]$
    If $w_d \cdot w_{rg} - w_{rg} \cdot w_{rg} = 0$
      $\Phi = 0$
    Else
      $\Phi = \frac{\sum_{w} w_d \cdot w_{rg}}{\sum_{w} (w_d - w_{rg}) \cdot w_{rg}} + \mu$
      If $\Phi > 1$
        $\Phi = 0$
      Else
        $\Phi = \Phi$
      End If
    End If
    $w_r = w_r + \sum_{x} \left[R + \gamma \max_{(x',u')} Q(x',u') - Q(x,u) \right] \left[\Phi \frac{\partial}{\partial w} \gamma \max_{(x',u')} Q(x',u') - \frac{\partial}{\partial w} Q(x,u) \right]$
    $w = w + \alpha \cdot w_r$
    $x = x'$
  End For
Until $E = 0$

4 EXPERIMENTAL RESULTS

In order to verify the effectiveness and performance of the algorithm, the proposed algorithm, Q-learning algorithm, Q-learning-Bellman algorithm are applied to the classic Windy Grid World and Pole-balancing problems.

4.1 Windy Grid World

We discuss the results of running FARQ, Q-learning, and Q-learning-Bellman algorithms on a $5*6$ Windy Grid World problem, as shown in figure 4. The agent always begins in the square marked ‘S’ and the episode continues until it reaches the square marked ‘T’. In any state, Agent can choose 4 actions $\{a_0, a_1, a_2, a_3\}$ which represents up, down, left and right as shown in the direction of the arrow in figure 4. When the column has no wind, the agent chooses an action according to a state transition, that is, if the agent chooses the right action, the state will move to the right state, and if the agent chooses the left action, the state will move to the left state. For example, the agent is in the state $(3,0)$, if the agent chooses the action to the right, then the state moves to $(3,1)$. When the column has wind, the resultant next states are shifted upward by the

wind. For example, the agent is in state $(2,2)$, the strength of the wind is 1(The dotted arrow in figure 4 indicates that the column has wind, and the value above the dotted arrow describes the strength of the wind, such as $0/1$, which means the strength may be 0 or 1.), the agent chooses the action to the right, then the state will move to $(1,3)$. In the process of state transition, agent can receives a reward of 10 until the goal state is reached, and in other cases, the agent receives a reward of 1. For this experiment, in order to ensure a certain degree of exploration, we adopt $\varepsilon-greedy$ method to choose actions. The maximum number of episodes is set to 250, and the maximum number of time steps of each episode is 2000. The episode ends means that the agent reaches the goal state or the time step reaches 2000. In addition, we choose regularization parameter $\gamma = 0.9$, $\alpha = 0.1$, $\varepsilon = 0.1$ and $\mu = 0.01$.

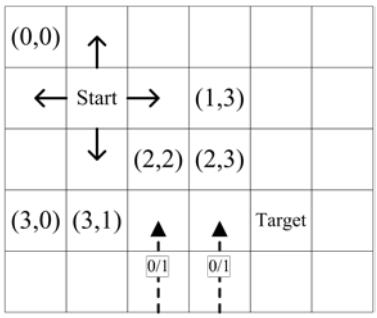

Figure 3: Windy Grid World problem

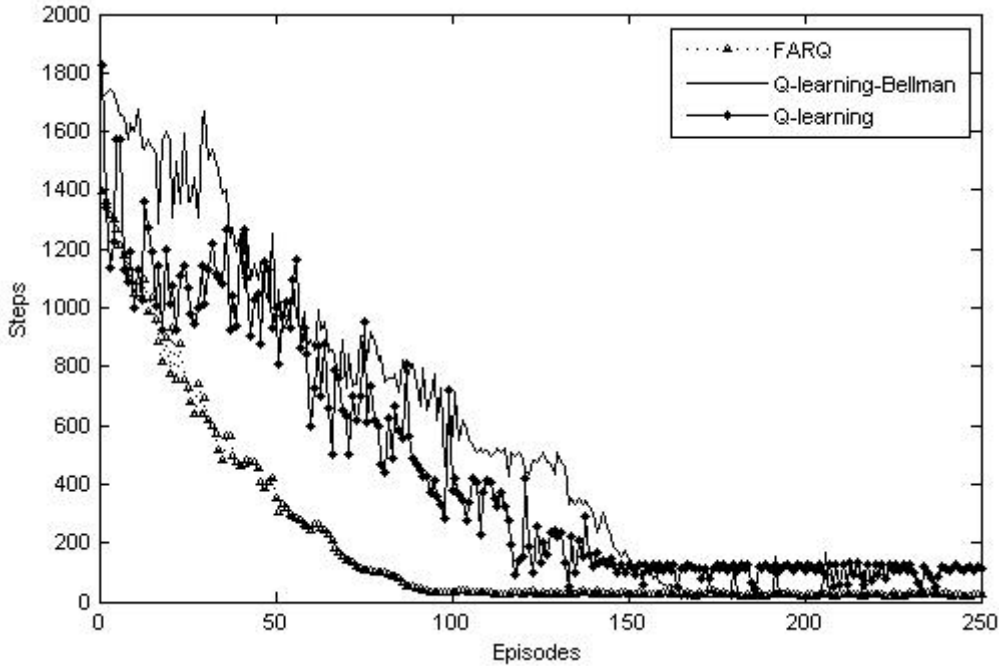

Figure 4: Performance analysis for different algorithms on Windy Grid World

Figure 4 shows the performance analysis of Q-learning, FARQ and Q-learning-Bellman algorithms on Windy Grid World problem. The horizontal axis is the episode, and the vertical axis is the steps for the different algorithms to the goal state. From figure 4, we can clearly see that the convergence performance of FARQ algorithm is better than the other algorithms. The three algorithms Q-learning, FARQ and Q-learning-Bellman converge at the 150th, 140th, and 30th episodes respectively. As a result, the FARQ algorithm has the fast convergence rate, possibly due to the introducing of forgotten factor. In the former episodes, the Q-learning algorithm learns faster than the Q-learning-Bellman algorithm, because the Q-learning-Bellman algorithm attempts to make each state match both successors and its predecessors, so the learning rate is slower. In the latter episodes, the Q-learning algorithm fluctuates heavily, and the Q-learning-Bellman algorithm fluctuates slightly, but the FARQ algorithm does not, that is because the FARQ algorithm combines the traditional Q-learning and bellman residual method, and introduces a new rule to update the function parameter vector. Evidently, FARQ algorithm has the best performance.

Figure 5 shows the performance analysis for different μ of FARQ algorithm on Windy Grid World problem. Figure 6 shows the performance of the latter 100 episodes with different μ. For each size of training episodes, the date point was calculated by averaging over 20 times. The horizontal axis is the episode, and the vertical axis is the steps for FARQ algorithm to reach the goal state. The values of μ for six lines in figure 6 are 0.001, 0.005, 0.01, 0.05, and 0.2, respectively. In figure 5, we can clearly see that when $\mu = 0.001$, $\mu = 0.005$, $\mu = 0.01$, $\mu = 0.05$, and $\mu = 0.2$ the algorithm converges at about the 125th episode, the 110th episode, the 90th episode, the 70th episode and the 30th, respectively. As a result, the

convergence rate of the algorithm is proportional to the value of μ. However, when $\mu = 0.2$, the convergence rate of FARQ is improved, but the number of the steps to reach the goal state is about 180. Moreover, In figure 6, we can clearly see that when $\mu = 0.001$, $\mu = 0.005$, and $\mu = 0.05$, the FARQ algorithm fluctuates more heavily than $\mu = 0.01$. From figure 6, we can know that when $\mu = 0.2$, the algorithm converges at a local optimum. Therefore, it is worth noting that the smallest μ is not the best μ. Evidently, an appropriate μ can make the algorithm have a good robustness and a faster learning rate.

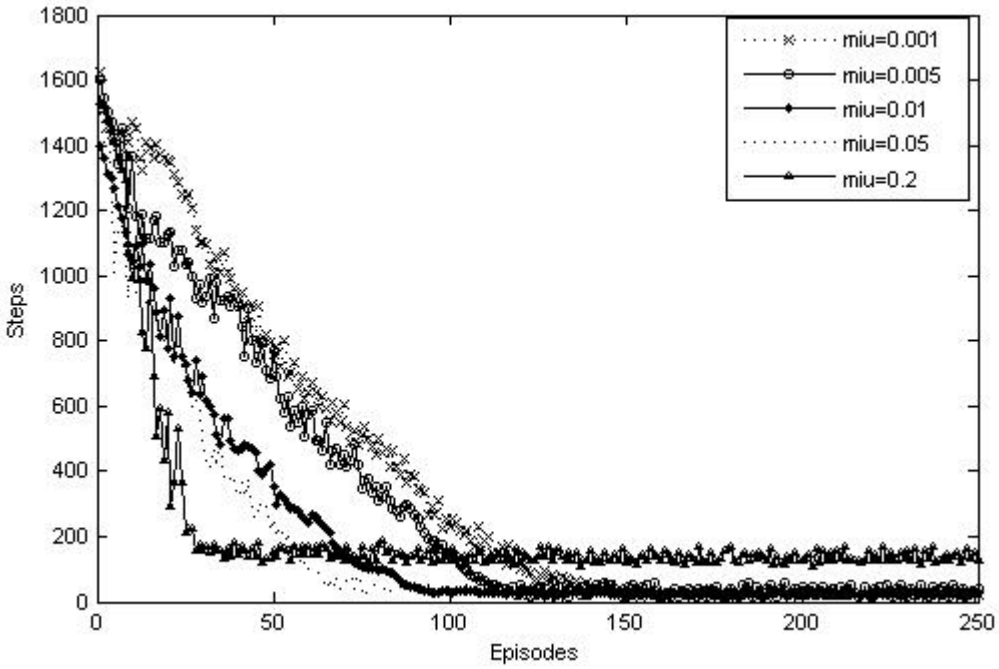

Figure 5: Performance analysis for different μ of FARQ algorithm on Windy Grid World

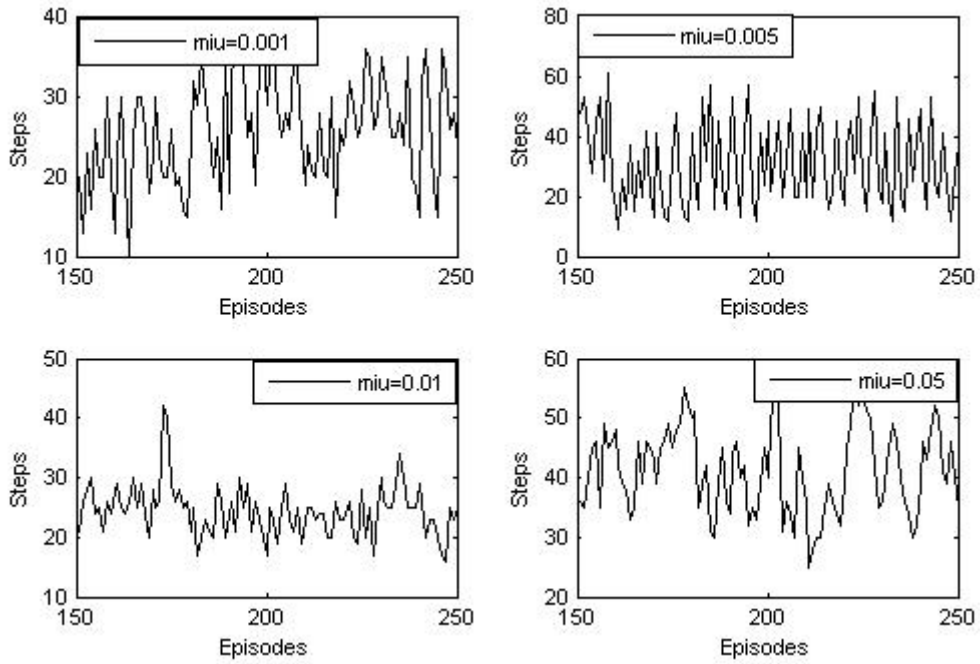

Figure 6: Performance analysis for different μ of FARQ algorithm on Windy Grid World

Figure 7 shows the performance analysis for different Φ of FARQ algorithm on Windy Grid World. For each size of training episodes, the date point was calculated by averaging over 20 times. Horizontal axis Phi represents the value of Φ, and the vertical axis is the steps required for FARQ algorithm to converge. In the simulation result, we can see that when Φ take different values, the algorithm has different performances. In figure 10, we can know that when $\Phi = 0.2$, the algorithm has the minimum number step to reach the goal state, and the performance of the algorithm is the best. Moreover, when $\Phi = 0$, the FARQ algorithm and Q-learning algorithm have the same performance, and when $\Phi = 1$, the FARQ algorithm and the Q-learning-Bellman algorithm also have the same performance. As a result, the Q-learning algorithm and Q-

learning-Bellman algorithm are special cases of FARQ algorithm, and the FARQ algorithm can be found that combine the beneficial properties of both.

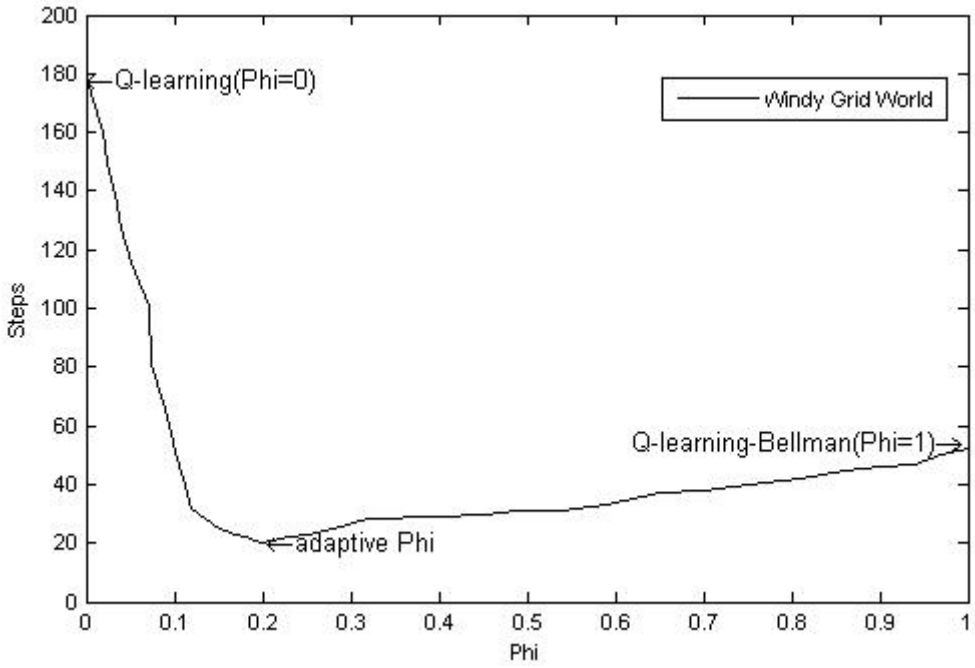

Figure7: Performance analysis for different Φ of FARQ algorithm on Windy Grid World

4.2 Pole balancing problem

The pole balancing problem (see Figure 7) requires balancing a pole of unknown length and mass at the upright position by applying force to the cart it is attached to. At each time step, if the angle m, between the pole and the vertical line is less than $\pi/4$ ($|\theta| \le \pi/4$), a reward of 1 will be given; otherwise a reward of -1will be received. The goal is to keep the pole balance ($|\theta| \le \pi/4$) for a total of 9000 steps during an episode. The maximal episode is 150 and an episode consists of 9000 time steps. For this experiment, we choose regularization parameter $\gamma = 0.95$, $\alpha = 0.1$, $\varepsilon = 0.1$ and $\mu = 0.01$. All the values of the parameters are hand-tuned to obtain the best performance.

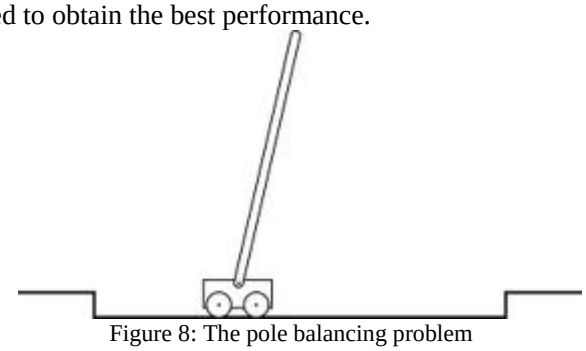

Figure 8: The pole balancing problem

The state consists of $(\theta, \dot{\theta})$, where $\theta \in [-\pi/2, \pi/2]$ is the angel between the pole and the vertical line and $\dot{\theta} \in [-2, 2]$ is the corresponding angular velocity of the pole. The action u is the force exerting on the cart and its range is $u \in [-50N, 50N]$. A uniform noise in $[-10N, 10N]$ is added to the selected action at each time step. At the start of each episode, the state of the pole is initialized as $(0, 0)$ with a uniform random perturbation. The dynamics of system is shown as equation (17).

$$\ddot{\theta} = \frac{g \sin\theta + \cos\theta \left(\frac{-F - ml\dot{\theta}^2 \sin\theta}{m+M} \right)}{l\left(\frac{4}{3} - \frac{m\cos^2\theta}{m+M} \right)} \tag{17}$$

Where the gravity constant $g = 9.81 m/s^2$, the masses of the pole and the car are $m = 0.1kg$ and $M = 1kg$ respectively, and the length of the pole $l = 1m$. Agent exerts the force F to the car, and the time interval is $\Delta t = 0.1$, then the state variables are $\dot{\theta} = \dot{\theta} + \ddot{\theta}\Delta t$ and $\theta = \theta + \dot{\theta}\Delta t$. The reward function is shown as equation (18).

$$\rho(x,u) = \begin{cases} 1 & |f(x,u)| < \pi/4 \\ -1 & |f(x,u)| \geq \pi/4 \end{cases} \tag{18}$$

We adopt radial basis function (RBF) to extract the features of the states. The center point of the angel θ and the angular velocity $\dot{\theta}$ locate over the grid points $\{-0.75,-0.5,-0.25,0,0.25,0.5,0.75\}$ and $\{-2,0,2\}$, which constitute a total of 21 center points. The variances are 0.2, 2 for θ and $\dot{\theta}$ respectively. In pole balancing problem, the performance of the algorithm is evaluated by the average steps in each episode, and the higher the average steps, the better the algorithm performance.

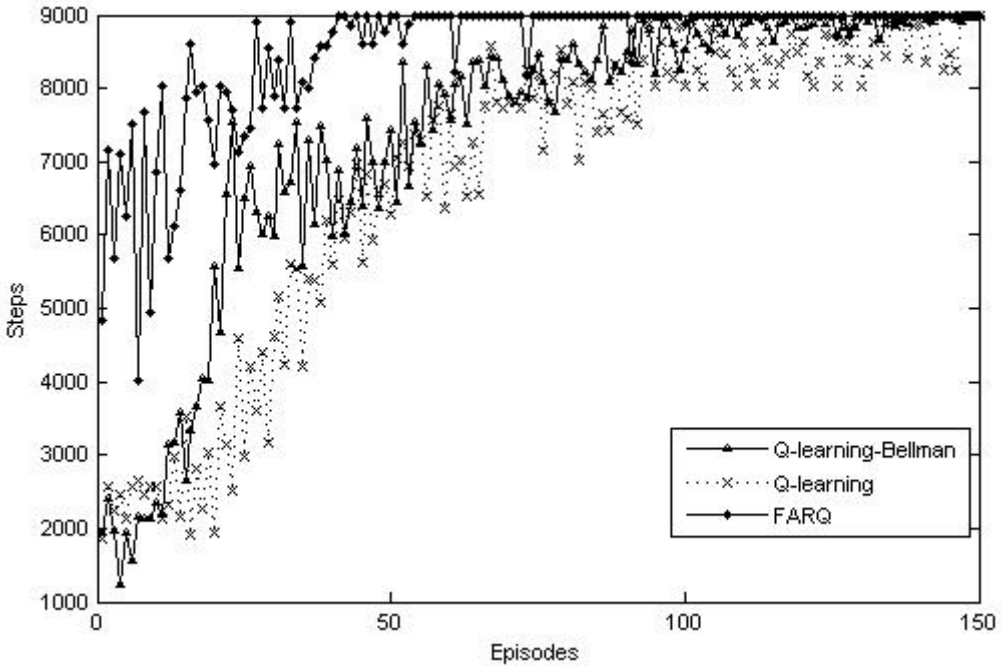

Figure 9: Performance analysis for different algorithms on Pole balancing

Figure 9 shows the performance analysis of Q-learning, FARQ and Q-learning-Bellman algorithms on Pole balancing problem. The horizontal axis is the episode, and the vertical axis is the steps for the different algorithms to the goal state. The state space is divided into 21 pieces in total, 7 pieces for the angel, and 3pieces for the angular velocity. From figure 9,

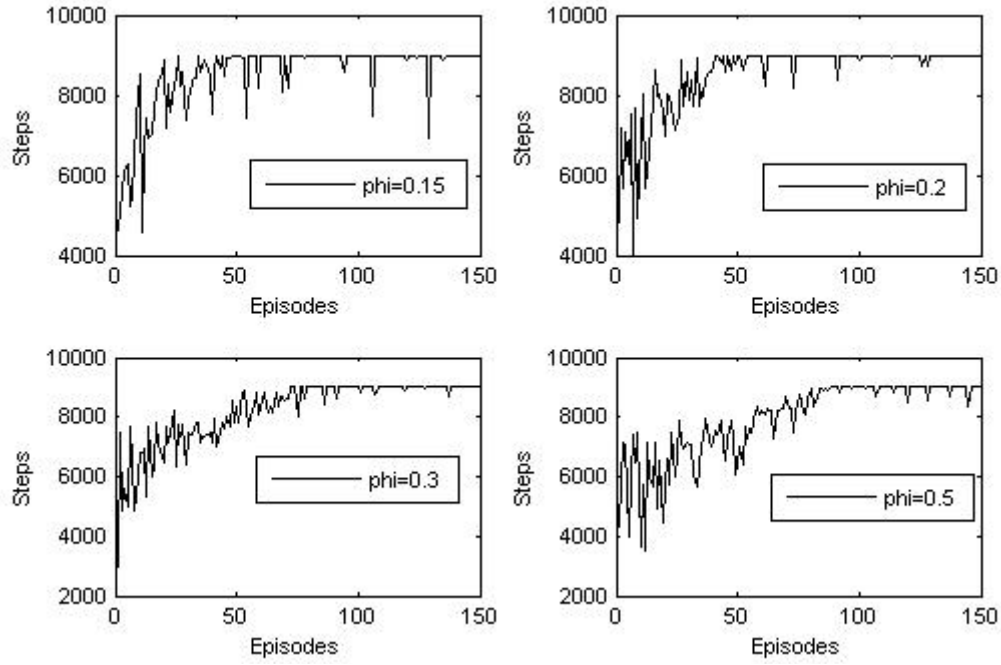

Figure10: Performance analysis for different Φ of FARQ algorithm on pole balancing

we can note that the performances of the three algorithms differ heavily in all episodes. FARQ tends to converge at the 50th episode and thereafter it fluctuates slightly. Q-learning and Q-learning-Bellman seem to converge at the 90th and 110th episodes, but they fluctuate heavily until the episodes are terminal. Evidently, FARQ algorithm performs best in terms of convergence rate and stability.

In order to evaluate the effects of Φ for FARQ algorithm on Pole balancing, we choose four different Φ to compare the performances of FARQ algorithm. the balancing steps are shown in figure 10 respectively. Phi in figure 10 represents the value of Φ. From four pictures, we can know that when $\Phi = 0.15$, $\Phi = 0.2$, $\Phi = 0.3$, and $\Phi = 0.5$, the FARQ algorithm converges at the 50th, 50th, 75th, and 90th episodes, respectively. Moreover, when $\Phi = 0.15$, the algorithm fluctuates more heavily than other three cases after convergence. As a result, when is Φ lager, the convergence rate of FARQ is slower, in contrast, the convergence rate is improved, but the stability is not the best. Evidently, an appropriate Φ can make the algorithm have a better performance.

Table 1: performance analysis for FARQ algorithm on Pole balancing

Φ	0.2				0.3				0.4			
μ	0.01	0.1	0.2	0.3	0.01	0.1	0.2	0.3	0.01	0.1	0.2	0.3
MSE	**0.28**	**0.296**	**0.315**	**0.342**	**0.29**	**0.305**	**0.323**	**0.358**	**0.3**	**0.318**	**0.359**	**0.385**
episode	**50**	**45**	**40**	**35**	**75**	**60**	**50**	**40**	**100**	**85**	**80**	**60**

Table 1 is the performance analysis for FARQ algorithm with different Φ and different μ on Pole balancing problem, where MSE represents the mean square error of action-value function after convergence of the algorithm, and episode means the number of episodes when the algorithm converges. From table1, we can clearly see that, when the value of Φ is constant, as the value of μ is higher, the convergence rate of the algorithm is faster, but the convergence performance is worse. When the value of μ is constant, as the value of Φ is higher, the convergence rate of the algorithm is slower, and the convergence performance is worse too. Evidently, an appropriate Φ and an appropriate μ can make the algorithm have a better performance.

5 CONCLUSIONS

This paper proposes an efficient residual Q-learning algorithm based on function approximation, which not only can guarantee the convergence but also has a fast learning rate. The algorithm combines the traditional Q-learning algorithm and Bellman residual, and adopts a new rule to update the action-value function parameter vector. Theoretically, the new rule for updating action-value function parameter vector can guarantee the convergence of the algorithm and solve the unstable convergence problem of the traditional Q-learning algorithm. To further accelerate the algorithm convergence rate, the algorithm introduces a new factor, named forgotten factor, which makes the algorithm have a faster learning rate and a good robustness. We verifies the performance of FARQ on the traditional Windy Grid World and Pole balancing problems, where we achieve performance exceeding that of both Q-learning and Q-learning-Bellman.

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SUIWML2016: Introductory talk to Soochow University International Workshop Series on Machine Learning and its Applications in Biomedicine.

Assist. Prof. Xiaoke Zhou

Assistant Professor of Computer Sciences School of Computer Sciences and Technology, Soochow University (SUDA), Suzhou, China

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Adaptive Exploration in Stochastic Multi-armed Bandit Problem

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Abstract:

The Multi-Armed Bandit (MAB) problem is a typical problem of the exploration and exploitation dilemma in reinforcement learning. As a classical MAB problem, the Stochastic Multi-Armed Bandit (SMAB) problem is the base of many new MAB problems. To solve the problems of insufficient use of information in existing SMAB methods, this paper presents an adaptive algorithm to balance exploration and exploitation based on the Chosen Number of Arm with Minimal Value, namely CNAMV in short. The upper bound of CNAMV's regret was theoretically proved, and our experimental results showed that CNAMV could yield greater reward and smaller regret with high efficiency than commonly used methods. Therefore, CNAMV is a cost-effective SMAB method.

Conclusions

In this paper, we proved the upper bound of CNAMV's regret theoretically, and discussed the influence of the key parameter *w* in CNAMV algorithm. Through three experiments, we provided the reference range of parameter *w* and compared CNAMV with classical algorithms and their variants in the random data set and the content distribution network dataset respectively. The experimental results showed that the CNAMV algorithm could yield greater reward and smaller regret than ε-greedy, ε-decreasing, SoftMax, decreasing SoftMax and UCB1. As a result, CNAMV algorithm is a cost-effective stochastic multi-armed bandit algorithm.

In future work, we intend to extend the CNAMV algorithm to more complex settings, such as budgeted multi-armed bandits and qualitative multi-armed bandits, and put forward practical application of new multi-armed bandit problem.

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Docking study of active flavonoids for Trypanosoma cruzi and *Leishmania* spp

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Abstract: Flavonoid compounds are normally present on nature being a common constituent of medicinal plants. These natural products have several health benefits, including activity against tropical parasites, such as *Trypanosoma cruzi* and *Leishmania* spp. Five flavonoid active against *Trypanosoma cruzi* and five active against *Leishmania* species were submitted to molecular docking with the enzymes cruzain and trypanothione reductase from *Trypanosoma cruzi* and *N*-myristoyltransferase, dihydroorotate dehydrogenase and trypanothiona reductase from *Leishmania* spp. Considering the binding energies and interactions between both, compounds and enzymes, the flavonoids chrysin dimethylether and tamarixetin against *T. cruzi*, and luteolin and ladanein against *Leishmania spp* presented the best results. This study hopes to contribute to existing research on these natural products against these tropical parasites.

Keywords: Flavonoids; Molecular Docking; *Trypanosoma cruzi*; Leishmania.

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1. Introduction

Flavonoid compounds are common constituents of medicinal plants and bring several health benefits, including cosmetic action[1], cardio protective effects[2], anti-inflammatory activity[3], in the treatment of cancer[1] and against tropical parasites, such as *Trypanosoma cruzi*[4] and *Leishmania* spp[5].

T. cruzi infects about 7 to 8 million people, mostly in Latin America where Chagas' disease is endemic, are estimated to be infected worldwide[6]. Leishmaniasis is an infectious disease that is prevalent in Europe, Africa, Asia, and the Americas, endemic in 88 countries, killing thousands, and debilitating millions of people each year[7].

The great social problems caused by these infections, the restricted number of drugs available, their serious side effects, and the emergence of new drug resistant forms, all motivate research for new antiprotozoal drugs. The aim of this study was to identify favorable characteristics occurring among those complexes which might be encouraging towards potential use of these flavonoids against parasites.

2. Results and Discussion

Table 1 shows the energies (kcal/mol) obtained from the interaction of the ligands and the enzymes cruzain and trypanothione reductase from *T. cruzi*. The compounds Chrysin dimethyl ether and tamarixetin have lower formation energy with both enzymes. Compared with the PDB ligand from cruzain, the hydrogen bonds and sterics with residues CYS25 and GLY66 contribute to a decrease in the energy of the flavonoids. Chrysin dimethyl ether had interactions equivalent to those of the ligand in the complex with trypanothione reductase from PDB: hydrogen bonds with GLY 16, and sterics with TYR 52 residues.

Table 2 shows the energies (kcal/mol) obtained from the interaction of the ligands and enzymes *N*-myristoyltransferase (NMT), trypanothione reductase and dihydroorotate dehydrogenase from *Leishmania* spp. Luteolin and Ladanein was the compounds with lower formation energy in these enzymes. We observed that the flavonoids forming the two lower energy complexes produced distinct interactions with the theoretical ligand 4-bromo-2,6-dichloro-n-(1,3,5-trimethyl-1H-pyrazol-4-yl) benzenesulfonamide, but similar to each other for their hydrogen bonds with the residue TRP 15, and sterics with PHE 14 and ARG 179. Using trypanothione reductase as the receptor for docking, the flavonoids show interactions almost equal to those of flavin-adenine dinucleotide, building interactions with the residues: GLY 127, ASP 35, and THR 51. We believe that this macromolecule is a good target for flavonoids against *Leishmania* spp.

Luteolin, and ladanein build many interactions with the receptor, similar to the PDB ligand. These interactions are hydrogen bonding (SER 45, LYS 44, and THR 273), electrostatic (ASN 195), and sterics (ALA 20).

Table 1. Energies obtained through docking between the flavonoids and enzymes from *T. cruzi*.

COMPOUND	CRUZIPAIN	TRYPANOTHIONE REDUCTASE
Chrysin dimethylether	**-50.9559**	**-93.651**
3'-Hydroxydaidzein	-21.1483	-91.594
5-Methoxyflavone	-43.2225	-76.855
Tamarixetin	**-56.0466**	**-104.551**
7,8-Dihydroxyflavone	-48.8028	-76.107

Table 2. Energies obtained through the docking between the flavonoids and enzymes from Leishmania.

COMPOUND	NMT	TRYPANOTHIONE REDUCTASE	DIHYDROOROTATE DEHYDROGENASE
Luteolin	**-63.469**	-101.527	**-75.638**
Galangin	-53.425	-110.430	-65.441
Ladanein	**-67.633**	**-116.541**	**-95.967**
3',4'-Dihydroxyflavone	-57.075	-93.587	-62.499
7,8-Dihydroxyflavone	-47.090	-97.467	-68.170

3. Materials and Methods

This work was based on the study reported by Tasdemir *et al*[8], where was tested a large series of flavonoids against *Trypanosoma brucei rhodesiense*, *T. cruzi*, *T. brucei brucei*, *Leishmania donovani* and L6 cells. We selected the 5 flavonoids compounds with high activity against *T. cruzi* (**Figure 1**) and the 5 most active flavonoids against *Leishmania major* (**Figure 2**).

Using the software Hyperchem v. 8.0, the chemical structures of the compounds were drawn and their geometry optimized using MM+ force field[9] and the semi-empirical method AM1 (Austin Model 1)[10].

Molecular docking was used to predict the binding orientation and investigate the molecular interactions between the flavonoids active against *T. cruzi* ligands and the enzymes cruzain (ID PDB 3IUT) and trypanothione reductase (ID PDB 1GXF) from T. cruzi, and the compounds active against *Leishmania* with the enzymes *N*-myristoyltransferase (NMT) (ID PDB 4A30), trypanothione reductase (ID PDB 2JK6) and dihydroorotate dehydrogenase (ID PDB 4EF8) from *Leishmania* spp.

The enzymes were imported from the PDB in the Molegro Virtual Docker 6.0 software. We created a template with the already complexed ligands, for the GRID. We selected the MolDock SE algorithm[11], with 10 runs for each ligand.

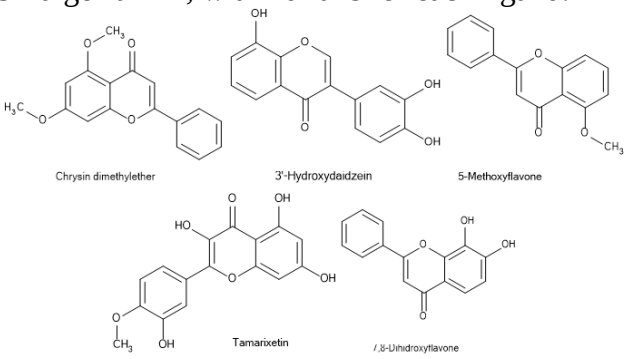

Figure 1. Active flavonoids against *T. cruzi*.

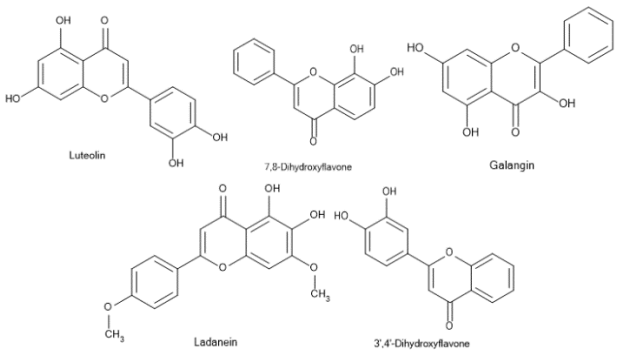

Figure 2. Active flavonoids against *Leishmania major*

4. Conclusion

Our results reinforce the experimental data observer by Tasdemir *et al*[8], demonstrating the potential of this flavonoids for the treatment of neglected diseases and contributing to future research.

Acknowledgments

The present work was carried out with the support of CNPq.

Conflicts of Interest

The authors declare no conflict of interest.

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A mixed ligand – Autogrid based pharmacophore model for the rational design of multi-kinase inhibitors

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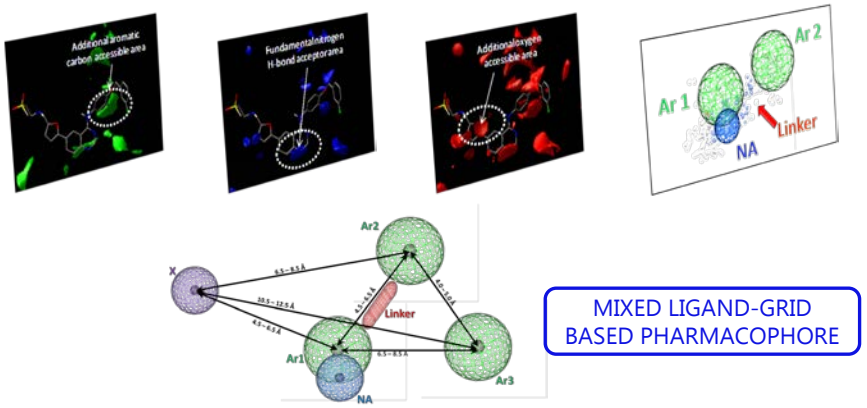
Abstract:
A number of *in silico* methods have been recently applied for searching and designing multi-target compounds. The simplest approach consists in docking the compounds into all the targets independently. Then, only those molecules that show a high score against all the targets at the same times are collected as hit compounds. This approach, however, is quite computationally expensive, particularly when more than two proteins are considered as targets. Moreover, it does not furnish any information on the structural features required for the multi-target potency, thus it is not suitable for the hit optimization process. Several authors circumvented some of these problems by combining pharmacophore models with docking studies. Do to our interest in multi-kinase inhibitor discovery, we decided to derive a multi-kinase pharmacophore model, facing a two stage approach. Firstly, starting from the structures of the ligands we extracted the features of an appropriate multi-TKI scaffold (*scaffold pharmacophore*). Then, we decorated this scaffold through information derived from the target structures (*multi-TKI pharmacophore*). The presented methodology for identifying pharmacophore model could be applied also to other interesting pharmacological models for which a multi-target activity would be valuable.

Keywords: *kinase inhibitors;pharmacophore; autogrid; multi-targeting compounds*

Graphical Abstract:



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Introduction. Alteration in the cell cycle regulatory system may cause cancer onset, progression and metastasis[1]. Among the proteins involved in signal recognition, transduction and amplification, tyrosine kinases (TKs) play a key role[2]. Despite the increasing interest in multi-TKIs discovery, the rational drug discovery methodologies are often applied only to the design of dual inhibitors. For example, several EGFR/src inhibitors or src/abl inhibitors have been discovered through docking studies[3,4]. However, since there is a complex relationship among the several TKs involved in the signal transduction pathway, it would be useful to find TKs inhibitors effective on more-than-two kinases.

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Results and Discussion

Among all the TKs involved in cancer, we considered EGFR, FGFR-1, VEGFR-2, abl and src. In almost all the cases, more than one tridimensional structure was available in the Protein Data Bank (PDB). For the purpose of the present work, the resolved structures relative to mutant proteins and those containing an irreversible inhibitor were not considered. Thus, a total number of 57 tridimensional structures were initially considered. The superimposition of all these structures revealed a high degree of structure similarity (mean RMSD obtained: 1.11 Å). In order to define the features of the appropriate scaffold for multi-TKIs development, all the atoms with a formal negative partial charge and all the carbon atoms common to the majority of the ligands have been selected. As depicted in Figure 1, two aromatic carbon atom allowed area connected by a linker chain and one nitrogen H-bond acceptor allowed area could be easily identified.

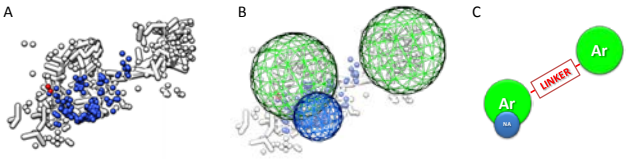

Figure 1. Scaffold pharmacophore derivation. (A) Atoms with negative partial charge (blue) and carbons (white) common to at least the 80% of the ligands. (B) Ligand based pharmacophore. (C) Schematization of the derived pharmacophore. NA = Nitrogen H-bond acceptor allowed area; Ar = aromatic carbon allowed area.

The second stage of the pharmacophore model generation has been faced employing the AutoDock (AD) 4.2 software. To compute the ligand-protein interaction energy and the geometry of interaction, AD makes use of several atomic affinity grid maps, calculated prior to the ligand docking. This method, also known as the “grid approximation” protocol, dramatically reduces the computational cost with respect to the continuous scoring function methodologies. Furthermore, as AD calculates one map for each desired ligand atom type, the affinity grids can be used to identify those regions in which a specific ligand atom favor the binding with the receptor.

The atom affinity maps for aromatic carbon, aliphatic carbon, H-bond acceptor nitrogen, non-H-bond acceptor nitrogen, oxygen, H-bond acceptor sulfur, non-H-bond acceptor sulfur, fluorine, bromine and chlorine atoms have been calculated in the ATP binding site of EGFR, FGFR-1, VEGFR2, src and abl. For each atom

type, the grids computed in the six kinases have been contemporaneously loaded in AD, setting the isovalue to about -0.60 Kcal/mol, thus permitting the determination of those ligand atoms allowed areas common to all the TKs. The grid derived information have been then overlapped to the *scaffold pharmacophore*, furnishing the final multi-TKIs pharmacophore model (Figure 2).

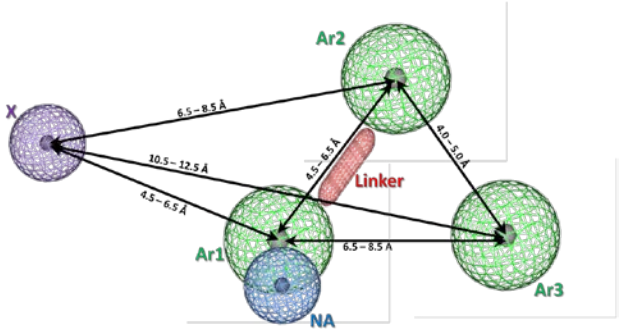

Figure 2. Multi-TKIs pharmacophore model.

Conclusions

Through the combination of a ligand-based and an AutoGrid-based approach we have identified a novel pharmacophore model potentially useful for the design of multi-kinase inhibitors. Interestingly, our recently reported multi-kinase inhibitors bearing biarylaminoquinazoline scaffold [5] perfectly fit the pharmacophore model, thus demonstrating its usefulness. The presented methodology for identifying pharmacophore model could be applied also to other interesting pharmacological models for which a multi-target activity would be valuable.

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

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Notes:
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Ilex guayusa: A systematic review of its Traditional Uses, Chemical Constituents, Biological Activities and Biotrade Opportunities

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Abstract:
Ilex guayusa is an emblematic tree of the Ecuadorian Amazon Region (EAR), widely used in folk medicine, ritual uses and household and industrial beverages. Despite the daily consumption only a few number of studies have been carried out and the species deserves a deepener bioprospecting activity, also in order to define a new Biotrade strategy for the EAR. This review summarizes the ethno pharmacological data and the researches concerning *I. guayusa*. Promising biological activities have been detected, especially as new source of antioxidant agents due to the presence of phenolic compounds. Also a preliminary study as antidiabetic natural product accounts for new researches

Keywords: *Ilex guayusa*, Ecuadorian Amazon Region, folk medicine, antioxidant, antidiabetic, Biotrade

1. Introduction

Ilex guayusa is an emblematic tree of the Amazonian region, widely present in the Amazonian region of Ecuador, Colombia, Peru and Bolivia. As reported by many authors (1 – 8) *I. guayusa* was mentioned in several researches regarding pre-Columbian archaeological collections, old historical manuals and ethnobotanical studies, legitimizing the fame of this specie as the most important plant in the daily life of Kichwa Amazonian communities (5) and local farmer from “colono” and “mestizos” communities. Despite the wide presence of ethnobotanical studies regarding several uses in folk medicine (6), there are only few researches about the phytochemistry and biological activities of *I. guayusa*, and this lack compromises a complete understanding about the concurrence between folk medicine and pharmaceutical applications. Moreover, deepener studies may propose new promising applications as nutraceutical or cosmetic ingredient. We aimed to compile an up to date and comprehensive review of *I. guayusa* that mainly covers the phytochemistry and pharmacology

information, in order to suggest new researches and to offer a complementary paper to the ethnobotanical research.

2. Results and Discussion

2.1 Botanical description, historical information and folk medicine

Ilex guayusa is an evergreen tree belonging to Aquifoliaceae family, native to the Amazon. The plant is dioecious and reaches between 6 to 10 meters tall. The leaves are simple, pinnate, glabrous, oblong, elliptic with serrate margin; they are 7-20 cm long and 2.5-7 cm wide (4). *I. guayusa* is distributed from 200 to 2000 m above sea level along the Andes and contiguous Amazonian piedmont (8). Historical information about *I. guayusa* was mentioned by Schultes (9). Describing an archeological finding from a shaman excavated tomb in Bolivia (Tihuanacoid culture), the author described the presence of dried and pressed leaves, a mortar and pestle. The finding probably describes the use of the species as snuff during ritual activities and it is feasible that the species has been used for at least 1,500 years. Since the XVI century until today, many authors described folk medicine and commercial activities related to *I. guayusa*. Even for Jesuit missionary in Ecuador the species was an important source of income (1,3) and, currently, a few companies from the EAR are selling beverages and infusions obtained from *I. guayusa*.

Regarding the folk medicine information, the Table 1 summarized several traditional uses which include ritual and magical application. According to the "cosmovisión" concept of the Amazonian ethnic groups, *I. guayusa* can be used for multiple porpoises, from human health remedy to the custom of cleansing the stomach daily as a ritual purification. *I. guayusa* tea is considered a "magical drink" and is also given to the hunting dogs, before a hunting expedition, in order to improve their abilities and skills. For indigenous people, the infusion can also provokes a soft hypnotic effect in which "little dreams" can inspire or dissuade in advance a hunting expedition (1).

2.2 Phytochemistry

Leaves contain caffeine, theobromine, phenolic compounds and flavonoids as the main components (10-13). Also guanidine was mentioned as an important component of I. guayusa leaves extracts (14,15). Another research performed by Ruiz and Roque (16), mentioned a phytochemical preliminary assay on ethanolic, methanolic and hydroalcoholic extracts of *I. guayusa*, the study revealed the presence of tannins, alkaloids, flavonoids, glycosides, phenolic compounds and quinones.

A study performed by liquid chromatography with tandem mass spectrometry (17) of a *I. guayusa* leaves extract detected several amino acids (Table 2), which provide an interesting information about nutraceutical profile and taste. From the same author (18), another research on *I. guayusa* leaves extracts, using gas and liquid chromatography and mass spectrometry, revealed the presence of two pentacyclic triterpenoid, oleanolic (1,18 mg/g) and ursolic acid (18,22 mg/g) respectively.

2.3 Biological activity

Stimulant and protective effect of caffeine and theobromine are extremely reported in literature (19-23). As reported by Jara *et al*. (13), dried leaves of *I. guayusa* were extracted with ethanol (EtOH) and ethyl acetate (EtOAc). The total phenolic content was determined spectrophotometrically according to Folin–Ciocalteu's phenol method and calculated as gallic acid equivalent (GAE). The total flavonoids content (TFC) was determined spectrophotometrically, the antioxidant activity was determined using free radical DPPH (2,2-diphenyl-1-picrylhydryzyl) scavenging method and the β-Carotene bleaching. Results are reported in Table 3.

Anyway, the presence of phenolic compounds and flavonoids may indicate a protection against cellular damage induced by free radical oxidative injury or reactive oxygen species. These antioxidant properties are associated with the presence of phenolic compounds and flavonoids.

Although guanidine was reported but wasn't quantified, its presence explains the preliminary hypoglycemic effect of *I. guayusa* leaves extracts in animal model. Guayusa may reduce hyperglycemia without affect the parameter of glucose homeostasis in non-diabetic mice (14).

Oleanolic and ursolic acid are recognized as antiviral and anti-inflammatory bioactive compounds and also were reported the in vitro inhibition activity of these molecules against various cancer cell type (18). Finally, estrogenic activity of ethanolic extracts from leaves of I. guayusa was tested in an *in vivo* model (female albino rats) shoving a remarkable increase of serum estradiol levels and ovaries and uteri weights. This finding is a preliminary but promising data in order to confirm the traditional use of *I. guayusa* against female infertility (24). In another study, it was observed that methanolic and hydroalcoholic extracts from I. guayusa have fungicidal action against *Candida albicans*, the hydroalcoholic extracts was effective also against *Microsporum canis* (16).
Moreover, toxicological study was conducted on ethanolic and water extracts using a Brine-Shrimp assay (25), in both cases the test shown respectively low (LC50 500–1000 µg/ml) and median toxicity (LC50 250–499 µg/ml), in according with safe traditional use, especially for the aqueous extract. Another research performed by Ames test and a chromosome aberration study in human lymphocytes demonstrated a no harmful effects (26).

2.4 Biotrade opportunities

In the EAR, ritual infusion, beverages and tea bags obtained from I. guayusa leaves are widely present in local market, restaurants and houses. Furthermore, there are some experiences about the development of a local and international Fair Trade market, based on social sustainability approaches and eco-friendly criteria. Actually, in order to valorize the Ecuadorian Amazonian biodiversity, the *I. guayusa* derivatives may be a sustainable alternative to design natural products, relevant for local economies, such as: tea (27), phytopharmaceuticals (12). In order to maintain national and international markets, it is necessary to design a bi-commercial business strategy that enhances the balance between conservation politics and entrepreneurs needs.
A recent study performed by Sidali and Garrido Pérez (8), focuses on a food tourism model, based on guayusa case, as a strategy of sustainable development for Kichwas communities in Napo (Ecuador). The qualitative research confirms as food tourism may be a viable strategy and a future trend for EAR. Moreover, the research identifies four principles of Kichwa communities' cosmovision (worldview) which are compatible with Western-based theory on niche tourism, respectively: mutual learning, empowerment, regulated access to intellectual property and community legislation**.**

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Table 1. Traditional, magical and ritual uses of *Ilex guayusa*.

Traditional, magical and ritual uses	Country	Ethnic group	Ref.
Glycemic effect	n.r.	Amaguajes	14, 15
Ritual uses	Brazil (outskirts of Manaus)	n.r.	11
Diabetes, venereal diseases, improving digestion and appetite, strengthening the body and treat pain, increase fertility, daily purging. Promoting conviviality, stimulant, stomach tonic, diuretic, and flu remedy. Increase fertility and libido. Can help to avoid insect and snakebites. Guayusa helps people to dream.	Ecuador, Colombia, Perú	Amazonian Kichwa, Shuar, Achuar, Cofán, Secoya, Awajún Mestizo and white people	6

Daily morning drink, can help to avoid insect and snakebites, improves hunting and fishing ability.	Ecuador	Amazonian Kichwa	5
Ritual uses, scatological purification, ailment, emetic, narcotic, hypnotic, stimulant or tonic, diaphoretic and diuretic, purgative. Increase woman fertility and helps people to dream for knowing in advance.	Ecuador	Several Amazonian communities	1
Health tonic, emetic, venereal diseases, improved the digestion and appetite, can cure dysentery and amenorrhea.	Ecuador, Peru	Amazonian communities	3
Use before and after drinking ayahuasca. Stomach trouble aphrodisiac.	Colombia, Ecuador, Peru	Amazonian communities	4
Emetic and stimulant tea	Ecuador	n.r.	9
Gastritis, relaxant, helping woman fertility.	Ecuador	Saraguros, Shuar	28
Energizing and stomach pain.	Ecuador	Kichwa, mestizo	7
Additives for hallucinogenic rituals and ritual snuff.	Ecuador, Peru	Shuar	29, 30

Table 2. Amino acids in *Ilex guayusa* extract (mg/g).

Gly	Asn	Ser	Asp	Gln	Thr	Ala	Glu	Pro	Lys
0.0100	0.2795	0.0107	0.0533	0.0502	0.0136	0.1069	0.0501	0.0253	0.0092
Val	His	Met	Arg	Tyr	Ile	Leu	Phe	Trp	Total
0.0174	0.0129	0.0052	0.0429	0.0129	0.0132	0.0125	0.0110	0.0794	0.8161

Table 3. Phenolic content, flavonoid content and antioxidant activity in Ilex guayusa extract

Sample	Total Phenolics (GAE mg/g)	Total flavonoids (RE mg/g)	DPPH IC_{50} (μg/mL)	β-carotene IC_{50} (μg/mL)
Guayusa EtOH	54.0±3.8	46.0±2.0	17.5±1.4	55.6±1.6
Guayusa EtOAc	36.0±2.2	20.0±1.8	52.7±4.3	85.7±3.7

3. Materials and Methods

The present systematic review was achieved adopting the following electronic databases: SciFinder, PubMed, Google Scholar, SciElo, Taylor & Francis and Scopus. Data were independently extracted from three reviewers and the final paper selections were completed avoiding duplication of data. The following keywords were selected: *Ilex guayusa*, guayusa. The reviewers selected articles were in English and Spanish language and were excluded data from patents. The above mentioned criteria allowed selecting 20 eligible articles; we also considered some additional key papers for introduction, discussion and result chapters. Anyway, it is deserved to remember that many authors mentioned remarkable letters and historical sources from XVI until XIX century

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4. Conclusions

Despite the widespread presence of beverages and commercial products obtained from *I. guayusa*, mainly ethnobotanical research has been realized in the last decades. All phytochemical researches known until today were developed exclusively on leaves extracts, without a deepener studies on other parts of the plant. A recent review about Ilex genus (31) reported wide information about active constituents and their biological activities, but

present basic information on *I. guayusa* regarding the presence of caffeine. For many others Ilex species were been identified many molecules as triterpenoids, saponins, flavonoids, alkaloids, anthocyanins and other phenolic compounds which can explain the mentioned biological activities. The lack of a deepener phytochemical research about I. guayusa is undeniable and the future trend may be to increase the researches about antidiabetic and estrogenic activity above-mentioned.

Furthermore, others studies about plants which contain caffeine (32,33) reported the effect of leaf age effects on the quantitative contents of caffeine, theobromine, methylxanthines and total phenolic compounds, essentially showing a decrease amount of mentioned active compounds in old leaves. Moreover was observed that caffeine presence seems to be cultivar-specific, tissue-specific, and season-dependent. In order to optimize nutraceutical and cosmetic formulations based on *I. guayusa* extracts, all these findings suggest a deepener research about caffeine presence and phenolic compounds focusing different parts of the plant, different plant ages and harvest seasons. Finally, *I. guayusa* represents a promising bio-active compound source and an alternative income wellspring for local farmers from the Ecuadorian Amazonian Region.

Acknowledgments

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Conflicts of Interest

State any potential conflicts of interest here or “The authors declare no conflict of interest”.

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Editorial: MOL2NET 2016, International Conference Series on Multidisciplinary Sciences

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The aim of this editorial is to inviting all colleagues worldwide to participate on a new International Conference Series. The official title of this conference series is MOL2NET International Conference Series on Multidisciplinary Sciences. The possibility of multidisciplinary collaborations in science between experimentalists and theoretical scientists inspired this title. MOL2NET (the conference running title) is the acronym of the lemma of the conference: **From Molecules to Networks**.

This is an International Conference Series to Foster Interdisciplinary Collaborations in Sciences with emphasis on Experimental Chemistry (all branches), Materials Science, Nanotechnology, Life Sciences, Medicine, and Healthcare, along with Data Analysis, Computer Sciences, Bioinformatics, Systems Biology, and Complex Networks Sciences.

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 research professors endowed by IKERBASQUE, Basque Foundation for Sciences, and/or 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).

In addition, researchers from abroad such as (to cite a few) Prof. Danail Bonchev from the Center for the Study of Biological Complexity of the Virginia Commonwealth University (VCU), USA, Dr. Subhash C. Basak, from the Natural Resources Research Institute, of the University of Minnesota, USA, or Assoc. Prof. Bakhtiyor Rasulev from Center for Computationally Assisted Science and Technology (CCAST), North Dakota State University (NDSU), Fargo, USA, along with many other are also founders and/or active supporters of this conference, please see full committee at: http://sciforum.net/conference/mol2net-02

The publication of communications will be online via the platform SciForum of the Editorial Molecular Diversity Preservation International (MDPI), with HQ in Basel, Switzerland, and Beijing -Wuhan, China. This year the second edition of MOL2NET is planed to be held from 2016-Dec-05 to 2017-Jan-25 (including interactive discussions, posts, comments, questions, and answers about papers in the online platform Sciforum). However, the online submission platform is open and the publication of communications will be asap upon acceptance, all the year. For more details, see Schedule & Program page and to submit a communication use the Submission link. Remember, these are the dates for the online conference and not for the face-to-face workshops associated to the conference.

MOL2NET Past Edition

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.

Face-to-Face Associated Workshops
(In person participation)

MOL2NET International Conferences Series on Multidisciplinary Sciences is a scientific conference that runs totally online at the SciForum platform promoted by the editorial of the Molecular Diversity Preservation Institute (MDPI), Basel, Switzerland. http://sciforum.net/conference/mol2net-02.

Consequently, no physical presence is needed saving traveling costs. However multiple workshops associated to the conference run in person (face-to-face) at their organizing universities. This year our conference is the online host of many of these 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, Chairperson Prof. David Quesada and Advisory Chairperson Prof. Humberto Gonzalez-Diaz (Online Publication).

IWMEDIC-04, IV International Workshop on Medical Imaging, Medical Coding, and Clinical Data Analysis of University of Coruña (UDC). The IWMEDIC-04 workshop will be 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). The chairman of this workshop is the Chair Professor and Director of Department of Computer Sciences, UDC, Coruña, Spain Prof. Alejandro Pazos.

UFI-QOSYC 1st Young Scientist Workshop hold at the Department of Organic Chemistry II, University of Basque Country UPV/EHU. This workshop have brought together early career researchers (post-docs and graduate students) from the area of organic chemistry and catalysis across the UFI QOSYC to exchange information and practice presenting their research work in a supportive scientific environment. Chairpersons Prof. Esther Lete, Prof. Esther Domínguez Pérez, and Prof. Jose Luis Vicario

SUIWCS-01, Soochow University International Workshop Series on Computer Sciences. The SUIWCS-01 workshop will be held presentially at the the School of Computer Science and Technology of Soochow University, PCR, China (Summer, 2016).

The chairman of this workshop is the Chief of Department of Software Engineering and Professor of Computer Sciences, School of Computer Sciences and Technology, Soochow University (SUDA), Suzhou, China, Prof. Quan Liu.

BMEICB-02 Second Bioinformatics Meeting of The School of Bioinformatics Engineering, University of Talca, Talca, Chile, (Oct, 13-14, 2016). Connection with MOL2NET conference Prof. Julio Caballero.

CIESABIO-01, 2016, the Workshop Series on Biotechnology and Zoonotic Diseases of the CIESA, Center for Invetigations and Advanced Studies on Animal Health of the FMVZ Faculty of Medical Veterinary and Zootechnique, of the UAEMEX Autonomous University of the State of Mexico. The Chairperson of this workshop is the Prof. Esvieta Tenorio.

MDPI JCR Journals Special Issues

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/). See, as example, the special issue on the International Journal of Molecular Sciences (IJMS), IF = 3.257), with 18 papers in total including papers from the conference, link: Special Issue on Data Analysis in Molecular Sciences. In order to send a proposal of associated workshop and/or special issue contact the chairperson of the conference and UPV/EHU Ikerbasque Professor Prof. H. González-Díaz .

People, Media, and Social Networks

We are uploading flyers and promotional videos (in different languages) to the MOL2NET accounts in different social networks such as: GOOGLE+ account with +50000 viewers; 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].

NOTES for participants

The MOL2NET conference is Totally Online; no physical presence is needed 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). Please, see the following instructions: (1) Read call for papers [Link], (2) Read [Instructions to Authors] and download template .doc file MOL2NET 2016 Microsoft Word template file, (3) Submit short communications (2-3 pages), reviews, papers, or videos: [Link]. For details about in person (face-to-face) participation on associated workshops contact the respective members of the local committees.

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Natural Product Inhibitors of Topoisomerases against cancer

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Abstract: Since ancient times, natural products have been used in treating various diseases effectively and safely. Nowadays, these natural compounds are submitted to sophisticated methodologies from isolation, computing, analytical, and even serving as pharmacophore suggestions for synthesis. The substances extracted from marine species, plants, and microorganisms present activities beneficial to our health, including protection against malignant tumors. The topoisomerase enzymes play an important role in DNA metabolism, and searching for enzyme inhibitors is an important target in the search for new anticancer drugs. We performed a docking study with our Brazilian diterpenes in topoisomerases I and II. The better compound, the trachylobane 1, forms one hydrogen bond when submitted to docking with Topo I (with the ASP533 residue) and two with residues in Topo II (THR213 and TYR188). The difference observed in the energy of formation can be attributed to hydrogen-bond interactions.

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Keywords: cancer; docking; terpenes; topoisomerases.

Mol2Net YouTube channel*:* *http://bit.do/mol2net-tube*
YouTube link: *https://www.youtube.com/results?search_query=luciana+scotti*

1. Introduction

Cancer is the set of chronic diseases caused by mutations in protein-coding genes leading to disordered growth and multiplication of abnormal cells to form tumors that destroy tissue and other organs [1]. Being caused by a genetic disorder, the disease development can be simple and fast. It was estimated that 18% of cancer cases reported in 2002 were associated with infections such as hepatitis B, C, and papilloma virus (present in 90% of patients with cervical cancer) [2–4]. In addition, about 30% of cancers are associated with tobacco smoking and inhalation of pollutants and another 35% to eating habits [5, 6].

During the processes of replication and transcription along a stretch in the anterior and posterior region of DNA, strands are separated due to the formation of spirals. Topoisomerases act on the control of spirals, relaxing the DNA, modifying its tertiary structure without changing the primary structure [7–10]. These macromolecules are classified according to the cleavage of DNA strands and the location of the covalent link between the enzyme and the DNA strands.

We selected six diterpenes isolated in our laboratory from Paraíbana flora (genus Xylopia (Annonaceae)) [11-14]. As we noted earlier, these diterpenes showed potential anticancer activity [15,16]. Here we selected, evaluated, and compared the topoisomerase inhibition of three atisane and three trachylobane diterpenes extracted from the roots of *X. langsdorffiana*, through Molecular Docking.

2. Results and Discussion

Table 1 shows the energies (kcal/mol) obtained from the interaction of the ligands and enzymes.

We observed that all compounds had the best interaction with Topo I. Compound T1 best interacted in both receptors; on the other hand A1 showed the highest energy with the two enzymes. We noted that compound T1 forms one hydrogen bond when submitted to docking with Topo I (with the ASP533 residue) and two with residues (between nonpolar atoms) with ARG364 Topo I and Topo II of TYR188. We believe that the stability difference observed in the energy of formation can be attributed to hydrogen-bond interactions. Other studies reported the same, as the observations of Laco et al, 2002 [17]; which H-bond interactions between the camptothecin and top1/DNA active site is reflected in the values of the energy scores.

Figure 1, we see the hydrogen bonds formed between compound T1 formed with the residues of Topo I and II.

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in Topo II (THR213 and TYR188) (Fig. 1). The atisane diterpene forms only steric interactions

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Table 1. Energy Values of ligand ad enzymes interaction calculated from Molegro Virtual Docker program

Compound	Topo I	Topo II
T1	–77.220	–73.918
T2	–66.860	–54.315
T3	–65.073	–62.102
A1	–48.066	–37.733
A2	–55.703	–48.965
A3	–65.204	–55.666

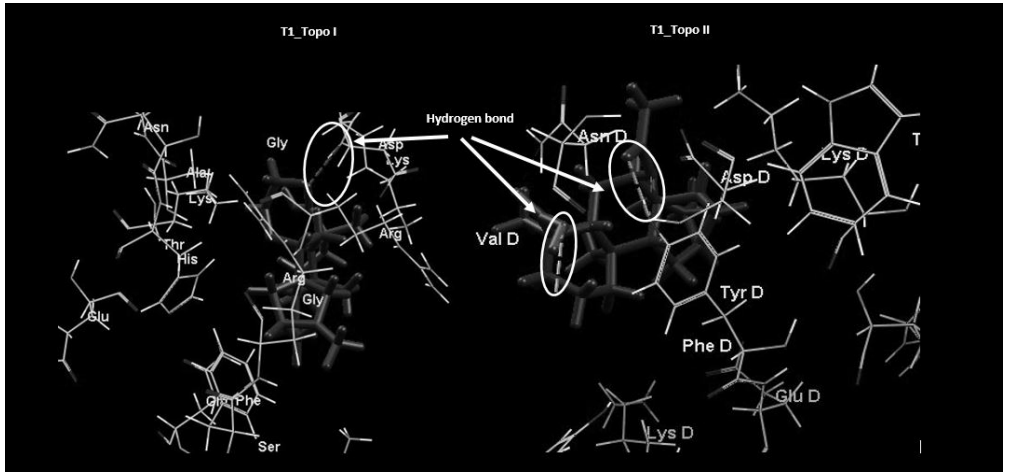

Figure 1. Hydrogen bonds between the diterpene T1 and Topo I and II.

3. Materials and Methods

The three-dimensional structures were drawn using HyperChem 8.0.3 software [18] and energy-minimized employing the MM+ [19] force field without any restrictions. Subsequently, we performed a new geometry optimization based on the semiempirical AM1 method [20]. The optimized structures were subjected to conformational analysis using a random search method [21,22]. Selected dihedral angles were evaluated by rotation in accordance with the standard (default) conditions of the Spartan program [23].

Docking

The six diterpene ligands were submitted to docking with two enzymes: topoisomerase I (ID PDB 1T81) [24], and topoisomerase II (ID PDB 2ZBK) [25]. The enzymes were imported from the Protein Data Bank (PDB) in the Molegro Virtual Docker 6.0 program [26]. We created a template with the already complexed ligands for the GRID. We selected the MolDock SE algorithm with 10 runs for each ligand.

4. Conclusions

Our docking study performed with Brazilian diterpenes and both enzymes showed that the trachylobane diterpene formed a more stable complex due to a hydrogen bond with Top I (with the ASP533 residue) and two hydrogen bonds with residues of Top II (THR213 and TYR188). With this study we intend to assist research for compounds that may belong to class II or drugs " catalytic topoisomerase inhibitors " , which interferes with the function of the topoisomerases.

Acknowledgments

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Conflicts of Interest

“The authors declare no conflict of interest”.

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AGRICULTURAL PRODUCTION UNITS (APUs) AT AMAZON REGION OF ECUADOR, PASTAZA PROVINCE AND DIVERSIFIED FARM AS EFFICIENT PRODUCTION SYSTEM

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ABSTRACT

The paper makes an analysis of the Agricultural Production Units (APUs) in Pastaza province, the largest of Ecuador (12% of the country) and of the Amazon Region (66%), where 56% of its population live in rural areas. The 88% of APUs in Ecuador are constituted by family farming (FF), defining this term as a farmer group that employs basically family workforce for their activity. In fact, 41% of total productive land in the country is grown as "family farms", which corresponds to a total of 618,685 APUs. However, an important part of agricultural production takes place in the "Chakras (farm)" which is the traditional way of Kichwa people grown their crops, combining the goal of family feeding opportunities for income generation. The chakra´s management is natural and is based on the ancient practice of Amazonian Kichwa, this is a family space for knowledge and to maintains a high biodiversity, which sometimes is affected by the increase of the commercial crop areas. At the Center of Research, Post-degree and Conservation of the Amazonian Biodiversity (CIPCA), belonging to the Universidad Estatal Amazónica a “diversified chakra” is developing in an area of 5000 m^2, with a high species diversity and managing agroecological principles, proposed as a productive model for the Amazonian region.

INTRODUCTION

Pastaza is the largest province of Ecuador and also from Amazon region, but meanwhile one of the greatest contrasts in the whole country. It has an area of approximately 29,773 km^2, equivalent to 66 % of the Amazon Region and 12% of the national territory. However, the extension of the territory is not concomitant with the richness of the zone and the income of their people.

Agricultural production in the region focuses on two forms of production: Agricultural Production Units (APUs), which can be framed in different sizes and farming activities and chakra, which is usually a small area and dedicated to agricultural crops. This work aimed to characterize both agricultural systems and to describe the species diversity and the agroecological principles carried out under diversified chakra at Center of Research, Post-degree and Conservation of the Amazonian Biodiversity (CIPCA), belonging to the Universidad Estatal Amazónica.

MATERIAL AND METHODS

Before the experimental research, we characterize the APUs from Ecuador and from the Amazon region. We also made a comparison from the several agricultural systems implemented in the region, focusing in the chakra, as the center of species diversification and to propose it as a productive model Ecuadorian Amazon Region.

To perform the investigation, we visited an important number of APUs and chakras at the 4 cantons of Pastaza province, where the diversity of cultivated species and conditions thereof were assessed. We also obtained information from the Ministry of Agriculture, Aquaculture and Fisheries (MAGAP) of the province, from the Agricultural

Group of Decentralized Self-Government of Pastaza (GADPPz) and publications of the National Autonomous Institute for Agricultural Research (INIAP). Finally we participate as advisor in the Workshop of Analysis and Definition of the Productive Plan of the province for the period 2014-2025. With these activities, we obtained important information that allowed us to characterize the production systems of the province. At the same time, we begin to "rescue" an area of 5000 m^2 from CIPCA to develop a diversified chakra and to design, organize and establish spatial and chronologically the crops to ensure a high species diversity. An analysis of the species diversity, production levels, income and health status of the crops was done, for which pests of different crops every seven days were evaluated, measuring six plants of each point having a total of 30 plants. All pests present in each crop were scored and counted.

RESULTS AND DISCUSSION

Characterization of Agricultural Production Units (APUs) in Ecuador

The 88%of agricultural production units in Ecuador are of family farming (FF), defining this term as a farmer group that employs basically family workforce for their activity, but not exclusively (Wong 2006). The 41% of total productive land in the country is grown as "family farms" with 618,685 UPAs.

Family farming, according to the temporary employment contract and type of labor for agricultural activities, can be classified as subsistence FF, transition FF and consolidated FF, being the consolidated agriculture that which employ contracted workforce permanently. By contrast, the transition FF is that does not hire permanent workers, although it may have temporary workers. The family subsistence agriculture is the activity that does not hire labor, or permanent or temporary, only use workforce from family members.

From the total FF of Ecuador, 456,108 (62%) belong to subsistence agriculture, 274,064 (37%) for transition agriculture and 9,780 (1%) to consolidated agriculture. Most of APUs are classified as "subsistence" FF. The average size of each classified APU in Ecuador (including all types of APUs) is approximately 14.66 ha, according to the Third National Agricultural Census, while APUs classified as "subsistence" reach 5.5 ha, and so called transition APUs have an average size of up to 7 ha. Finally, it is assumed as consolidated APUs those whose reaches up to 65.5 ha in average (Wong 2006).

The production system in the chakras

The chakra is a shifting agricultural system, which includes in its production space several agroforestry systems, spatial or temporal, developed in a forest clearing or enhancement, which deliberately conditioning the families to combine marketing and subsistence strategies. Their attributes define it as a priority system of sustainable land use in the Amazon Region.

This shifting cultivation way is the most widespread traditional system in the Amazon region. It is based on slash and burn areas of mature or secondary forests (1-5 ha), the implementation of crops for a short period (2-5 years), followed by long periods of rest or fallow (over 15 years). It is characterized by the exclusive use of human labor and the use of simple tools (Clarke, 1971).

In Kichwa production systems, which is the chakra, soil rotation is done every so often, allowing to grow for a few years and then is abandoned, and a new chakra is set to a new lot. The soil rotation period according to some authors is diverse, two to three years (Gonzales and Ortiz de Villalba, 1998) or three to five years (FEPP, 2002). The aim of rotation is addressed to soil fertility regaining.

Nowadays to establish a chakra by Kichwa, the first activity is to define an area or a lot in the natural forest, where they perform the slash and burn and then grow mainly cassava and plantain. In the chakra, the same crop is grown twice, and then a different crop the previous one, due to that three cycles of crops are done on a lot set, then this chakra is abandoned for a few years until the soil recover its fertility, which means that a new area of natural forest is deforested, beginning a new cycle. Each year one family open from 1.5 to 2.3 ha of natural forest to perform the chakras and leave trace of 0.8 to 1 ha, what means that in a year a family opens about one additional hectare in the forest.

Kichwa produce in their chakras banana and cassava as staple food, which permanently planted at any time. The proteins source are from hunting and fishing. Part of their tradition is also the collection of non-timber forest resources as lianas, fibers, vines, seeds, leaves, bark, herbs, oils and resins, among others, which are used for food, medicine and crafts (FEPP, 2002).

Kichwa from the Amazon Region takes into account summer and winter times and the lunar cycle to the planting and harvesting of agricultural products. Usually in the summer months (less precipitation) perform clearing (slash and burn) and prepare the ground until the first rains, which are exploited for growing crops. No work or activity are performed until harvest (Gonzales and Ortiz de Villalba, 1998).

The chakra is set up in many ways, beginning with annual or seasonal crops and then planting cocoa. Other way is the planting of cocoa and after annual or temporary crops, or turn both at the same time. The size usually ranges between 0.50 and 1.25 ha.

It is expected that the cocoa cultivation will implanted for over 10 years and in intercrop annual species are rotated temporal or spatial in small areas by way of mosaics and permanently associated with woody species such as: timber, fruit, medicinal and craft, among other species. This chakra structure is typical of the Kichwa communities, while settlers use simultaneously the whole area planted with cocoa to plant annual crops, practicing greater the intensification than in the Kichwa chakra.

Currently, the Kichwa communities and settlers have made progress in the knowledge and application of good production practices and postharvest of national fine and flavor cocoa, which is. the main revenue of income generation, allowing them to produce and sell in domestic and international markets. However, production is still insufficient to meet a growing demand for this product, reason why it is required to improve the supply and distribution of appropriate technology to their condition.

Today in the chakra low diversity of agricultural and forest products is observed. Forest products are at the limit of agricultural crops or roads to the chakra. Agricultural products for home consumption (cassava, plantain, maize and rice) are grown on averaged 1.56 ha surfaces and agricultural products intended for sale (cocoa, coffee) are grown in larger areas of 3.44 haaverage.

Conclusions:

Characterization of APUs in Ecuador and in Ecuadorian Amazon Region clarify the importance of the studies of several agricultural systems that can be implemented in the region. Our results highlight the chakra as a model for harmonic production in the forest and focus that the diversified chakra is an efficient alternative system for indigenous and settlers families at Ecuadorian Amazon Region.

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Designing, cloning and amplification of pDream2.1/MCS/CII-6 recombinant plasmid which includes a mexican scorpion *Centruroides limpidus limpidus* CII-6 gene

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Abstract:
Biologically active molecules present in the venom of several species of scorpion, have shown potential against various diseases, including cancer. It has been reported that several toxins can block ion channels present in the membrane of several cancer cell lines, through action potential, altering their cell function, cell cycle arresting, and inducing apoptosis death pathway. The use of

the sequence of Cll-6 gene present in the genome of the Mexican scorpion *Centruroides limpidus limpidus* encoding a beta-toxin-locker of Na + channels by action potentials is proposed. This molecule has not been evaluated to determine if it has potential as an anti-cancer agent. In this paper the *in silico* design of recombinant molecule pDream2.1/MCS/CII-6, including the CII-6 gene into the polylinker site and identification by molecular biology techniques. Our results confirm a fragment of 316 base pairs, by digestion with BamH1 and Kpn1 enzymes, PCR, and the sequenced of amplicon, the alignment with CII-6 sequence reported in the GenBank database, showed 99.6% similarity and identity. The recombinant plasmid, could be used to assess potential as anti-having cancer agent on several cancer cell lines.

Keywords: CII-6 toxin; *Centruroides limpidus limpidus*; pDream2.1/MCS; Cloning; Transformation; *Escherichia coli*; canal ionic; cancer

Mol2Net YouTube channel*: http://bit.do/mol2net-tube*
YouTube link: *please, paste here the link to your personal YouTube video, if any.*

1. Introduction

Studies with different venom toxins scorpions have shown anticancer property, to inhibit the cell proliferation, arrest the cell cycle and induce death cancer by apoptotic on several cancer cell lines (Diaz *et al.,* 2010); (Ilhem *et al.,* 2011); (Yue-Jun *et al.,* 2011, 2007); (Kievit *et al.,* 2010); (Fan *et al.,* 2010); (Gupta *et al.,* 2010): (D'Suze *et al.,* 2010); (Deshane *et al.,* 2003); (Soroceanu *et al.,* 1998, 1999).

It has been reported that the growth and invasion of several cancers is associated with dysregulation of ion channels (Le Gueneec *et al*., 2007), a necessary mechanism that requires Na+, K+ and Cl- ions to facilitate growth and invasion of a tumor cell (Mao *et al*., 2008), therefore it is suggested that, related toxins to ion channels in the membrane cell can be used to treat certain types of cancer (Mamelak *et al*., 2007)

A potential resource is the *Centruroides limpidus limpidus* scorpion species (CII), the second in importance in Mexico by medical reports of scorpionism (Ponce and Francke, 2004). The components of the scorpion venom are molecules physiologically active, the most important are toxins that interact selectively and specifically with Na^+, K^+ (Rodriguez de la Vega and Possani, 2004; 2005), $Ca2^+$ and Cl^- ion channel, presents in the cell membrane of mammalian, insect and crustaceans.

CII-6 is a beta-toxin of 85 aminoacids, with a molecular mass of 9,323 Daltons, CII-6 belongs to the superfamily of scorpion toxins long chain (4 C-C), inhibitors of Na^+ ion channels, which is expressed in high concentrations and secreted in the venom gland of this scorpion, this toxin binds independently of the voltage on the site-4 of Na^+ ion channel (NAV), and changes the voltage activation to more negative potentials, affecting the activation of Na^+ ion channel and promoting spontaneous and repetitive shots (Coronas and Possani, 2002; Uniprot, 2016).

To date, not been explored the CII-6 molecule as anti-cancer agent, however, there are reports of toxins from the venom of different scorpions with affinity to Na^+ ionic channels, can inhibit, control and interact with various stages of metastatic cascade, as has been evidenced in several aggressive for its high levels of expression carcinomas (Gellet *et al*., 2009).

In the present work, is reported the *in silico* design of recombinant molecule pDream2.1/MCS/CII-6 containing the CII-6 sequence, present in the genome of *C. l. limpidus*, as well as their cloning, restriction enzyme digestion, amplification by PCR and sequencing.

2. Results

A schematic figure of pDream2.1/MCS/CII-6 is shown, is an expression vector of 7475 bp including the sequence of the gene CII-6 of 316 bp of *C. l. limpidus*, this sequence is flanked by sites for restriction enzymes EcoRI and Kpn1 (Figure 1).

The integrity of pDream2.1/MCS/CII-6 as DNA, is shown in figure 2B, meanwhile, enzymatic digestion of the recombinant molecule pDream2.1/MCS/CII-6 with enzymes EcoRI and KpnI shown a fragment of approximately 316 bp (Figure 2A).

The Chain Reaction of Polymerase (PCR) carried out, shown in electrophoresis, an amplified sequence of approximately 316 bp (Figure 3) and correspond to the size of the sequence for the CII-6 gene reported in GenBank with access number AF491132.

The amplicon sequenced and aligned with the sequence reported in the database GenBANK corresponding to the CII-6 gene of *Centruroides limpidus limpidus* encoding for a beta-toxin, Na+ channel modifier. Both show 99.6% of identity and similarity (Figure 4)

This molecule, may be used to test effect on several cancer cell lines, which is the very purpose of the investigation. It is noteworthy that the experiments were already started and show promising results.

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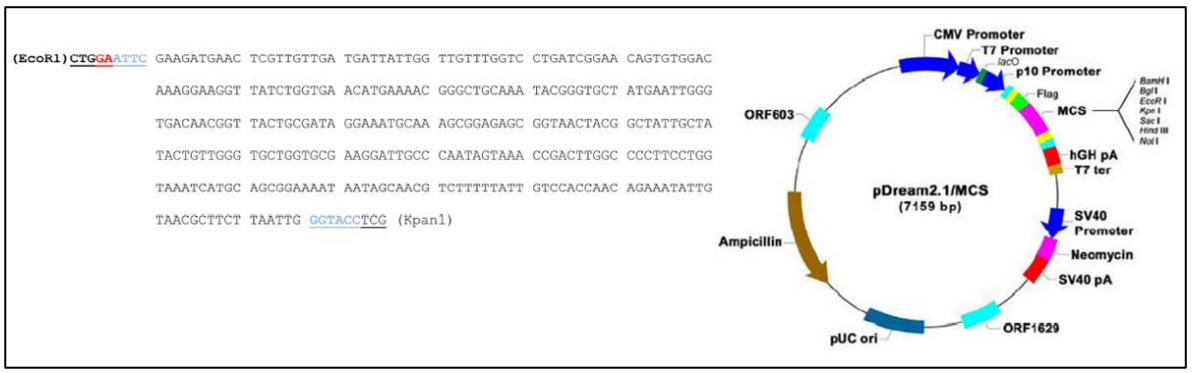

Figure 1. Design of pDream2.1/MCS/CII-6, as from the expression vector pDream2.1/MCS and sequence of CII-6 gene of *C. l. limpidus.*

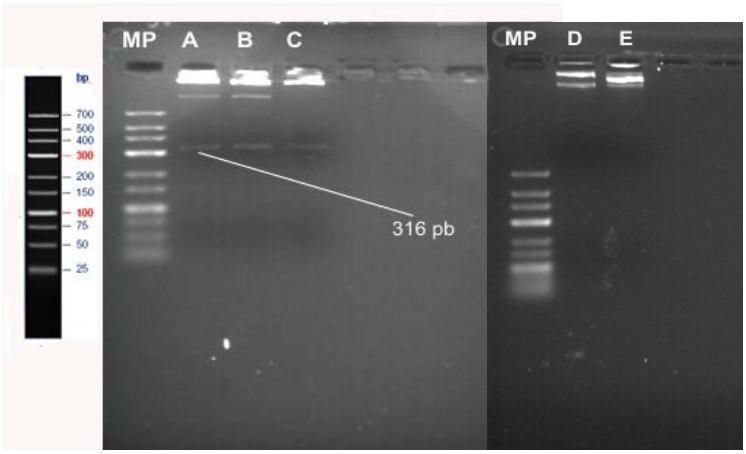

Figure 2: Electrophoresis showing a fragment of 316 bp, corresponding to the sequence of the gene CII-6 post-cloning in E. coli DH5a strain; MP: Marker weight (25-700 bp); A, B and C: Digestion of plasmid pDream2.1/MCS/CII-6 with restriction enzymes EcoR1 and Kpn1 of; D and E: plasmid DNA.

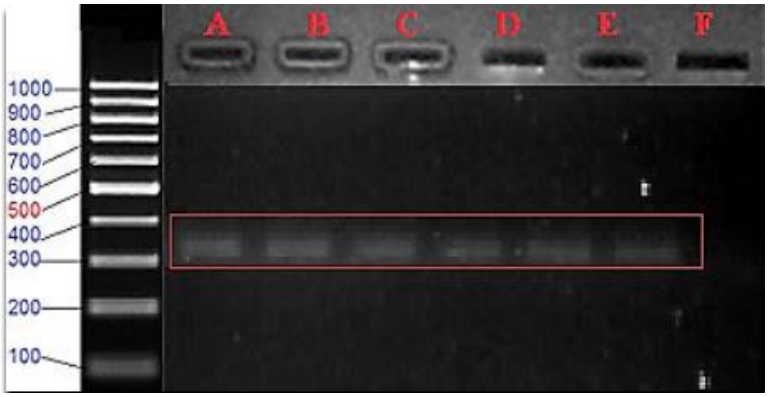

Figure 3. Electrophoresis showing amplicon of approximately 316 bp. A: molecular weight marker (100-1000 bp); B, C, D, E, F amplicons of CII-6; F: nuclease-free water.

```
########################################
# Program: water
# Rundate: Wed 30 Dec 2015 20:35:45
# Commandline: water
#    -auto
#    -stdout
#    -asequence emboss_water-I20151230-203543-0991-98371257-es.asequence
#    -bsequence emboss_water-I20151230-203543-0991-98371257-es.bsequence
#    -datafile EDNAFULL
#    -gapopen 10.0
#    -gapextend 0.5
#    -aformat3 pair
#    -snucleotide1
#    -snucleotide2
# Align_format: pair
# Report_file: stdout
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 250
# Identity:     249/250 (99.6%)
# Similarity:   249/250 (99.6%)
# Gaps:           0/250 ( 0.0%)
# Score: 1241.0
#
#
#=======================================

EMBOSS_001         1 GAAGATGAACTCGTTGTTGATGATTATTGGTTGTTTGGTCCTGATCGGAA
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001         1 GAAGATGAACTCGTTGTTGATGATTATTGGTTGTTTGGTCCTGATCGGAA

EMBOSS_001        51 CAGTGTGGACAAAGGAAGGTTATCTGGTGAACATGAAAACGGGCTGCAAA
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001        51 CAGTGTGGACAAAGGAAGGTTATCTGGTGAACATGAAAACGGGCTGCAAA
```

Figure 4. Alignment of sequence CII-6 and sequence GenScript by EMBOSS software.

3. Materials and Methods

Design of pDream2.1/MCS/CII-6

Was designed *in silico* the recombinant molecule pDream2.1/MCS/CII-6, incorporating into the multicloning site of commercial expression vector pDream2.1/MCS (GenScript, USA), the sequence of CII-6 gene (GenBank : AF491132) flanked by two sites for restriction enzyme EcoR1 and Kpn1, it was sent to company GenScript, for synthesis. The product freeze-dried was resuspended in 200 µl of nuclease-free water at 42 ° C.

Preparation of competent cells

Bacteria frozen Escherichia coli strain DH5-α was inoculated into 20 ml of LB medium (Luria-Bertani, Sigma SLBB9243) without antibiotic and incubated for 16 hours at 37 ° C with constant stirring (200 rpm), later, 1 ml of bacterial growth was cultured in a Falcon tube of 50 ml (Axygen Scientific, Cat. 431621) containing 10 ml of LB medium and incubated for 4 hours at 37 ° C with stirring, and was measured optical density in a spectrophotometer (Epoch: 1209284) at 450 nm.

The bacterial culture was transferred to two 50 ml Falcon tubes prechilled and centrifuged at 3000 rpm x 10 min at 0 ° C, the supernatant was decanted, and the pellet was resuspended in 1 ml of cold CaCl2 (0.1M), the tubes were centrifuged at 3000 rpm x 10 min at 0 ° C. Subsequently was added again 1 ml of cold CaCl2 (0.1M) and aliquoted into sterile vials precooled (Sambrook and Fritsch, 1989).

Transformation of competent cells by heat shock

Was added 1 µl of recombinant plasmid pDream2.1/MCS/CII-6 to one vial of competent cells and it was incubated for 30 minutes on ice placed, immediately in water bath at 42 ° C for 45 seconds, finally in ice for 2 minutes. Was added 900 ml of LB medium and was incubated for 1 hour at 30 ° C with stirring (220 rpm) (Sambrook and Fritsch, 1989).

Cloning and replication of Escherichia coli strain DH5-α/pDream2.1/MCS/CII-6

30 µl of cells transformed, were grown in dishes LB-agar with ampicillin (100 mg/ml, PentrexilR Bristol-Myers Squibb) and incubated at 37 ° C for 16 hours (Sambrook and Fritsch, 1989). A colony from plate with transformed bacteria growing, was inoculated into 5 ml of LB medium with ampicillin and incubated at 37 ° C for 16 hours with constant stirring.

Extraction and purification of pDream2.1/MCS/CII-6

The extraction of plasmid DNA was performed with the kit GeneJET Plasmid Miniprep (Thermo Scientific Cat. # K0502) following the manufacturer's instructions. Briefly, Escherichia coli strain DH5-α/pDream2.1/MCS/IIC-6 after 16 hours in culture, was centrifuged at 3000 rpm for 10 minutes and the supernatant was discarded, the pellet was suspended with 250 µl of the solution resuspension and transferred to a sterile vial, then, were added 250 µl of Lysis Solution and mixed by inversion 5 times, after, were added 350 µl of Neutralizer Solution and again mixed by inversion. The tube was centrifuged at 13,500 rpm for 5 minutes and the supernatant was transferred to a GeneJET column, and centrifuged at 12,500 rpm for 1 minute, the filtrate was discarded, subsequently, 2 washed were performed, with 500 µl of Wash Solution and centrifuged for 1 minute at 13,500 rpm. The filtrate was discarded and one additional step of drying by centrifuging for 1 minute was performed. Finally, the column was transferred to a new microcentrifuge tube and 50 µl Elution Buffer was added, allowed to incubate for 5 minutes at room temperature and centrifuged for 2 minutes at 14,000 rpm. The extraction yield of DNA was quantified in spectrophotometer (Nanodrop Quawell 5000) at 600 nm.

Enzymatic digestion and electrophoresis

Double digestion of pDream2.1/MCS/CII-6 was performed, mixing 12.8 µl of nuclease-free water (Cat. R0581 Thermo Scientific), 2 µl of MµLTI-

CORE Buffer (Cat. R999A, PROMEGA), 0.5 µl of restriction enzyme EcoR1 (Cat. R601A, Promega), 0.5 µl of restriction enzyme Kpn1 (Cat. R634A, Promega) and 4 µl of plasmid DNA, and incubated for 1 hour at 37 ° C, later, the fragment was visualized by electrophoresis (Sambrook and Fritsch, 1989).

PCR of pDream2.1/MCS/CII-6

For amplification of the gene CII-6, primers were used: CII-6/F 5'CTTCTACTTGAGCAACAACTA3' and CII-6/R: 3'CAATTAAGAAGCGTTACAATA5' a Master Mix as follows: 12.5 µl of PCR Master Mix (Promega, Cat M7502), 10 µM of each primer (10 µM), 5 µl of DNA and 4.5 µl of nuclease-free water. The reaction was performed in a thermocycler (Techne TC-512 USA) with the following parameters: Initial denaturation: 95 ° 5 min; 95 ° C for 30 seconds; 35 cycles at 40 ° C for 30 seconds; initial extension at 74 ° C for 30 seconds and final extension at 74 ° C for 5 minutes, finally cooling at 4 ° C for 5 minutes. The amplicons were visualized by electrophoresis.

Sequencing of amplicons (CII-6)

The PCR products were sequenced by the Sanger method by the company Genscript (Genscript Corporation, Piscatway, NJ, USA;) the received sequence was aligned with MEGA program (6.06) with the sequence CII-6 gen of the scorpion *Centruroides limpidus limpidus* reported in GenBank.

4. Conclusions

The design and assembly of pDream2.1/MCS/CII-6, were performed correctly, was verified a fragment of 316 bp approximately by digested with restriction enzymes after cloning. PCR and sequencing of amplicons, confirm the insert of CII-6 gen of *C. l. limpidus*, such molecule therefore can be used for various experiments.

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Author Contributions

JMECO**:** Design of pDream2.1/MCS/CII-6, write; MAEG: cloning and transformation; MCRA: Extraction of DNA, Enzymatic digestion; EGD: PCR and Sequence analysis; ABP: Design of pDream2.1/MCS/CLL-6, Suggestions and article revision; JCVC: Suggestions and article revision.

Conflicts of Interest

The authors declare no conflict of interest

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Design and Evaluation of cruzipain gene using *Saccharomyces cerevisiae* as a vaccine vector against *Trypanosoma cruzi* experimental infection

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Abstract:
Chagas disease, or American trypanosomiasis, is caused by *Trypanosoma cruzi*, a hemoflagellate Trypanosomatidae family parasite, widely distributed in the American tropics and subtropics. Cruzipain (Cz) is a crucial parasitic proteases belong to the papain-like CA C1 family and have close structural mammalian homologues and considered to be a good vaccine candidate for Chagas disease. Cz full gene sequence was obtained from GenBank Acc. No. AY099317.1 and redesigned in silico, sequences were sent to be synthesized and cloned into Sc expression plasmids (pYES-2, Invitrogen) in Genscript Company (USA). pYES-CZ recombinant plasmid was transformed into DH5α *E.coli* strain for its selection and production. Plasmidic DNA was extracted and transfected to INVSc1 *Saccharomyces cerevisiae* strain, then cultivated and selected through ura3-52 deficient medium. Finally, transfected Sc were evaluated as a potential vaccine against Chagas disease in 8 weeks female BALB/c mice. Results showed that modified cruzipain gene construct was successfully stable during its transfection in bacteria then in Sc, moreover amplification results showed a unified linear DNA with the desired size. Data showed that transfected recombinant Sc elevates lymphocyte responses and gives remarkable results. Lastly Sc and Cz are promising vaccine candidates against *T. cruzi* experimental infection, and further investigation would be required for establishing a strong therapy for the population at risk of the infection.

Keywords: Cruzipain, *Saccharomyces cerevisiae*, Chagas disease, recombinant vaccine.

1. Introduction

Chagas disease or American trypanosomiasis, is caused by *Trypanosoma cruzi*, a hemoflagellate parasite, widely distributed in the American tropics and subtropics, including over 50% of the Mexican territory. There are different prevalence estimates of the disease in Latin America. On the other hand, it is estimated that 18 million people are infected and 100 million at risk of infection in endemic areas of the Americas (WHO, 2008).

Chemotherapeutic treatments such as nifurtimox and benznidazole, have been partially successful in controlling infected patients and are currently the only option to reduce the parasite load and disease severity during the acute infection (Venegas, *et al.*,1997). However, the use of these agents is limited by the drug high toxicity, low therapeutic efficacy, long treatment duration, its high cost and limited availability (Urbina, 2002; Molina *et al.*, 2000).

Until now No vaccines are currently available. Several investigators have documented that protective immune mechanisms against *T. cruzi* are constituted of a strong lytic antibody response, cytotoxic T lymphocytes activity, and Th1 cytokines (Franchin, 1997), Towards identifying the potential vaccine candidates, Immunological studies in mice infected with the parasite in host cell secreted proteins were capable of entering the class I and class II pathways of antigen presentation and eliciting antibody and T cell responses, and this protein is considered to be the best choice for making vaccine proteins of *T. cruzi* have been identified, and their immunogenic potential examined in mice. Many of the selected antigenic targets provided variable degree of resistance to *T. cruzi* as DNA or protein vaccine *T. cruzi* antigen TcG2, TcG4 and other antigens, (Gupta *et al.*, 2010) which give us the attitude to complete what they have begun by using the cruzipain gene in a new vector the *Saccharomyces cerevisiae* (Sc) yeast.

Moreover, (Cazorla *et al.*, 2008) reported a series of combinations in vaccine treatment involving the use cruzipain gene vector alone or used as a strain of *Salmonella typhimuriumaroA* cruzipain gene and found that this treatment was much more effective.

Sc has been used primarily as a bioreactor of the recombinant proteins expression from pathogenic bacterial and viral microorganisms' infections, which have been able to promote a protective immune response against these agents. The heat-killed Sc, complete or derivatives thereof has been used as a vector vaccine for preventing bacterial or viral infections

(Stubbs *et al.*, 2001, Shin *et al.;* 2007 Ardiani *et al.*, 2010).

Among the benefits offered by yeast are a vaccine vector that a) can express more than one antigen, b) economical large-scale production, c) it expresses cell surface ligands which are seen as danger signals by the body, which leading to maturation of dendritic cells without the need for additional adjuvants, d) presents antigens through both major histo-compatibility complexes (MHCI and MHCII) e) lacks neutralized immune response by the host, so it can give reliably reinforcement vaccine (Liu *et al.*, 2001; Edwards *et al.*, 2002; Ardiani *et al.*, 2010).

Immune response produced by Sc gather the cellular and humoral and therefore is an excellent candidate vaccine vector for the production of a vaccine against *T. cruzi*.

2. Results and Discussion

Cruzipain gene was successfully designed and modificated, we were able to transform select the gene to bacterial cells with a high efficiency (80% of total colonies) and was verified with enzymatic digestion using BamH1 and NoT1 restriction enzymes, agarose gel showed two bands (14 kb for the gene construct and 5.9 kb for the pYES2 plasmid) figure 1, moreover

conventional PCR was realized using specific primers for cruzipain gene (data not shown). A minor challenge was found during the transfection of pYES2-Cz to Sc competent cells, a small transformation process was detected (2-3 colonies appeared after selection in a deficient ura3 medium. As a result, the expression of the gene of interest was also low using the indicated induction medium (Easy comp kit protocol, Invitrogen, USA) figure 2. Which indicate that the translation process was with low efficiency, the problem may be in the gene design and the promotor selection which do not permit a high protein expression or may be the Sc strain rejects somehow the plasmid transfection or the integration of the plasmid with the gene of interest to the whole Sc genome. That results hindered the proper extraction of the expressed cruzipain gene (Ashty S. Karim *et al.*, 2013) .

Interestingly, recombinant Sc showed a proper elevation of the mice immune system by elevating the specific lymphocyte to cruzipain gene which was expected as found in a previous research

(Cazorla *et al.*, 2008). Lymphocyte proliferation assay showed a higher response to recombinant Sc group versus control group which explains that Sc is capable of introducing the gene of interest to the antigen presenting cells which is explained with the mechanism of Sc for presenting its proteins on the outer cell membrane so that they would be easily recognized, that's considered to be one of the benefits of using pYES2 plasmid. Finally, Sc also permits a good expression process due to its self-antigenic characteristic and probiotic effects.

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Figure 1. 0.7% agarose gel showing plasmid and cruzipain gene after digestion

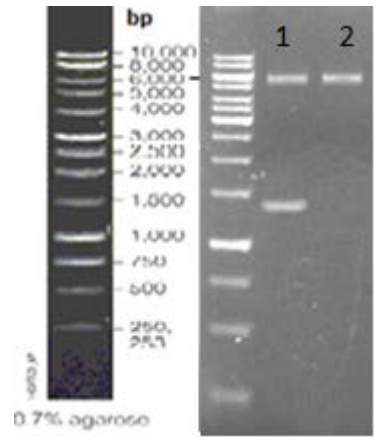


Figure 1: Shows 0.7% agarose gel with 1kb gene lader in the primer lane. Lane No.1 shows digestion of pYES2-Cz plasmid into two bands with 5.9kb for pYES" plasmid and 1.4kb for cruzipain gene of interest.

Figure 2. Poly acrylamide gel showing cruzipain protein expression.

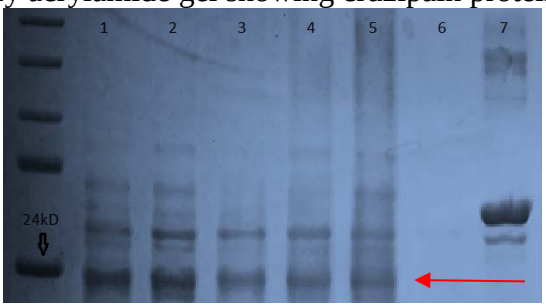
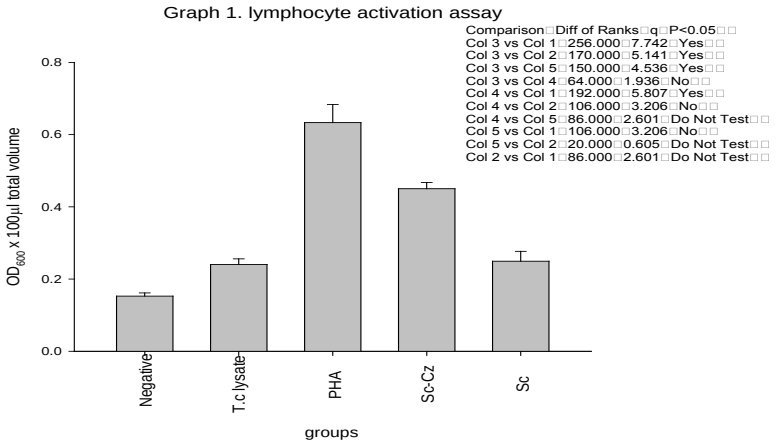


Figure 2: Showing poly acrylamide gel with cruzipain protein expression, lane (1-5): showing Sc proteins with a slight expression of Cz gene at 24kD size shown by the red arrow at different lysate concentration, lane (6): shows negative control, lane (7): shows positive control (BSA).

Graph 1. Lymphocyte proliferation assay for Sc-Cz and Sc mice groups



Gragh 1: Shows lymphocyte activation to recombinant Sc-Cz antigen, negative (medium alone), T.c lysate (*T. cruzi* parasite lysate), PHA (Phytohaemaglutinin, is used to stimulate mitotic division of lymphocytes), Sc-Cz (recombinant Sc with the gene of interest), Sc (empty Sc without the gene of interest). The statistical analysis showed a significant difference between treatment groups vs control $p<0.05$.

3. Materials and Methods

The studies will be conducted in facilities CEISA Autonomous University of Mexico State and operated under international standards and bioethics commission and FMVZ Animal Welfare.

Vaccine production

The gene sequence obtained from Gene Bank, sequence will be modified by removing the start codon, and adding the sequence 5'-TAAAATGTCTCATCACCATCACCATCAC-3. It contains a region AAAATGTCT allowing translation initiation (Invitrogen protocols). Also contains the N-terminal His6 tag CATCACCATCACCATCAC encoding a polyhistidine which will facilitate the purification of the recombinant protein. Designed in silico sequences will be sent to synthesize and cloned into expression plasmids Sc (YES-2, Invitrogen) in Genscript Company (USA), and the plasmid will be created Sc pYes-Cz.

For recombinant protein production will be used Saccharomyces cerevisiae expression vector pYES2 (Invitrogen), which allows for high levels of expression by galactose induction.

For plasmid Sc-pYES2-cz transformation of the will be used competent cells of INVSC1 strain auxotrophic for uracil (Ura-) according instructions of EasyComp kit (Invitrogen).

Animals

Experimental groups: 4 groups of 8 weeks old female BALB/c mice are formed with 12 animals each Group; 1- gp-cont (saline), 2- rProt (pure recombinant protein 25µg antigen, 3- Scra (combination Sc active recombinant protein),4- Sca (not active recombinant Sc).

Serology

Serum will be extracted from blood samples obtained by cardiac puncture (at the time of sacrificing the animal) and immediately processed by centrifugation (10 min at 800g) the serum is stored at -20°C until analysis.

Clinical evaluation.

Mice will be examined routinely every 2 days during the first 30 and then weekly until the end of the experiment.

Lymphocyte proliferation analysis

Peripheral blood mononuclear cells (PBMCs) were separated directly from mice spleenocytes using Histopaque®-1077 sterile-filtered, density: 1.077 g/mL (Sigma), (Garg et. al., 2010) then the PBMCs were collected, washed and cultivated in RPMI-1640 medium (Product. No. R0883, Sigma). With 10% Fetal Bovine Serum. The 96 plates were prepared with antigens which corresponds to the vaccine, Reagent Grade Phytohaemagglutinin is used to stimulate mitotic division of lymphocytes maintained in cell culture and facilitate cytogenetic studies of chromosomes was used as a positive control, finally saline

solution is used as a negative control for all experimental analysis. The results were statistically analyzed and graphed depending on the optical density obtained from the cultivated lymphocytes at 495 nm using EPOCH microplate reader.

Statistical analysis

Data are expressed as means with SEM (standard error of the mean), and derived from at least triplicate observations per sample (n=6 animals/group). Results were analyzed for significant differences using one way analysis of variance ANOVA procedures, and presented as $p \leq 0.05$.

4. Conclusions

Recombinant Sc-cz could be a good vaccine candidate for future investigation.

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Author Contributions

M in Sc. WHHM and Ph.D. JCVC. drafting the article and analysis and interpretation of data, Ph.D. ABP and Ph.D. ETB substantial contributions to conception and design, acquisition of data; Ph.D. JCVC final approval of the version to be published.

Conflicts of Interest

"The authors declare no conflict of interest".

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EVALUATION OF ANIMAL WELFARE DURING TRANSPORT OF SHEEP FOR SLAUGHTER.

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During transport, loading and unloading sheep are subjected to stressors that affect their welfare and the quality of the meat, in addition to producing significant economic losses to the producers. Objective: To evaluate the animal welfare practices during the terrestrial transport of sheep for slaughter. The information collection was carried out by means of a (transport stress survey on animal welfare of sheep). Demographic variables, animal management and vehicle design conditions, transport risks, type of animals, such as feeding, perception of animal welfare were evaluated. The transport of sheep by people aged between 29 and 48 years (63.30%), the drivers own the vehicle for the transport of sheep (63.30%). Drivers do not suffer from any chronic illness (86.7%) because they cannot perform their activity. The drivers do not monitor the animals' physical conditions during the trip, and they use them with methods such as electrical current (16.70), sticks (8.30%), shouts (58.30%), and other methods (15.00%) which do not guarantee animal welfare. In the transportation of passengers (45.0%); Causes of the accident (55.56%), shock (40.74) and mechanical failures (3.70), the accidents occurred every day (33.33%) and night (66.67%). The transport has a mortality of (1-2%) and the conditions of the vehicles comply partially with the sanitary requirements. Conclusions the improvement of sheep transport infrastructure in the study area, the training of drivers, and the promotion of animal welfare practices in all chain users and the development of research in the area are required.

Keywords: welfare, electrical current, mortality,

1. Introduction

Animal welfare (BA) has become an important attribute in the concept of sensory and ethical quality of food of animal origin, and an issue of interest in international trade in sheep meat, because of its importance and contribution to the Animal health and livestock productivity, which is why the World Organization for Animal Health (1), establishes principles of well-being in its legislation, thus reaffirming animal health as a component of BA (2,3). A growing concern on the part of consumers who claim that the animals are produced throughout the agri-food chain (from the farm to the profit) under acceptable welfare standards and managed in a humane way (2,4). In South America, the

implementation of BA practices in meat production chains is not a generalized priority due to socio-economic and cultural situations; However, the main meat exporting countries: Brazil, Argentina, Uruguay and Chile, have found in the BA a differentiating element for the commercialization of their products and as an opportunity to include these aspects in their programs of safety assurance and in Health legislation (4).

Mexico has a sheep marketing system, which requires the transfer of the animals from the production areas to the profit centers, being necessary to travel great distances and to face different geographical conditions, which implies times of transport and of prolonged fasting, factors that generate Stress and have an impact on the quality of meat (4,5). Stress is considered to be an indicator of BA loss; Its appearance is related to the change in the physiological behavior of some hormonal bioindicators such as cortisol, and blood variables such as glucose, creatine phosphokinase and urea, among others (5-7).

Transport, embarkation and disembarkation are stages that generate high levels of stress in the sheep, causing economic losses related to seizures by contusions of different grades, animal mortality, low yield of the carcass and alteration of the organoleptic variables of the meat, among others Aspects (8).

It has been suggested that the attitude and training of animal handlers, as well as the characteristics of facilities and vehicles, are directly associated with BA loss (4,5). BA is closely related to food safety, meat quality and economic losses in the sheep meat chain (6). Stress during pre-slaughter (stages from loading on the farm to desensitization and bleeding) favors the migration of intestinal microorganisms to the deep muscle mass and subsequent microbial growth; (Eg E. coli O157: H7), and through cross-contamination of the meat during the benefit, increases the biological risk for the consumer (7,8).

Poor transport can reduce 1.5 to 9% of live weight, increase risk of fall, death and bruising, increase in economic losses due to the elimination of blunt tissue, lower carcass yield and decrease in the classification category Of the channels, among others (2,6). Exhausted and stressed animals provide a meat called "dark" cut or DFD (Dark, firm, dry) meat, which because of their organoleptic and physicochemical characteristics, are unattractive to consumers, reduce their useful life, favor microbial growth, limit The possibilities of export and are not apt to pack them in the vacuum (9,10).

Given the importance of BA and its implications on meat quality, this article aims to discuss the stress factors during transport, loading, unloading, and their relationship to the quality of the sheep meat.

2. Results and Discussion

The transport of sheep is carried out by people aged between 29 and 48 years (63.30%), the drivers own the vehicle for the transport of sheep (63.30%). The drivers do not suffer from

a chronic disease (86.7%) for which they can not perform their activity. The drivers do not monitor the physical conditions of the animals during the trip, and they use methods such as electrical current (16.70), sticks (8.30%), shouts (58.30%), and other methods (15.00%). Do not guarantee animal welfare (Table 1).

The vehicles used to transport animals are varied. The service is not specialized and the infrastructure of the vehicles partially complies with the sanitary requirements, since they only have 1-2% mortality of transported sheep (Table 2). Type of lesions present to sheep when transported is: fractures 16.00%, hematomas 20% and dehydration 64%.

The use of a truck of a certain size will depend on the type of cattle to be transported, the specific market demand, the length of the usual routes and the geographical regions where it will operate. In the national cattle truck inventory of the United States of America and Canada, 30% are single trucks, 45% are trucks with semi-trailers and the remaining 25%, trailers with trailer.15

In Europe, the most commonly used trucks are single trucks and semi-trailer trucks.18 In Australia, however, trucks are usually trailers, which in some cases even have three trailers because of the long distances they take on highways predominantly straight lines.

There are three key elements in driving quality: skill, style and attitudes. The skills the ability of a driver to control the vehicle, for example during the change of direction or when braking with a motor. The age of drivers influences driving styles: young people (18-33 years) are often more reckless, and those over 55 are more distracted due to chronic diseases related to the craft.17

The widespread use of horse radish (16.7%) can generate stress and pain in cattle, as well as injuries in muscular masses and loss of quality of the canal, aspects that produce economic losses by seizures and decrease in the commercial value of the carcass , In the profit plant (4,6).

The widespread use of electric elements for mobilization of livestock is presented in the same way in Spain, and it is suggested that it may be probably the result of poor facilities for loading and unloading, and the lack of knowledge about BA practices.).

The duration of the trip is one of the most discussed topics in terms of animal welfare, because it is assumed that long distances affect the physiological and behavioral status of animals, which is why the European Community.18

3. Materials and Methods

Collection of information

The study was carried out between June and September 2016 in Capulhuac, State of Mexico, where 40 to 60 thousand sheep are slaughtered from Querétaro, Guanajuato, Jalisco, San Luis Potosí, Zacatecas, Chihuahua, Coahuila, and from the same State from Mexico.

The data collected in relation to the displacements have been, origin and destination of the trip, mode of transport used, duration, number of stages, number of sheep transported, risk e in the transport and handling of the animals. Along with this, a series of data characterizing the introducers (age, level of studies, occupation) have been obtained. How important is it to introducers the animal welfare of the sheep when being transported?

It was applied to 60 surveys, with a willingness to participate through informed consent.

Statistic analysis

The descriptive analysis of the variables was performed with the spss program version 21.0.
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4. Conclusions

It is concluded that in the study area, the adoption of infra structure is required that allows an optimal handling of the animals during the trip, as well as the training of the transporters, the promotion of BA practices in all users of the meat chain Sheep and the development of investigations that evaluate the impact of the BA on the quality and safety of the meat, as well as the economic losses due to the lack of its implementation.

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Table 1. Demographic characteristics and attitudes of drivers on the welfare of animals during transport to the municipality of Capulhuac, State of Mexico evaluated, 2016

Variables		n	%
Drivers	Owner	38	63.3
	Employee	14	23.3
	Partner	8	13.3
Age	18-28	8	13.30
	29-38	17	28.30
	39-48	21	35.00
	49-58	10	16.70
	59 >	4	6.70
Years of experience handling the livestock truck	1-3	11	18.30
	4-6	6	10.00
	7-10	6	10.00
	10 > age	37	61.70
Suffer from chronic illness	Yes	8	13.3
	Do not	52	86.7
Optimal Intervals in Vehicle-Based Transport	Transport< 90	24	40.00
	90 <= Carry< 300	22	36.7
	300 <= Carries	14	23.3

Table 2. Accidents in transport, causes, mortality and injuries, regarding the welfare of the animals during the transport to the municipality of Capulhuac, State of Mexico evaluated, 2016

Variables		n	%
Has suffered an accident in the transport of animals	Yes	27	45.00
	No	33	55.00
Causes of Accident	Turn	15	55.56
	Shock	11	40.74
	Mechanical failure	1	3.70
Time of the accident	Day	9	33.33
	Night	18	66.67
In the most significant accident that happened to the surviving and injured animals?	Re-transported	8	33.33
	Abandonment	4	16.67
	Empty	12	50.00
Does the transport have any mortality?	Yes	41	68.30
	No	18	30.00
Sheep are injured when transported	Yes	25	41.70
	No	30	50.00
What kind of injuries do sheep have when they are transported?	Fractures	4	16.00
	Hematomas	5	20.00
	Exhaustion	16	64.00
Methods to mobilize sheep during loading and unloading when transported	Electrical current	10	16.70
	Sticks	5	8.30
	Screams	35	58.30
	Other	9	15.00

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Identification molecular and clinical characterization of CPV-2c in dogs from the state of Mexico.

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Abstract: Canine parvovirus type 2 (CPV-2) is the main etiological agent of viral enteritis in dogs. Actually in literature, CPV-2 has been reported with clinical signs that vary from the classical disease. In this study, we evaluated the clinical signs presented in 50 dogs infected naturally with CPV-2. All the infected dogs were analyzed by PCR and sequenced. Our data indicate that the CPV-2c is the most frequently genovariant in Mexico the 50 dogs belong to the CPV-2c, through the amino acid change 426 Asn-Glu and concerning clinical signs, the presence of either vomiting or enlarged lymph node was observed in all virally infected patients.

Keywords: Canine Parvovirus, Clinical signs, PCR

1. Introduction

CPV-2 is the main etiological agent of viral gastroenteritis in dogs. It is a member of the *Parvoviridae* family, belonging to the *Protoparvovirus* genus and *Protoparvovirus type 1* species. It is a non-enveloped virus with a single stranded DNA genome, which encodes for two capsid proteins, VP1 and VP2, required for the assembly and packaging of the viral genome, as well as for NS1 and NS2 nonstructural proteins, which aid in controlling DNA replication, assembly and regulation of genes expression [11].

Over the past years, CPV-2 has developed new antigenic variants. In 1980, CPV-2 original strain was replaced by the variant designated type 2a (CPV-2a), in 1984, CPV-2b was identified [10], and in 2001, CPV-2c was detected and reported in Italy. The last variant has also been identified in Asia, Africa and America. The first reports seemed to account for a low pathogenicity of CPV-2c, experimental data and field observations now indicate a more severe clinical course and higher mortality rates associated with CPV-2c infection, as well as its ability to infect and cause disease in adult dogs, even if repeatedly vaccinated [4].

The aim of this study was to identify genetically isolated of CPV-2c present in Mexico through the molecular characterization of segment of VP2 gene and characterize clinically infected patients.

2. Results and Discussion

The 100% of dogs belong to the CPV-2c genovariant, through the amino acid change 426 Asn-Glu, 96% were pure bred; 88% of the patients were between one and seven months old, and 12% were older than one year. Concerning their vaccination status, 58% were vaccinated at least once to prevent CPV-2 infection.

Frequency of clinical signs showed by these dogs was as follows: 72% displayed vomiting and diarrhea (catarrhal or hemorrhagic); 14% showed only diarrhea; 7 displayed only vomiting; Leukopenia was observed in 46% of the dogs (Table 1). The analysis of the clinical signs performed through the use

of a chi-squared distribution and the model of logistic regression showed the relationship between PCR and 14 independent variables. The adjusted model equation is:

PCR = -618.314 - 16.6157*VAC - 0.246372*CF + 2.66763*BF + 16.8336*T - 7.93061*AP - 2.14908*LEUK + 1.63731*ED - 53.6041*VOM=0 - 12.9519*DIA=0 - 56.9484*LN=0 - 14.6088*MUC=0 + 57.7868*AP=0 + 17.458*CFT=0 + 38.704*RC=0

As the P-value of the Deviation Analysis is less than 0.05, there is a statistically significant relationship between the variables, with a confidence level of 95.0%. In addition, the P-value for residues is greater than or equal to 0.05, indicating that the model is not significantly worse than the best possible model for these data with a confidence level of 95.0% or greater.

Gastroenteritis caused by CPV-2 is considered one of the main viral diseases that affect dogs. Although clinical signs of canine parvovirus infection may vary, the most common signs reported were: anorexia, depression, lethargy, fever [6,8], mucoid and hemorrhagic diarrhea and leukopenia [1,3,9]; in subclinical cases, some of these signs may or may not be present [5,7]. Clinical variability for this disease has been reported previously [2,5] and some authors have discussed factors, such as age, immune status, exposure route, viral dose, virulence of strains and co-infection with other infectious agents as possible causes [2,7]. Concerning clinical signs, the presence of either vomiting or diarrhea was observed in all virally infected patients, while other clinical signs were not considered relevant to the infection.

3. Materials and Methods

Dogs with clinical enteritis hospitalized in the Veterinary Hospital for Small Animals of the Universidad Autónoma de Estado de México, were screened for this study and selected based on tested positive or negative CPV-2 using PCR. As a result, 50 dogs that tested positive were selected. The 50 dogs were clinically examined by veterinarians to obtain the clinical diagnosis and Information regarding age, breed, vaccination status and clinical outcome of disease were recorded for all dogs.

Dog's stool samples were obtained using rectal swabs, which were suspended in nuclease-free water and 200 µ*l* of the homogenates, and were used for DNA extraction. The procedure was performed

using the QIAamp® DNA Stool DNA extraction kit (QIAGEN, Mainz, Germany), following the manufacturer's instructions. All DNA samples were quantified using a Q5000 Quawell spectrophotometer (Quawell Technology, Inc. San Jose, CA, U.S.A). 100 *ng* of DNA of each sample were used for PCR reactions with 50 µ*l* of final volume. Previously, a pair of primers was designed in our laboratory to amplify a 275 bp fragment, ParvoInt2FB (5'-TCAAGCAGATGGTGATCCAAG-3') and ParvoInt2CR (5'-GGTACATTATTTAATGCAGTTA-3') located at nucleotides 1,107-1,130 and 1,360-1,382 of the VP2 gene (GenBank accession number FJ0051962c).

PCR reactions were performed using 2 µ*l* of each primer (200 *n*M), 12.5 µ*l* of GoTaq® Green Master Mix (Promega, Madison, WI, U.S.A) containing DNA polymerase, reaction Buffer (pH 8.5) and 400 µM of each nucleotide (dATP, dGTP, Dctp and dTTP); 3 mM of $MgCl_2$. and 28.5 µ*l* of nuclease free water. All reactions were carried out under the following amplification conditions; 1 cycle at 94˚C for 5 min for initial denaturation, followed by 35 cycles at 94˚C for 30 sec, 52˚C for 1 min, 72˚C for 1 min and a final extension cycle at 72˚C for 5 min.

All the amplification products were identified through horizontal electrophoresis in 2% agarose gels stained with 0.5 μg/m*l* of ethidium bromide and visualized with a UV transilluminator. Subsequently 25 µl of PCR product were submitted to Macrogen USA to be purified by ExoSAP-IT® (Affymetrix) and sequenced by BigDye® v3.1 Life Technologies, Applied Biosystems. All sequences were aligned using the Software MEGA 6.0 (Tamura et al., 2007). The analysis of the clinical signs was performed through the use of a chi-squared distribution and the model of logistic regression.

4. Conclusion

Our data indicate that the CPV-2c is the most frequently genovariant in our Mexican dogs and concerning clinical signs, the presence of vomiting and enlarged lymph node was observed in all virally infected patients, while other clinical signs were not considered relevant to the infection.

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Conflicts of Interest

The authors declare no conflict of interest.

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Table 1. Clinical signs and characteristics presented by the patients (50 dogs tested positive to CPV-2 PCR).

N°	Age	Sex	Vaccines	CF	RC	CFT	BF	AP	Mucous	T°	LN	Diarrhea	Vomiting	Leukocytes $6x10^3/\mu L$
1	10 m	F	0	100	-	> 2 Sec	28	Pain	Pink	38.5	N	-	+	29.9
2	1 y	F	4	120	-	> 2 Sec	30	Pain	Pink	38	↑	+	+	17.2
3	3 m	M	1	140	-	> 2 Sec	22	Pain	Pink	38.4	↑	+	+	8
4	5 y	M	4	120	-	> 2 Sec	40	Pain	Pink	39.3	N	+	-	19
5	7 m	M	3	112	-	> 2 Sec	45	Pain	Pink	38.8	↑	+	-	10.3
6	4 m	F	0	150	-	> 2 Sec	18	Pain	Pink	38.6	↑	+	+	8.1
7	10 m	F	3	100	-	> 2 Sec	44	Pain	Pink	39.2	N	+	-	9
8	4 m	M	1	120	-	> 2 Sec	33	Pain	Pink	39.3	↑	+	+	5.3
9	3 m	M	2	50	-	> 2 Sec	30	Pain	Pale	38.2	↑	+	+	9
10	2 m	M	0	120	-	> 2 Sec	20	Pain	Pale	37.1	↑	+	+	3
11	1 y	M	0	150	+	> 2 Sec	22	Pain	Pale	34.5	↑	-	+	18
12	1 m	M	0	140	-	> 2 Sec	36	Pain	Pink	38.4	N	+	+	13.4
13	5 m	F	2	160	-	> 2 Sec	44	Pain	Pink	39.6	↑	+	+	10.2
14	5 m	M	2	120	-	> 3 Sec	20	Pain	Pink	40	N	+	+	3
15	1 m	F	0	100	+	> 2 Sec	30	Pain	Pink	38.7	N	+	+	11.1
16	5 m	F	1	124	-	> 3 Sec	64	Pain	Pink	40.8	N	+	+	4
17	5 m	M	0	92	-	> 2 Sec	20	Pain	Pale	38.8	↑	+	+	3.1
18	3 m	F	0	136	-	> 3 Sec	20	Pain	Pale	38.8	N	+	+	6.1
19	1 y	M	2	140	-	> 3 Sec	20	Pain	Pale	39.6	N	+	+	1.2
20	5 m	F	3	110	-	> 3 Sec	20	Pain	Pale	38.7	↑	-	+	8.2
21	2 m	F	2	160	-	> 2 Sec	20	Pain	Pink	38	↑	+	+	4.1
22	2 m	M	2	180	-	> 3 Sec	30	Pain	Pink	37.9	↑	-	+	4.2
23	4 m	M	0	204	-	> 3 Sec	24	Pain	Pink	38.8	↑	+	+	2.2
24	3 m	M	1	200	-	> 3 Sec	30	Pain	Pink	39.5	N	+	+	2.9
25	2 m	F	1	110	-	> 3 Sec	20	Pain	Pink	37.1	↑	+	-	23.6
26	3 m	F	0	138	-	> 2 Sec	22	Pain	Pale	38.1	↑	+	+	2.7
27	4 m	M	1	176	-	> 2 Sec	28	Pain	Pale	37.8	↑	+	+	0.95
28	3 m	M	0	132	-	> 2 Sec	56	Pain	Pinks	39.9	↑	+	+	1.2
29	3 m	M	0	120	-	> 2 Sec	30	Pain	Pinks	38.6	N	+	+	3.4
30	2 y	M	0	170	-	> 3 Sec	28	Pain	Pinks	38.3	↑	+	+	3.4
31	7 m	F	3	146	-	> 2 Sec	28	Pain	Pinks	38.6	↑	+	-	3.2
32	4 m	M	0	140	-	> 2 Sec	20	Pain	Pale	36.4	N	-	+	3.7
33	2 años	F	4	146	-	> 2 Sec	26	Pain	Pink	37.8	↑	+	+	24
34	4 m	M	0	126	-	> 2 Sec	20	Pain	Pink	40.3	↑	+	+	1.2
35	5 m	M	0	160	-	> 2 Sec	28	Pain	Pink	37.7	N	+	+	2
36	5 m	M	1	160	-	> 3 Sec	24	Pain	Pale	37.7	↑	+	+	3.3
37	3 m	M	3	140	-	> 2 Sec	20	Pain	Pale	38.4	↑	+	+	9
38	4 m	M	0	140	-	> 2 Sec	24	Pain	Pink	39.6	↑	+	+	8.2
39	3m	M	3	120	-	> 3 Sec	26	Pain	Pale	38.8	↑	+	+	14.9
40	4 m	F	2	100	-	> 2 Sec	24	Pain	Pink	39.2	N	+	+	6.2
41	5 m	M	0	127	-	> 2 Sec	28	Pain	Pink	38.1	N	+	+	11.9
42	4 m	F	0	216	-	> 2 Sec	28	Pain	Pale	38.1	↑	+	+	16.1
43	2 m	M	2	140	-	> 2 Sec	32	Pain	Pale	38.7	N	+	-	14.2
44	3 m	M	0	100	-	> 2 Sec	46	Pain	Pale	38.5	↑	+	+	3.5

45	4 m	M	1	140	-	> 3 Sec	40	Pain	Pale	39.5	↑	+	+	5.8
46	4 m	F	3	128	-	> 2 Sec	18	Pain	Pale	37.6	↑	+	+	1.3
47	3 m	M	1	142	-	> 2 Sec	25	Pain	Pink	39.8	↑	+	-	6.5
48	3 m	F	0	180	-	> 2 Sec	24	Pain	Pale	37.4	↑	-	+	4.2
49	4 m	F	3	180	-	> 2 Sec	38	Pain	Pink	40.1	↑	-	+	7.9
50	7 m	M	4	187	-	> 2 Sec	26	Pain	Pale	38.9	↑	+	+	5.3

(m) months; (y) years; (F) Female; (M) Male; (CF) Cardiac Frequency; (RC) Reflex cough; (+) Positive or present during the study; (-) Negative or absent during the study; (CFT) Capillary filling time; (BF) Breathing frequency; (AP) Abdominal palpation; (T°) Temperature °C; (LN) Lymph node; (N)Normal; (↑) Increased in size.

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The milk industry seen from the farms of producers in the Ecuadorian Amazon.
La industria de la leche vista desde la finca de los productores ecuatorianos en la amazonía

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Abstract:
The food industry is important in Ecuador ’s Amazon Region, so it is necessary to characterize the processes that intervene in the quality and quantity of milk production. A survey was carried out, in which 82 milk producing farms and 35 variables related to the processes of feeding, reproduction, production and animal health were analyzed using an analysis of Principal Components. This analysis showed that the efficiency of the production of milk is affected by three factors that influence 71% of the variance explained in the system and that relate variables to the productive and reproductive processes. The first component related to the variables, number of cows, calves, heifers, total births, births rate, load capacity of system, explains the 47.53% of the accumulated variance, the second component inferred in the variables service period, calving-calving, and age at incorporation into reproduction. It is concluded that if production of milk in quantity and quality is desired for the industry, attention must be paid to processes related to production and reproduction on the farms of producers.

Keywords: Keywords: Milk production, Prnicipal Components, industry, farms

Mol2Net YouTube channel*: http://bit.do/mol2net-tube*
YouTube link: *please, paste here the link to your personal YouTube video, if any.*

1. Introduction

In recent years, the trend towards increased milk production for the industry is around 30%, which generates a promotion and consolidation of the dairy industry, according to (Rocha y Rocha, 2014; Chimarro, 2016) the industry has exported to Venezuela and is looking for new Spaces for the commercialization of the product in Central America and Russia. The largest milk production in the equator

is concentrated in the Sierra. However, the Amazon has a positive trend in the development of industry and milk production, which already reaches 8%.

The knowledge of man in animal breeding and the application of technologies, play an important role in achieving milk production in quantity and quality, so that production is subject to different processes that occur on farms of producers governed mainly by Management of the herd, feeding, genetics and animal health, all linked to the process of industrialization of this food. For the reasons explained above the objective of this work is to characterize the factors that are influencing the production of the raw material for the milk industry and its commercialization.

2. Results and Discussion

The milk production as a raw material for the development and consolidation of dairy production in the Amazon has important challenges to depend on the management, reproduction, genetics and health processes of the animals; that occur in the producers, where technical knowledge are not achieve to increase quantity and quality milk production.

Table 1 shows the factors that affect the milk production efficiency studied in 82 farms in the eastern Amazon region. The 35 variables analyzed, only 12 related to the herd structure, reproduction and births conformation processes and the area devoted to the cattle breeding and the totality of mass were selected as the main exponents. The Principal Components analysis showed that there are three important factors in the milk production efficiency and that explain the 71.75 of the accumulated variance, the first factor is related to the efficiency in the herd and births structure, where it stands out that a good herd structure is important to guarantee replacement and production. Authors such as Herrera, Barrios and Flores (2013) and Grijalva (2014) considers that birth rates above 89% have a positive impact on milk production, although this can be adjusted by understanding and achieving quality in the reproductive process.

In component second it find the reproductive variables that present the greatest variability, among which is the inter-part period and the period of service with a factor of weight of 0.77 in both, is related to the management system where breeding prolongs the Weaning up to 12 months. The calf exerts a negative sensory effect to the reincorporation of the reproductive activity, increasing the service periods and inter-partal, coupled to the feeding system that does not supply the energetic-protein requirements, the females diminish their somatic and corporal development upon incorporation. Results in the reproduction were similar to indicate by Orantes-Zebadúa et al. (2014)

Component three grouped the variables total area the farm and total of animals with weight factor of 0.68 and 0.64 respectively. These variables present great variability, considering that there are family farms from 5 ha considered small to medium and large that can reach 233 ha with slopes from 7 to 80%.

Similar behavior presents the animals number in the herd and its structure. That does not depend in many cases on the size of the farm since in general the number of animals only reached 102 achieving in many cases that the capacity of the system is underutilized and in others overpower it, because of the large amounts of land incompatible with livestock activity. Therefore, these indicators are important to take into account to correct design the farm production system.

Nader (2011) point out that one of the most important factors that influence the definition of the typology of the farms is undoubtedly the size of the farm and the herd, on which production alternatives depend heavily.

Table 1. Factors that affecting milk production Efficiency for industry.

Component	Related variables	Weigh factor	Own value Cumulative	Cumulative variance explained, %
Efficiency in the structure of the herd and births	Cows, heads	0,94	4,50	47,53
	Calves, heads	0,83		
	Heifers, heads	0,78		
	First calving cows, heads	0,82		
	Animal load, UGM.ha-1	0,94		
	Total deliveries in the year	0,87		
	Birth rate, %	0,90		
Efficiency in the reproduction process	Period of service, days	0,77	1,53	66,94
	Inter-part period, days	0,77		
	Age of incorporation into reproduction, months	0,80		
Size of farm and herd	Total area, %	0,68	1,39	71,75
	Total animals, heads	0,64		

3. Materials and Methods

The province of Napo was a reference for the milk production from the Ecuadorian Amazon. For this purpose, 82 milk farms were evaluated where 60% of the farms have Holstein and Jersey breeds the rest of the breeding of these breeds and the Brows swiss. A survey was applied in which 35 variables related to the feeding, reproductive, health processes, application of technologies in the farms were measured. The Multivariate Analysis of Principal Components was applied to decant the variables of these processes that are influencing the obtaining of the raw material for the milk industry. We used the SPSS program version. 19

Conclusions

The factors that affect milk production for the industry are expressed in three components that explain the 71.75% of the accumulated variable and are related in 12 variables that represent the Farms efficiency processes in the structure of the herd, birth, reproduction and size.

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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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Low productivity and quality of the primary link of the cattle production chain as an input for the industry in the Ecuadorian Amazon Region.

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Abstract:
The objective of the study was to evaluate the main causes that affect the low productivity and quality of the primary link of the cattle production chain as an input for the food transformation industry and related to other destinations in the Ecuadorian Amazon region (RAE). Questionnaire were conducted with 399 variables from each of the Amazonian provinces to evaluate behavior in the productive, environmental, economic and social dimensions of livestock systems and animal behavior of the different breeds. The results were analyzed once the livestock systems were typified in each productive purpose, we analyzed the discrete variables that affect the behavior of the systems, related to the productive dimensions (individual milk production and birth rate) and ANAVA for breeding breeds more frequent in each of the provinces. The composition of the systems in the RAE showed that the most frequent breeds are Criollo (35.9) and mestizos without records (63.3%) with a specialized breed deficit that means that the agroindustrial sector is almost non-existent. The Norman races; Holstein and Brown Swiss present births of 82.47; 78.19 and 66.47% respectively, however they do not express their milk production potential due to the management system used. It is concluded that the link that presents the greatest vulnerability is the primary one (livestock farmers) mainly because in the RAE are producing with important productive deficiencies.

Keywords: amazon; Livestock efficiency; Breeds, bovine.

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1. Introduction

Although not contributing large amounts of milk and meat to the country, it does have one of its main agricultural activities, which means that a significant proportion of the population is engaged in the management of livestock systems Cattle. However, this represents large levels of advancement of the agricultural frontier, in addition to impacting the ecosystem (MAGAP, 2014 and Jarrín et al, 2016).

The pre-selection of the meat chain of standing cattle as the object of analysis responds to the priorities established at national level to tax food security. The country's economic and social policy raises the need to promote this area (GAP, 2012). It is for this reason that in the criteria evaluated for the scope of industrialization for deboned meats; Cuts of meat, sausages, pasteurized milk and its by-products, base the selection of the productive chain cattle standing and, at the same time of the products that derive from this line, that is why the objective of this work was to evaluate The main causes that affect the low productivity and quality of the primary link of the cattle production chain as an input for the food processing and related industries in the Ecuadorian Amazon Region.

2. Results and Discussion

Table 1 shows the composition of breeds of the first productive link of bovine cattle in the whole RAE, for which a deficit of specialized races is observed and the lack of registration of other races that implies that, the meat agroindustrial sector in the RAE is Almost non-existent, slaughtering purebred beef cattle only in order to supply the local market, similar results noted (Vargas et al, 2015). The product with higher added value (boneless meat) that can be traded at 5,0 USD / kilo does not take advantage of its potential. Therefore, the main vulnerability is the primary producers, mainly because in the RAE they are producing with cost structures above the price they receive per kilo of live meat (2,3 USD / kilo[1]), (Ríos and Benítez , 2015). Similar in behavior with producers who dedicate to milk production, farmers provide milk through intermediaries that carry the transport, the price they perceive ranges from 0,35 to 0,39 USD / liter.

The reproduction is considered the most important cattle-raising process in any livestock operation, since it defines the structure of the herd, the relative potential of production that is expected from the livestock system, the feeding program that must be established to obtain high and stable yields (Viamonte, 2010; Benitez et al.). Table 2 shows the races, individual milk yields and reproductive efficiency measured by the birth rate of the different races in the RAE, where it can be seen that the Norman races; Holstein and Brown Swiss present the highest birth rates, however they do not express their milk production potential due to the management system, the reproductive capacity of the herds is the last physiological priority of the animals, it is complemented when all the food and physiological requirements Are covered, hence the importance of maintaining the highest possible efficiency in this process in livestock systems.

The RAE cattle herding specialization has a clear trend. Morona Santiago is the province with the highest presence of cattle (43,8%) followed by Zamora Chinchipe (25,7%); Napo (9,7%) and Sucumbíos (9,58%). Pastaza participates only with 5,1% of the cattle population. In the province of Pastaza, the existing management systems are characterized by small farms, especially family farms.
Table 3 shows that RAE milk production represents only 7,9% of the milk produced at the national level. Morona Santiago is the province that produces the largest quantity of milk (36,3%), however, the yields of liters / cows are lower, due to non-milk specialties. Followed by Zamora Chinchipe (32,0%), however 46,5% of the production destined for the industry is processed by local companies and its products are marketed in stores mainly in the form of fresh cheeses, because of their low levels of production.

Ríos, (2016), referring to the destinies of milk have different behaviors: first, those provinces that allocate over 60% of their milk production for sale in liquid, in this group is Napo and Sucumbíos. Second, those provinces that allocate between 30 to 40% for sale in liquid, is the case of Pastaza and Orellana. Finally, the provinces that allocate less than 30% of the milk to be sold in liquid being processed in the relevant UPA, here are Morona Santiago and Zamora Chinchipe; these results demonstrate the need to industrialize dairy products derived from livestock production Bovine.

The production alternative, with rare exceptions, is based on grazing to tied up paw, where animals remain confined in a certain area, a small number of producers not specified, use electric fencing to control the animals, leaving one grazing area per animal Similar to that used in the tied up paw. As a complement to the pasture we use balanced or concentrated, which are supplied without following a rational pattern, varying the frequency of consumption from two to three times per week, to once a day. The supplement is supplied equally to all milked animals, regardless of the production potential of the animals, the state of pregnancy or duration of lactation, and the potentialities of the staple food. Rarely, shrubs, protein plants or legumes are used to supplement the diet of the herds. Vargas et al. (2000) reported that 78,76% of the total mineral salts were considered as a necessity, using popular formulas in the market, but without considering the soil limitations or the environmental characteristics of the Amazon. Factors that determine the productive

efficiency of livestock systems dedicated to the production of milk and meat in the RAE is the size of the herd in operation and the volume of production. Similar results showed Orantes et al. (2014), in the Chiapas region of Mexico in the bovine production system of DP, where their feeding is based on grazing, with a minimum of supplementation and limited to the seasonality of fodder in the dry season, affecting weight and commercial value of the price of milk and meat.

Table 1. Composition of breeds of the first productive link of cattle in the whole RAE.

Provinces	**Napo**	**Orellana**	**Pastaza**	**Sucumbíos**	**Morona Santiago**	**Orellana**	**RAE**	**%**
BREEDS OF CATTLE BOVINE, cbz	**Cbz**	**Cbz**	**Cbz**	**Cbz**	**Cbz**	**Cbz**		
Creole	29,154	21,281	4,133	22,841	57,126	53,184	**187,719**	35.9
Half-breed without registration	21,468	13,106	22,529	26,150	171,604	76,425	**331,282**	63.3
Half-breed With registration	130	139	82	386	255	367	**1,359**	0.3
Pure meat blood	23	937	*	52	11	101	**1,124**	0.2
Pure milk blood	70	328	*	41	21	150	**610**	0.1
Pure blood double purpose	139	151	*	122	186	449	**1,047**	0.2
TOTAL	50,984	35,942	26,820	49,591	229,205	130,677	**523,219**	100

[1]Average unit cost of production for beef cattle considering family labor estimated for this research, (Ríos, 2015).

Table 2. ANAVA results for the most frequent breeding breeds.

Breeding Breeds		**Brown Swiss**	**Holstein**	**Half-breed Charoláis**	**Half-breed Brown Swiss**	**Half-breed Holstein**	**Normando**	**Otros Half-breed**	**Sign**
Females in reproduction	$\overline{X}$	25,38	18,61	12,4	16,91	17,44	25,6	17,92	NS
	EE	3,62	1,97	6,47	3,09	2,26	6,47	4,01	
Milk yields, l/v/ day	$\overline{X}$	5,77	7,98	4,6	7,13	6,34	5,54	7,34	NS
	EE	0,97	0,53	1,73	0,82	0,60	1,73	1,07	
Birth rate,(%)	$\overline{X}$	$66,47^{abc}$	$78,19^{bc}$	$58,24^{a}$	$62,52^{ab}$	$66,87^{abc}$	$82,47^{c}$	$57,22^{a}$	*
	EE	0,41	0,22	0,73	0,35	0,25	0,73	0,45	

Table 3. Yields of milk production in the provinces of the RAE.

Provinces	**TOTAL**		**DAILY DAIRY PRODUCTION**		**Performance, liters. cows -1**
	UPAs	**Heads**	**Heads**	**Liters**	
Napo	2,394	50,984	7,764	36,476	4,7
Orellana	2,705	35,942	4,876	17,806	3,7
Pastaza	2,145	26,820	3,245	13,281	4,1
Sucumbíos	4,117	49,591	6,699	24,246	3,6
Morona Santiago	10,918	229,205	31,064	105,086	3,4
Zamora Chinchipe	6,725	130,677	22,742	92,655	4,1
RAE	29,004	523,219	76,390	289,550	3,8

3. Materials and Methods

The determination of the scope of the analysis of the beef chain in the Ecuadorian Amazon Region (RAE) was carried out from the database derived from surveys with 399 variables from each of the Amazonian provinces to evaluate the Behavior in the productive, environmental, economic and social dimensions of livestock systems and animal behavior. The data obtained during the sampling were tabulated in data matrices organized in Excel spreadsheets, where the cattle systems visited were located in the rows and the variables studied were in the columns. Given the size of the database, it was divided for productive purposes, the first with the information of the farms dedicated to the production of milk, the second corresponding to the herds dedicated to the breeding, most of which also feed part or the totality of the males they generate and the third to the herds specialized in the fattening of males or females for the slaughter that their animals acquire from what is available in the market. Once the livestock systems were typified in each productive purpose, we analyzed the discrete variables, which affect the behavior of the systems, related to the productive dimensions.

4. Conclusions

- The production costs of each link in the primary cattle production chain are not known.
- The link that presents the greatest vulnerability is the primary one (cattle ranchers), mainly because in the RAE they are producing with important productive deficiencies in the management of livestock systems and the non-use of breeds suitable to the Amazonian ecosystem.

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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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AMAZONIA, HEALTHY FOOD AND RURAL COMMUNITIES IN PASTAZA-ECUADOR

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Abstract: *The current situation and perspective of feeding with a focus on a healthy diet with probiotics in animals are evaluated in the Ecuadorian Amazon conditions. Surveys were conducted in the Ecuadorian Amazon communities to determine the current and prospective situation in organic and healthy foods production. In the Amazon region, diagnoses indicate that, despite the large amount of natural resources, an important part of this population is subject to problems of availability, economic access and use of food that affecting their food security. The levels of agricultural production, with benefit, are very low and agro industrial processes for getting products, through processing, are scarce. It was evaluated Lactobacillus salivarius and Bacillus subtilis probiotic cultures as probiotic in broilers. These probiotic got better many indicators in the animals. In Lactobacillus salivarius, coliformes count was higher in the groups without probiotics, while total anaerobes were higher in the animals with probiotic. Bag of fabricio and the spleen weight were greater in those treated with the probiotic. The Newcastle vaccine antibodies did show differences among treatments where probiotics were applied. Cecal content pH was lower in the treatments with probiotics. Total fatty acids showed higher values in broilers with the probiotics, however, the acetic, butyric and propionic acid did not provide differences between treatments. Bacillus subtilis show a higher total anaerobes count in treatments with the cultures, while the coliform count decreased in these one. Lactobacillus spp and endospores were superior in the treatments Bacillus subtilis.*

Keywords: *Functional feeding, indigenous peoples, probiotics, Lactobacillus salivarius, Bacillus subtilis.*

Introduction: *A food may be considered "functional" when it is demonstrated that, in addition to its nutritional effects, beneficially influences functions of the organism in a way that improves health or well-being or reduces the chronic degenerative disease risk [1].*

The main components of functional foods are intestinal microflora balancers (probiotics, prebiotics and symbiotics), antioxidanets and dietary fiber [2]. Probiotics are non-pathogenic microorganisms that, when ingested, exert a positive influence on the health or physiology of the host [3]. It proposed to define probiotics as "a preparation of a product containing viable, defined and sufficient microorganisms which alter the microflora in a host compartment and therefore exert beneficial effects on the host health" [4]. Antioxidants are compounds whose

primary function, in the body, is to protect against the oxidative damage caused by molecules known as free radicals. This oxidative damage is responsible for important degenerative diseases of the circulatory system, cardiovascular and cancer, which are today the leading cause of death in society [5]. Dietary fiber is part of a healthy or functional diet. There is still no single definition that encompasses the different dietary fiber components and its functions. The major factors of fiber are complex carbohydrates and lignin, although new products may be included in the fiber concept in the future. The objective of the present study was to evaluate, in the conditions of the Ecuadorian Amazon, the current situation and perspective of feeding with a focus on a healthy diet with results of the probiotics use in birds.

Materials and Methods:

Surveys were conducted in the Ecuadorian Amazon communities to determine the current and prospective situation in the organic and healthy foods production. In relation to the biopreparates evaluated in broilers with of Bacillus subtilis culture, 5 l of product with three cultures in modified media were prepared. The microbial count was performed according to the standards of Microbiology of Food for Human and Animal Consumption according NC-ISO and [6]. For the evaluation of the probiotic activity, 200 chickens from the female EB34 reproductive line of 1-42 days of age were used according to the methodology of [7] and to Bacillus subtilis and [8] to Lactobacillus salivarius. Microbiological, related to the immune response, fermentative and hematological indicators were studied in the broilers according to Technical Instructions for chicken feed (1998).

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Results and Discussion:

In the Amazon region, diagnoses indicate that, despite the availability of natural resources, an important part of this population is subject to problems of availability, economic access and use of food, affecting their food security. The levels of agricultural production are very low and the agroindustrial processes for the products production with benefit, through processing, are scarce.

Today there is an irrational and extractives use of biodiversity: Illegal woodcutting and trade in birds and other animals. Little is taken of the opportunities offered in various sectors (pharmaceutical, cosmetic, food and ecotourism) as a generation of fair income for families. In summary, the studies conclude that food security can be achieved in the Amazon, to the extent that increases in the availability of adequate food, in a sustained and permanent manner, through successful use of natural resources. Substantial increases in agricultural production and productivity; the local markets expansion, to expand demand and stimulate production, increasing in exportable production and the food conservation techniques development, at the family level.

Biopreparates evaluation with Lactobacillus salivarius in broilers.

Chickens used in the experiments showed the higher coliform population ($P < 0.01$) in the control group than in the treated animals, while the lactobacilli presented higher proportions ($P < 0.001$) in the groups treated with biopreparates respect to the control. For the total anaerobes counts, differences between treatments at 42 days ($P < 0.001$) were observed with an increase of these microorganisms in the animals where biopreparations were applied. Yeasts were observed at 42 days and their presence, but with no differences between treatments.

The immune system of the birds undergoes significant changes, which appear in the different stages of development. According to Giambrone (1996) in a first stage of life, the Fabricio bulsa and the thymus are presented as the main organs of immunity. Fabricio bag produces B-lymphocytes, which are responsible for humoral immunity (antibodies production by B lymphocytes) and the second, generates cell-mediated immunity (cytokines production and by T lymphocytes). With the use of Lactobacillus salivarius biopreparates, at 42 days, the effect of the biopreparates on the immunological indicators was superior. In the Fabricio bag and the spleen weight, no differences were observed in the animals treated with the biopreparates, but there was differences with the control group ($P < 0.01$). The HI titres for the Newcastle vaccine do not show differences between the treatments where the biopreparates were applied. However,

the data differed (P <0.001) with those of the control group, when higher values were obtained in the animals. Gut content pH was lower (P <0.05) in the treatments where the biopreparates were applied respect to the control group. Total CFAs showed higher values (P<0.05) in the broilers treated with the biopreparates in relation to the control; however, the values of acetic, butyric and propionic acid separately did not provide differences (P<0.05) between treatments. In the poultry digestive system, there is a relationship between the pH and the bacteria that are established, since an acidic pH inhibits growing of harmful bacteria. Newborn chicken maintains an almost sterile TGI, with ideal conditions for the of pathogenic bacteria proliferation; however, young birds do not have the capacity to produce enough hydrochloric acid to maintain acidic pH [9]. Therefore, the application of these beneficial bacteria, from the first hours of life, contributes to decrease the pH of the blind, due to the fermentative processes that these microorganisms develop in the ecosystem with the organic acids production [10].

Evaluation of biopreparates with Bacillus subtilis in broilers.

For total anaerobes, a higher count (P <0.001) was observed in broilers treatments with Bacillus subtilis cultures. Coliforms number in the treatments with Bacillus subtilis cultures decreased (P<0.001). Lactobacillus and endospores, at 42 days, had higher values (P <0.001) in treatments with Bacillus subtilis. The probiotic products application based on Bacillus spp. cultures, in sporulated form, as microbial balance is informed by [11]. Studies carried out by Rondon define one of the probiotic cultures actions from Bacillus spp endospores as the decrease in enteropathogens [8], caused by an increase in the population of Lactobacillus spp, the decrease of pH levels and the increase of levels of AGCC and lactic acid. Decrease in coliforms and the increase of the populations of total anaerobes and Lactobacillus spp is related to the action of the endospores and acetic and lactic acids that occur at the level of the intestinal tract. It is observed that the Fabricio bag, spleen and HI Titers for the vaccine response measured by the Newcastle vaccine are superior (P<0.001) in the treatments with Bacillus subtilis. Acetic acid and propionic acid levels (P<0.05) were higher for all treatments with Bacillus subtilis. It is important the role of these acids in the control of bacterial growth of intestinal enteropathogens [12]. In addition, a trophic effect on the intestinal epithelium is manifested, which favors the processes of digestion and nutrient absorption with a favorable response in the productive indicators of live weight and feed conversion [13]. However, butyric acid, which contributes with greater weight to the trophic effect [14], does not express differences between treatments, in any of the stages evaluated.

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Conclusions:

For the biopreparates with Lactobacillus salivarius the coliform population was higher in the groups without biopreparates, in total anaerobes differences between the treatments were observed with an increase of these microorganisms in the animals with biopreparates. With the use of biopreparates in the PR of the Fabricio bag and PR of the spleen, no differences were observed in the animals that were treated with the biopreparates, but with the control group, which showed lower values. The HI titres for the Newcastle vaccine did not show differences between the treatments where the biopreparates were applied, however, the data differed with those of the control group, when higher values were obtained in the treated animals. The pH of the cecal content was lower in the treatments where the biopreparates were applied with respect to the control group. Total CFAs showed higher values in broilers treated with biopreparates, however, acetic, butyric and propionic acid values separately did not provide differences between treatments.

In the case of biopreparates with Bacillus subtilis in total anaerobes, a higher counting was observed in the treatments with the cultures, the count of coliforms decreased in the treatments with the cultures. On the other hand, Lactobacillus and endospores, presented superior values in the treatments with the

cultures studied. The Fabricio bag, spleen, and HI titers for the vaccine response measured by the Newcastle vaccine are superior in Bacillus subtilis treatments. Higher AGCC values were found for treated chickens, while acetic and propionic acid levels (P <0.05) were higher for all bioprepared treatments..

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Nestedness between aphids and parasitoids populations in plants associated with an organic citrus grove

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Abstract: In the present study the nestedness between bipartite networks of plants, aphids and parasitoids species of an organic citrus grove located at La Selva del Camp (Tarragona, NE Spain) was determined to analyze the dynamics of the plant-aphid-parasitoid system and establish the possible reservoir of citrus aphids and their rates of parasitism. The results showed a low nested in plant-aphid assemblages, because the aphids are specialized forms to exploit certain types of plants, while the aphid-parasitoid assemblages were clearly nested because there are species of parasitoids generalists; such is the case of the parasitoid *Lysiphlebus testaceipes*, one of the most common and abundant on this type of crop, which interacts with various species of aphids. This parasitoid produced a high rate of parasitism in two species of aphids on two species of plants associated with citrus, *Dysaphis pyri* on *Pyrus communis* and *Sipha maydis* in *Avena barbata*. This positive nesting is also due to the heterogeneity of aphids attacked, because if there are few species of aphids, would lead to a competitive exclusion and therefore fewer species of parasitoids.

Keywords: bipartite networks; biological control; Lysiphlebus testaceipes

1. Introduction

The use of vegetation cover creates numerous benefits for agro-ecosystems such as an increased abundance of natural enemies, which limits the effect of some pest insects (Domínguez Gento et al., 2002). This is the case for aphids which can become very numerous on plantations

and can serve as an alternative food for several beneficiary insects (Bugg et al., 1990). These species are controlled by numerous parasites (Belliure et al., 2008), which greatly reduce populations in different crops, mainly citrus (Kavallieratos et al., 2004). On the other hand, the study of networks between different populations allows one to determine symmetrical or asymmetric relationships between them. One example is the bipartite networks that illustrate the connections between species from two distinct groups (Borgatti and Everett, 1997), which is very useful in the study of parasite-host interactions. A common pattern in these interaction networks is the nestedness that occurs when the specialist species interact only with the generalists, but the generalists interact also amongst themselves, giving rise to a marked asymmetry of the specificity of the interactions (Bascompte et al., 2003). Consequently, the aim of this study was to examine the plants associated with citrus cultivation in order to analyse the dynamics of the plant-aphid-parasite system through the use of bipartite nets and the degree of nestedness among the species involved, in order to determine possible aphid reservoirs characteristic of citrus fruits and their rates of parasitism.

2. Materials and Methods

Study Area

Sampling was carried out in the associated vegetation inside and outside an ecological citrus plantation in "La Selva del Camp" (Tarragona, Northeast Spain, 41° 13 '07' 'N, 1° 08' 35"E). The citrus plantation consists of about 300 clementine trees (*Citrus clementina* var. *clemenules*) grafted on Hybrid Citrange Carrizo [*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osb.]. The crop has met all organic agriculture standards since 2004.

Sampling of aphids and parasites and determination

Amongst the most abundant herbaceous species were individuals with colonies of aphids, both in the perimeter area and in the intertidal of the mandarin tree plantation. Colonies of aphids were also looked for in the existing fruit trees within the crop site. Finally, one selected vegetation from the periphery of the plantation that had aphids (up to 10 m). From each selected plant, the associated species and aphids were determined and their abundance was estimated using a semi-quantitative scale of 1-5, 6-25, 26-100, and >100 individuals per shoot, branch or leaf. In order to calculate the abundance, each of the previous density scales was considered taking 3, 15, 60, and 250 individuals respectively (Piñol et al., 2009). The parasite aphids (mummies) and empty mummies were then quantified to determine the rate of parasitism in each plant and in each tree (1 to 3 branches). This procedure was carried out in mid-June 2013for a duration of one week. Later parasite aphids were collected and stored in a nursery until their emergence. The aphids were identified in the field by taxonomic codes (Nieto-Nafría et al., 2003). The parasites were separated by morphotypes and sent to specialists for their determination.

Data analysis

First, the most abundant species of each community (plants, aphids and parasites) were quantified. The rate of parasitism associated with each plant species was estimated as the ratio between the number of mummified aphids and the total number of aphids (aphids plus mummies). Subsequently the degree of nestedness in these species was analysed, for which two qualitative bipartite networks (of presence-absence) were established between plants and aphids and between aphids and parasites. To calculate the degree of nestedness, a non-parametric, permutational statistical test was carried out with the BINMATNEST (binary matrix nestedness temperature calculator) programme (Rodríguez-Gironés and Santamaría, 2006).

3. Results and Discussion

Community of plants, aphids and parasites

A total of 22 plant species associated with mandarin cultivation were sampled. The most abundant herbaceous plants containing colonies of aphids were *Sonchus oleraceus* L., 1753 (44% of the branches selected from each plant had one or more species of aphids) and *Avena barbata*

Pott. Ex Link (19%). Regarding trees, the only ones that harboured aphid colonies were pomegranate trees (*Punica granatum* L., 1753) and this was the species containing the highest number of aphids (1,350 individuals (adults + nymphs)). There were 8,469 aphids in the total of selected plants, corresponding to 27 species. The most abundant species were *Aphis punicae* Passerini, 1863 (16% of individuals counted in all plants) and *Thelaxes suberi* Del Guercio, 1911 (14%) associated to the *Punica granatum* and *Quercus ilex* L. plants respectively. Depending on the time of year and their reproductive cycle, aphids select different species of plants to start or end their biological activities. The most common aphid species in the *Aphis spiraecola* and *A. gossypii* citrus plants (Belliure et al., 2008) were not very abundant in the accompanying plants (arable and arboreal) in the citrus plantation. It has been stated that these species prefer leguminous, cucurbitaceous, composite and other herbaceous plants as secondary hosts (Blackman and Eastop, 2006). In fact, four of these species were found in our samples but with very few individuals, perhaps because the vegetation existing during the sampling period was not attractive or appropriate to complete their cycles or to become secondary hosts, mainly for the *Aphis* Species. Of the 1,852 mummies controlled in the breeding chamber, a total of 468 parasites emerged, corresponding to 25 species and associated with 13 aphid species. The most abundant were *Lysiphlebus testaceipes* Cresson, 1880 (43% of the emerged parasites), a species with a very wide range of hosts in different cultures (Michelena et al., 1994). This was followed by *Adialytus ambiguus* Haliday, 1834 (18%). In fact, our results showed a not very high species richness in the three analysed communities, especially in terms of aphids and parasites.

Rates of parasitism

A total of 1,155 mummies distributed in 14 plant species were recorded. *Dysaphis pyri* Boyer de Fonscolombe, 1841, was the aphid species with the highest parasitism rate on *Pyrus communis* L. (100%), followed by *Sipha maydis* in *Avena barbata* (50.2%) (Table 1). The first species of aphid was parasitised by *Lysiphlebus testaceipes* and *Lysiphlebus* sp. whilst the second was parasitised by *L. testaceipes* and *Adialytus ambiguus*. Given that *L. testaceipes* is one of the most important primary citrus parasites, it can be concluded that this species easily attacks the aphids in any other host plant because the aphid - parasite relationship is almost independent of the plants on which colonies of aphids are found (Michelena et al., 1994). It is also known that *L. testaceipes* can withstand higher temperatures than other parasites (Belliure et al., 2008), a fact that would justify its abundance on the dates when the sampling was done.

Table 1. The highest parasitism rates (%) of aphids, whose host plants contained mummies in the field.

Plants	Aphid	% of parasitism
Pyrus communis	*Dysaphis pyri*	100
Avena barbata	*Sipha maydis*	50,2
Hedera hélix	*Aphis gossypii*	25,0
Carduus pycnocephalus	*Brachycaudus cardui*	21,1
Malus domestica	*Dysaphis plantaginea*	20,6

Nestedness of bipartite networks

The plant-aphid network was clearly non-nested (P> 0.05 for all null models) (Table 2). Consequently, one did not find any plants that had been attacked by many species of aphids or many generalist aphids, which attacked many plant species. Only the taxon *Spartium* sp. was affected by 4 species of aphids. Therefore, a dispersed interaction between the two communities is clearly present. This behaviour could be due in part to the non-random selection procedure of the host plants (Wright et al., 1998) and sampling time: in the month of June, aphidological populations begin to diminish or disappear.

On the other hand, few generalist species of aphids were detected, perhaps because most are associated with a certain type of plant; this factor is called "reciprocal specialisation" and is the possible cause of non-nested networks (Joppa et al., 2010) and statistically non-significant results (Bascompte et al., 2006).

The aphid-parasites network presented nestedness (P <0.001 in all cases and null models; Table 2). The most significant interaction was that of the *Hyperomyzus lactucae* L., 1758 aphid with 10 species of parasites, followed by *Brachycaudus cardui* with 8 species. Regarding the species of parasites, the most generalist was *Lysiphlebus testaceipes* that parasitised with 11 species of aphids and *Pachyneuron aphidis* with 4 species. This is perhaps due to the presence of generalist parasites that attack similarly to diverse aphids, which are affected by many parasites. This is what Lewinsohn et al. (2006) found for a set of generalist species that interact with other communities of generalists forming a dense network of interactions. In our case we believe that the heterogeneity of attacked aphids is the most important factor, because if there were few species of aphids, competitive exclusion would be produced and thus there should be fewer species of parasites.

Table 2. Nestedness temperature (T) of each studied matrix and its statistical significance (*P*)

MATRIX	*P*	Average T	Variance
MATRIX 1			
Null model 1	0.05	14.92	9.18
Null model 2	0.08	14.72	10.61
Null model 3	**0.07**	**14.69**	**9.29**
MATRIX 2			
Null model 1	< 0.001	14.56	8.19
Null model 2	0.003	9.95	8.35
Null model 3	**0.001**	**10.44**	**10.23**

4. Conclusions

The common aphids in *Aphis spiraecola* and *A. gossypii* citrus had a very low presence in the plants associated with the plantation, possibly because at the time of sampling the existing vegetation was not appropriate for them to complete their biological cycles.

The *Lysiphlebus testaceipes* parasite proved to be the most significant species for this type of crop because of the large number of its individuals and its high rate of parasitism.

The degree of nestedness of the aphid species and their parasites was positive, as opposed to the plant-aphid network, due to the presence of generalist species. This added to the heterogeneity of aphids attacked by the parasites.

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Conflicts of Interest

The authors declare no conflict of interest

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Design of an industrial process focused on the elaboration of cosmetics from Amazonian oils: a Biotrade opportunity.

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Abstract:
In Ecuador, the biodiversity offers a wide variety of plant species that, due to their characteristics, represent an alternative to produce cosmetics with high added value, environmental friendly and respecting Biotrade concepts.
The objective of this research was to design an industrial process in order to producing a cosmetic emulsion using as ingredient Amazonian vegetal oils, analyzing the mass and energy balance for a 10Kg turbo-emulsifier equipment and determining the capacity according to future productive development needs.

Keywords: Biodiversity, emulsions, Biotrade, mass and energy balance, capacity based on demand

1. Introduction

Ecuador is a privileged country in terms of biological diversity. It is included within the 17 megadiverse countries, considering that its territory covers only 0.2% of the Earth's land surface (1). Its biological richness is reflected in a variety of organisms, 18% of bird species, including 50% of those in South America, and 7% of amphibians (2). Within its surface is 7% of the vascular plant species that exist in the world, 11% of ferns, 20% of orchids (3) In addition to having a great biological wealth, it is important to emphasize that Ecuador is also a multi-cultural and multiethnic country, within it we find 14 nationalities and 10 of these are located in the Amazon Region (4).

Local biodiversity offers a huge range of natural products suitable for Biotrade activities (5), for example oils and plant extracts useful for skin care products. Plant extracts, due to antioxidant activity, are able to prevent or reduce oxidative damage. The bioactive compounds are usually flavonoids, polyphenolic compounds and

anthocyanidins, mainly (6). The design of technology for the manufacture of emulsions has been intensifying with the passage of time. Today we have what is known as vacuum turbo-emulsifiers; These equipment are suitable for all preparations which require temperature and vacuum parameters controlled for example in the manufacture of: creams, milks, gels, oils, ointments, make-up, detergents and emulsions, for the cosmetic, pharmaceutical, chemical or food industry. For this study, we was supported by the Production department of the Chankuap Foundation (7), which mission is to create Biotrade cosmetic and to support Shuar and Achuar communities developing Fair Trade projects. Finally, the objective of this research is to design an industrial process in order to producing a quality cosmetic emulsion based on the use of Amazonian vegetable oils and in compliance with the Biotrade concepts.

2. Results and Discussion.

2.1 Product and technology

An O/W cosmetic emulsion has been obtained as described in the Figure 1. Figure 1 shows the flowsheet of the emulsion production process. This diagram has fundamentally two parts, the lipid phase mixer and the aqueous phase mixer.

2.2 Emulsion flowsheet

Both mixers carried out different operations such as adding raw materials, heating, adding the rest of the raw materials and cooling. These operations are performed as shown in Figure 2.

2.3 Plant capacity estimation

The Chankuap foundation started its activities in 2005, initially had a production capacity of 448 kg/a. In 2007 it increased its capacity to 1120 kg/a due to the increase of the demand of the product. In 2016 demand increased to 4144 kg/a. With this information of the increase of the demand was projected what could be in 10 years the increase of the demand and the capacity of production from 2016.

Figure 3 shows the tendency to increase the demand for the product up to approximately 7504 kg in the year 2026. This value could be estimated using the expression: y = 336x - 673232.

The production capacity is 7504 kg/a (40 kg/d) and can ensure to the Chankuap Foundation the market demand needs for the established time period.

2.4 Mass and energy balance

According to the capacity (40 kg/d of emulsion) and following the order of the technological scheme (figure 2) the mass and energy balances were realized. The results are shown in Table 1. These are divided into indicators of raw material, water and energy consumption and dumping of solid, liquid and gaseous waste.

Water is the fundamental component of the formulation as can be seen in Table 1. It represents more than 70% of the consumption of raw materials. The energy consumed in the heating process is 0.124 kW.

2.5 Viable economic alternative

The income was calculated from the sales prices of the emulsion. For the determination of the Net Present Value (NPV) and the Internal Rate of Return (IIR), 10 useful years are taken from the Chankuap Foundation, increasing to USD 202795.46 and 12.9%, respectively, with Payback (PB) before of 2.0 years. Figure 4 shows the behavior of the NPV in the 10 years, as well as the IIR.

Table 1. Consumption and emissions indicators.

Indicators	Items	Amount	Unit
Raw material consumption	Cacao Butter	0,08	kg/kg product
	Ungurahua OIL	0,08	kg/kg product
	Emulsifying agent	0,08	kg/kg product
	Preservative	0,015	kg/kg product
	Thickener	0,007	kg/kg product
	Parfume/essential oil	0,002	kg/kg product
water consumption	Process	0,74	kg/kg product
	Cooling emultion process	0,11	kg/kg product
Energy consumption	Water phase heating	0,21	kJ/Kg product
	Oil phase heating	5,4	kJ/Kg product

Figure 1. Emulsion flowsheet

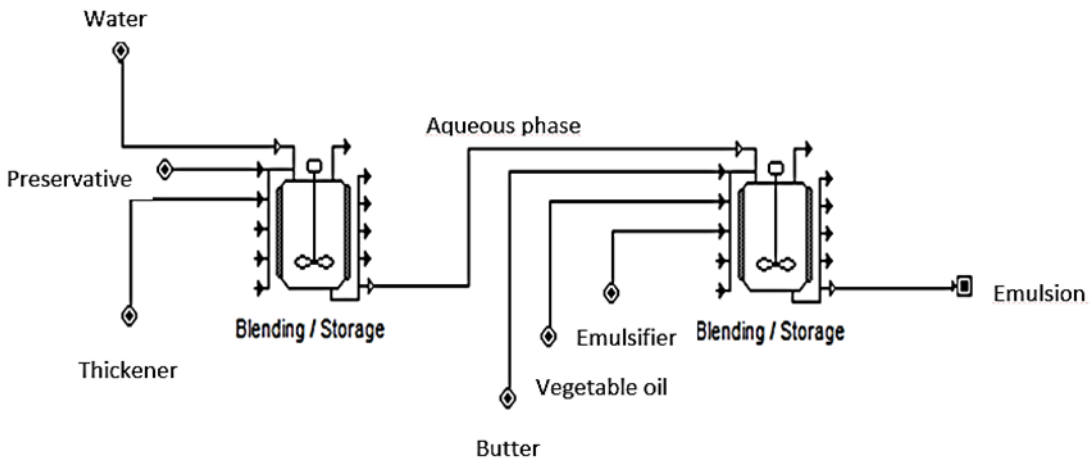


Figure 2. Technological procedure

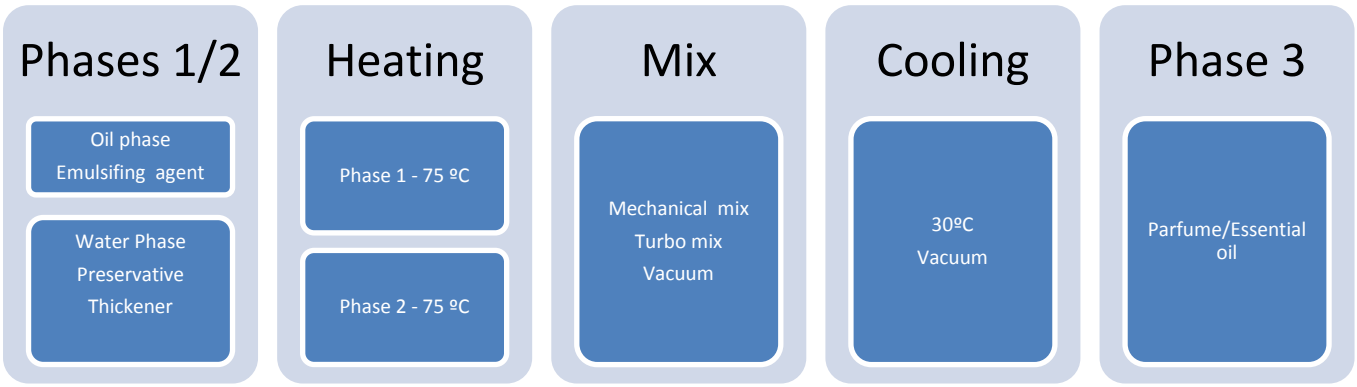


Figure 3. Plant capacity estimation

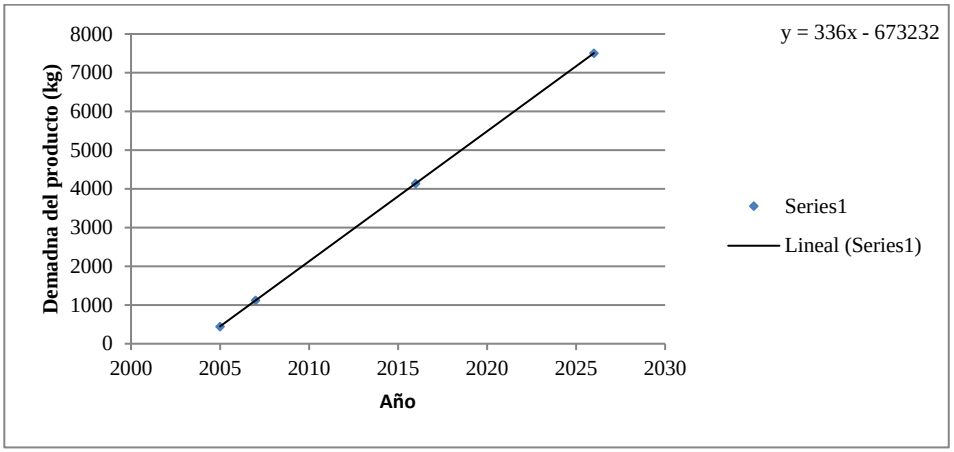
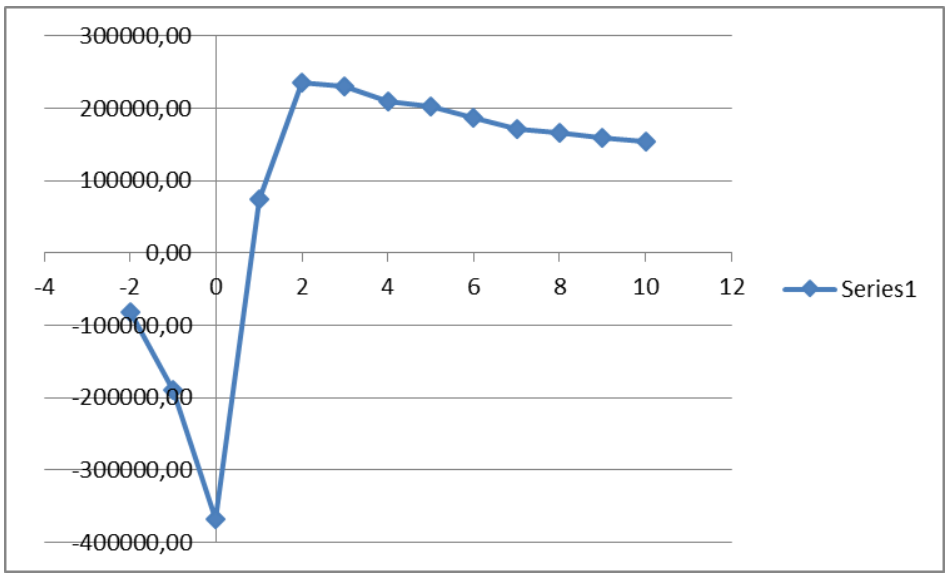


Figure 4. Economic indicators Fluctuation, NPV and PB



3. Materials and Methods

The procedure used in this research was proposed by Pérez-Martínez *et al*., (8). This procedure, although its utilization proposal is for the sugar cane industry and its derivatives can be used in other design scenarios. The method is based on the following steps: selection of technology, definition of the technological scheme, estimation of capacity and economic parameters, investment feasibility.

4. Conclusions

The local biodiversity offers a huge range of natural products suitable for Biotrade activities, Chankuap Foundation presents this kinds of alternative production as a business model for cosmetic market.

Finally, thanks to the dynamic investment indicators, VAN, TIR and PB, it has been possible to demonstrate favorable results regarding the analyzed cosmetic emulsion process.

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Conflicts of Interest

State any potential conflicts of interest here or "The authors declare no conflict of interest".

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ENVIRONMENTAL IMPACT OF LIVESTOCK SYSTEMS IN THE ECUADORIAN AMAZON

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Abstract.
Converting natural ecosystems into livestock agroecosystems often reduces soil organic carbon content by decreasing its supply source, as well as by increasing erosion losses and organic matter decomposition rate. In this sense, this study aimed to evaluate carbon sequestration and soil structure in livestock systems at Ecuadorian Amazon. The study was carried out in livestock areas of the Pastaza province, under rainforest and livestock systems with and without trees. We collected disturbed an undisturbed soil samples within depths from 0-10 and 10-30 cm. From these samples, we determined total organic carbon and some structural indexes, such as bulk density and soil porosity. The results suggest that the land use with forest, sequestered more total carbon in comparison with non-trees management, with an average value of 515 Mg C ha^{-1}. The silvopastoral systems stored total average from 55 to 103 Mg C ha^{-1}, which was affected by the number of trees. The structural indexes showed suitable values in all land uses, highlighting the role of the organic matter as an enhancer of the soil structural conditions, which favors aeration, root penetration and a greater rainwater uptake.

Keywords: Carbon sequestration; agroforestry systems; ecosystem services; structural indexes.

1. Introduction

The Ecuadorian Amazon region represents 45% of Ecuador territory. It is one of the most important biosphere reserves and provides a complex network of ecosystem services. In tropical areas, the extensive conversion of forests to pasture lands and the expansion of the agricultural frontier might be identified as the most important promoters of land use changes. These facts lead to consequent loss of environmental quality and biodiversity (Vallejo-Quintero, 2013). The unsustainable use of soils in deforested area at the Amazonian border, is one of the greatest threats to the rainforest. Among the causes of soils degradation in humid tropics are phosphorus (P) depletion, decrease of soil organic matter (SOM) and the loss of basic cations

(Ferreira Aguiara et al., 2016). This scenario shows the extreme fragility and vulnerability of soil resource to degradation, deforestation and the advance of the agricultural frontier in the Ecuadorian Amazon, which are problems requiring evaluation (Bravo et al., 2015). Agroforestry systems are believed to have a higher potential to sequester C than pastures or field crops. For example, the estimates of carbon sequestration potential in agroforestry systems are highly variable, ranging from 12 to 228 Mg ha^{-1} (Mutuo et al., 2005; Dixon ,1995) and for the different carbon compartments such as aboveground biomass (70 Mg ha^{-1}) and soil (25 Mg ha^{-1}) (Jadan et al., 2012). As can be expected, these values are a direct manifestation of the ecological production potential of the system, depending on a number of factors including site characteristics, land-use types, species involved, stand age, and management practices (Ramachandran et al., 2009). In this context, the objective of this work was to assess the environmental impact of land use change on carbon sequestration and soil structure in livestock systems in an experimental area of Pastaza province at Ecuadorian Amazon Region.

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2. Results and Discussion

Structural parameters and carbon sequestration under different land uses systems are showed in Table 1. The results suggest that the soil use with forest, sequester more total carbon in comparison with management without trees, with average values of 515 Mg C ha^{-1}. The silvopastoral systems stored an average from 55 to 103 Mg C ha^{-1}, which were affected by the number of trees. As expected, these values are concomitants with ecological production potential of the system, depending on a number of factors including: site features, land-use types, species involved, stand age and management practices (Ramachandran et al., 2009).

The structural indexes presented suitable values in all land uses, highlighting the role of the organic matter as an enhancer of the soil structural conditions, which favors aeration, root penetration and a greater rainwater uptake. We elucidate that land use changes with the adoption of agroforestry systems (AFSs). Proper management practices promote carbon sequestration in both, soil and biomass in Amazon region. Our results reinforce the role of good soils structural conditions as regulator of the ecosystem and its contribution to the mitigation of global climate change (Lal, 2008).

Table 1. Structural parameters and carbon sequestration under different land uses systems.

	Land Use				
Structural parameter /Carbon Stock	AFSs GGWFT	AFSs GGWAT	AFSs DGWGT	AFSs MGWAT	Forest
Bulk density (BD)	0,33**b**	0,30**b**	0,91**a**	0,72**a**	0,56**b**
Total porosity (TP)	92,13**a**	88,02**a**	71,42**b**	78,39**b**	77,13**b**
Aeration porosity (AP)	14,94**a**	15,09**a**	16,20**a**	13,86**a**	18,29**a**
Retention porosity (RP)	77,19**a**	72,94**a**	55,22**b**	64,53**b**	58,84**b**
Soil C stocks	56,87**b**	56,89**b**	35,74**c**	58,30**b**	60,86**a**
Total biomass ha^{-1}	54,85**c**	82,41**b**	39,75**d**	58,56**c**	909,39**a**
Mg C in aboveground biomass Ha^{-1}	27,42**c**	41,20**b**	19,88**d**	29,28**c**	454,70**a**
Mg CO_2 Ha^{-1}	100,65**c**	151,22**b**	72,94**d**	107,46**c**	1671,12**a**
Total Carbon Stock	84,30**b**	98,08**b**	55,62**c**	87,58**b**	515,56**a**

Agroforestry systems (AFSs); GGWFT: Gramalote grass with few tree s; GGWAT: Gramalote grass with abundant trees; DGWT Dali Grass with Guayaba trees. Significant differences of the means according to Tukey's adjustment ($P< 0.05$) in the same row are indicated with different letters.

3. Materials and Methods

The study was carried out under livestock areas in Pastaza province, Amazon Region, Ecuador. The climate of this area is typical of an humid tropical forest, with average altitude within 500 and 900 m.a.s.l, average annual rainfall of 3500 mm, annual evapotranspiration of 150 mm, average annual temperatures within 23.4 and 25.4 °C and relative humidity of 87%. (Nieto and Caicedo, 2012). The assessed soils belonged to the *Inceptisols* (Soil Survey Staff, 2014; Nieto and Caicedo, 2012) order and are characterized by acidic conditions, low natural fertility, low potassium, calcium and phosphorus contents and high iron content. The forest and pastures systems, with and without trees, were compared among them. We collected disturbed an undisturbed soil samples at 0-10 and 10-30 cm depth. From these samples, total organic carbon (TOC) bulk density and soil porosity were determined. TOC was analyzed by Walkey-Black method (Nelson and Sommer, 1982), bulk density (ρ_s) by cylinder method (Blake and Hartge, 1986) and soil porosity by the tension table method (Pla, 2010).

4. Conclusions

Identifying potential land uses for carbon sequestration and enhancing soil structural conditions, can restore the functionality and productivity of the soil resource. These environmental services can reverse degradation due to land use changes, diminishing CO_2 emissions into the atmosphere and increasing soil water uptake as corrective measures to global warming.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest".

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Study of the functional properties of the corn flour proteins (*Zea mays*), barley (*Hordeum vulgare*), quinoa (*Chenopodium quinoa*), potato (*Solanum tuberosum*), and wheat (*Triticum aestivum*) national and imported intended for use in baking and noodles

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Abstract: Proteins are not only sources of amino acids, but because of their polymeric nature, their presence directly influences the rheological characteristics of the food, which makes it more acceptable to the consumer. A protein can have a high nutritional quality and yet not possess functional properties suitable for incorporation in a particular food system or process. The functionality of proteins is therefore of great technological importance, and there is a great interest to gain insight into the mechanisms involved in the functionality itself, in order to be able to modify them and extend their range of applicability. Several physical chemical analyzes of each sample demonstrated that the product presents the most adequate protein characteristics to obtain a flour that is better adapted to the technology of baking and noodles. It was proposed the use of five flours from different cereals and tubercle (wheat, maize, barley, quinoa and potato), where a randomized single factor design was applied . The parameters that were used as indicators of the adaptation of the flour for baking technology and noodles were the solvent retention capacity and the determination of disulfide and sulfhydryl groups. The main objective of the present research is to study the functional properties of flour proteins of different products in order to intend their use for baking and noodles.

Keywords: functional properties, cereals, tubercle, solvent retention capacity

1. Introduction

The proteins are not only sources of amino acids, but because of its polymeric nature. Its presence strongly influences the rheological characteristics of the food, which makes it more acceptable to the consumer [1].

The protein maybe have a high nutritional quality and not possess functional properties that are unique to obtain a viscoelastic and cohesive mass capable of retaining gas and suitable for incorporation into a given food system or process. The functionality of the proteins is accordingly of great technological importance, being of great interest to know the mechanisms involved in the functionality of the same, in order to be able to modify them and extend its applicability range [2].

One of the functional properties of proteins is due to their ionizable carboxyl groups, amino, disulfide and others. The amino acids are able to change from one charge to another according to the pH in which they are. In other words, their amphoteric character confers the ability to receive and donate electrons, this situation induce a chemical condition known as an isoelectric or double ion point [2].

The wheat is used primarily in the manufacture of various bakery derivatives, since it presents the peculiarity that during its fermentation a swelling occurs. This capacity of swelling is mainly due to proteins, wheat flour contains 10 to 12 percent proteins that as the corn, are basically glutelins and prolamins [2].

The glutelins of the wheat receive the name of glutenins, while prolamins with name of gliadins, both account for 85 percent of the protein fraction, this along with lipids and water form the so-called gluten, who is the responsible for the properties of cohesiveness and viscoelasticity of the bread bulk [3].

[4] mentions that the functional role of wheat proteins, especially of gluten in bread quality is well defined. The specific viscoelastic properties of the bread mass are usually explained by the presence and interaction of the thiol and disulfide groups.

[5] mentions that wheat flour is the only one that has proteins that when mixed with water or water containing liquids form a firm, gummy and elastic substance called gluten. This protein is a determining factor in the technological characteristics of wheat, both in quantity and in quality in bread manufacturing. In quality, reserve proteins, especially gliadins and glutenins, play a relevant role, since during the mixing thank by the action of water form gluten. Both contribute to the viscoelastic properties necessary for a good mass behavior during baking, by the formation of a continuous three-dimensional network called gluten.

The objective of the present work is search for a substitute of the wheat in the elaboration of bread and noodles

2. Results and Discussion

Determination of the percentage of gluten

[2] mentions that the gluten have an amino acid composition of about 6% ionizable, 45% polar and 49% non-polar, the same which is characterized by its high content of proline and glutamine.

Figure 1 shows the difference in the percentage of gluten from samples of imported wheat flour (CWRS # 1 and Hard Red Winter) with the national wheat flour (Cojitambo), the latter showing a value equal to half of that is reported for imported wheat flour. The percentage of gluten of the national wheat flour Cojitambo is due to the fact that it has a smaller amount of proteins, therefore a lower amount of glutamines and prolamines, which are responsible for the formation of glutenin and gliadin respectively, this proteins are responsible for the formation of gluten. The prolamins are responsible for the viscosity and extensibility. The glutenins of the elastic characteristics of gluten. In the case of national wheat Cojitambo which has an excess of gliadin (proline) relative to glutenins, gluten is weak and permeable.

Determination of Sedimentation Volume

One parameters associated with the wheat protein quality is the sedimentation volume what hydration capacity and expansion gluten protein in medium light acid.

Figure 2 shows the sedimentation volume index, consisting of measure the volume of the particles that sediment (principally swollen proteins that have absorbed water) in one acid solution of water and lactic acid. The sedimentation volume is conditioned by the quantity and quality of proteins which when is denatured by lactic acid, the flour with best quality link more water, it will float and it will precipitate slowly. The sedimentation volume of imported wheat flour is considerably high because of its content in glutenins (glutamines) which upon

contact with the solution of lactic acid and isopropyl alcohol are denatured and absorb water [4]. National wheat flour contains about half of glutamines than imported wheat shows so that is less denatured so the action of lactic acid is lower and therefore the sedimentation volume tends to fall.

The flours of barley, corn, quinoa and potato, have high sedimentation values since the absorption of water by denaturation of the proteins is measured by the acid hydrolysis to which it is subjected.

But especially in samples of potato and quinoa flour the volume of sedimentation that it presents is due to the amount of starch that they have, since the content of glutamic acid would not contribute to the volume of sedimentation.

In these flours the starch plays a very important role, since in the process of grinding it undergoes modifications, provoking a greater content of damaged starch and justifying in this way the volume of sedimentation that they have.

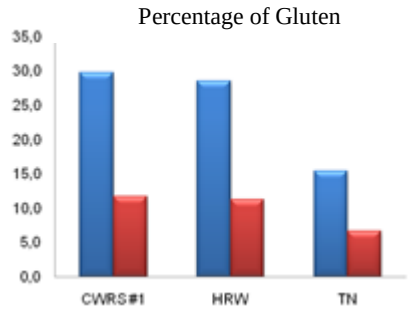

Figure 1. Percentage of gluten of wheat samples. In blue percentage of wet gluten, in red percentage of dry gluten.

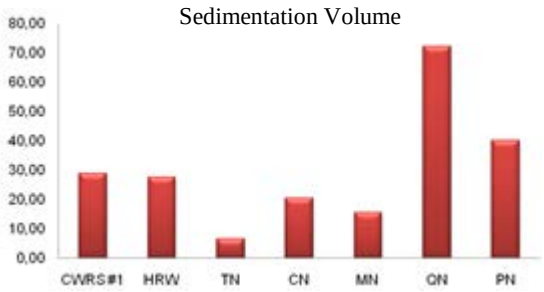

Figure 2. Sedimentation volume of cereal and potatoes flour.

3. Materials and Methods

It was used samples the imported wheat flour CWRS # 1 (control) and Hard Red Winter, samples from National Cereal Flours such as maize, barley, wheat, quinoa and variety potato Gabriela.

For the analysis in the flours, physical - chemical analyzes were taken into account, such as: Percentage of Gluten[6], Sedimentation Volume

4. Conclusions

It was possible to know the functional properties of cereal flour proteins (maize, quinoa, barley, wheat national and imported) and potatoes concluding that from the functional point of view the best flours for use in baking and noodles were barley and national wheat the same ones that, when substituted, improved the characteristics of bread and noodles.

After a study of the functional properties of proteins of cereal and tuber flours it maybe suggested that the flour suitable for partial use in baking and noodles is barley flour thanks to its pentosans content which considerably improves the masses to be formed.

The national wheat flour improves the characteristics in the noodle and derived products, such as volume, extensibility, elasticity and water absorption.

Author Contributions

Main text paragraph.

Conflicts of Interest

State any potential conflicts of interest here or "The authors declare no conflict of interest".

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Thyme and rosemary essential oils as an alternative control of plant-parasitic nematodes.

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Abstract: Since ancient times, essential oils have been used as an alternative against pests. Recently, the respect for the environment and public health has increased and has motivated the search for natural, biodegradable and with little or no toxicity nematicides. In this study, the nematicidal activity (in vitro) against parasite nematodes isolated from plants was evaluated. The essential oils evaluated were thyme and rosemary, both of them applied at three different concentrations. The nematicidal activity was evaluated during 48 hours and it was determined at different time intervals. The best results were obtained using the lowest concentration of thyme essential oil, after 8 h of exposure, where the number of nematodes was reduced by about 90%. Rosemary essential oil showed a similar effect (70.2% mortality), but at a higher concentration. These results demonstrate that essential oils could be considered as an alternative for pest control with a promising application in agriculture.

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Keywords: Essential oil, thyme, rosemary, nematicidal activity.

1. Introduction

Phytoparasitic nematodes have proven to be natural competitors of agricultural crops, and cause economic losses in a wide variety of crops. The plant-parasitic nematodes reduce the normal development of plants and make them susceptible to attack by pathogens such as fungi or bacteria (Hooks). In Ecuador, crop losses due to these pest range from 11% to 14%, and in interaction with *Fusarium* spp., the losses can reach values up to 90% of production (Ramírez, Grijalva, Navarrete, & Guerrero, 2016). Although phytoparasitic nematodes can be controlled by synthetic pesticides (carbamates and organophosphates), the excessive use of these pesticides produce resistance in nematodes and may be persistent in the environment and highly harmful to human health (Opperman & Chang, 1990).

The effort is carried out in the search for natural products with nematicidal activity, which represent an alternative and replace the synthetic chemicals and do not cause toxicological effects on the environment and the human being (Gupta, Sharma, & Naik, 2011). It has been found that several essential oils and their main compounds possess biological, antioxidant, antimicrobial, antifungal, insecticidal and nematicidal activity (Batish, Singh, Kohli, & Kaur, 2008; Espitia Yanes, 2011; Oka et al., 2000). However, only a few essential oils have been evaluated for their nematicidal effects. The objective of the present study was to evaluate the in vitro nematicidal activity of thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) essential oil at three concentrations (0.5, 1.0 and 1.5% v/v), against phytoparasitic nematodes.

2. Materials and Methods

Preparation of tested samples: Thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) essential oils were purchased from Isabrubotanik S.A (Ambato-Ecuador). Essential oils, at three different concentrations (0.5, 1.0 and 1.5% v/v) were diluted in aqueous solution of Tween 80 (0.5%).

Nematode: The juveniles used in the experiment were provided by the Plant health laboratory (Department of Agriculture, Technical University of Ambato), using roots of tomato tree (*Solanum betaceum* Cav.) disease-infected by *Meloidogyne* sp., which were isolated by using the Baermann funnel Technique (Gonzales, 2013).

Nematicidal activity: two milliliter suspension containing mean number of ~ 60 juveniles of root-knot nematodes were added to the Petri dishes with the essential oil suspension at the rates of 0.5%, 1% and 1.5%, respectively. Two-milliliter suspension containing an average of 60 juveniles, were kept in a Petri dish with 2 ml sterile aqueous solution of Tween 80 used as control and sterile water used as blank. The experiment was conducted at ~20 °C. The number of motile juveniles was recorded at 0.25, 0.50, 0.75 1, 2, 4, 6, 8, 24 and 48 h after treatment application, using stereoscopic microscope. Juveniles were considered paralyzed (non-mobile) if they did not move when probed with a needle. Data were corrected by Abbott formula (Kong, Lee, Moon, Lee, & Ahn, 2006). Effects of the EO in the nematode were observed by light microscopy (EVOS cell Thermo Scientific, USA.). The effectiveness of the EO's was assessed by comparing the different concentration by one-way ANOVA and Tukey's multiple comparison test, which was performed using GraphPad Prism version 6.01 for Windows.

3. Results and Discussion

Toxic effects of the essential oils against juvenile nematodes, after 48 h of exposure, are shown in Figure 1. The results varying according the type and concentration of the essential oil. In the present study, the thyme EO show higher nematicidal effect than the rosemary EO even among the concentrations assayed. The higher the concentration, the higher the mortality. These results could be related to the presence of thymol (~46.1%), p-cymene (~33.8%), gamma terpinene (~8.4%), caryophyllene (~5.4%), linalool (~3.4%) and carvacrol (~2.8%) as the main components (evaluated by GC-MS, data not shown) in thyme EO. Under the microscope, the control appear as a smooth surface (Fig. 2A) and the treated with the thyme EO appears wrinkled (Fig. 2B). (Gutierrez, Barry-Ryan, & Bourke, 2008) described that the p-cymene, a very weak biological activity compound, swells cell membranes to a greater extent than carvacrol (compound with high biological activity). The synergism between carvacrol and its precursor p-cymene probably enables carvacrol to be more easily transported into the cell and may improve the EO efficacy against nematodes. Similar results, using thyme and rosemary EO were observed by (Oka et al., 2000), were the thyme EO was more effective against nematodes than rosemary EO. Although some essential oils and their components have been reported to be phytotoxic at high concentrations, the thyme EO at 1.5% (v/v), in only 5 h, reach the 100% of mortality. No significant differences were observed between concentrations of 1.0% and 1.5% of thyme essential oil.

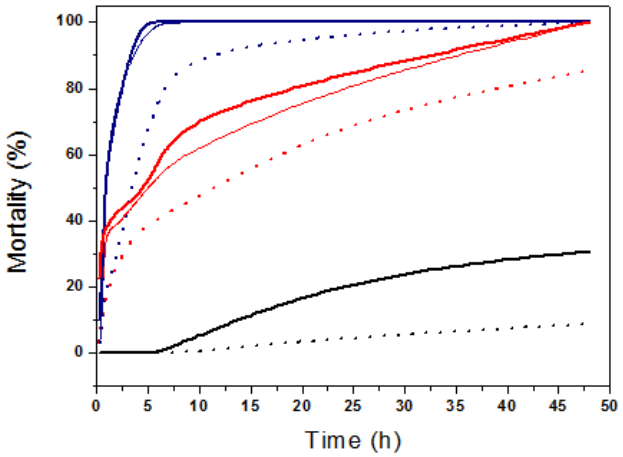

Fig. 1. Effect of thyme (blue) and rosemary (red) essential oils at different concentrations: 0.5% (dot line), 1.0% (fine line) and 1.5% (thick line), control (dot black line) and blank (black line) against *Meloidogyne sp*.

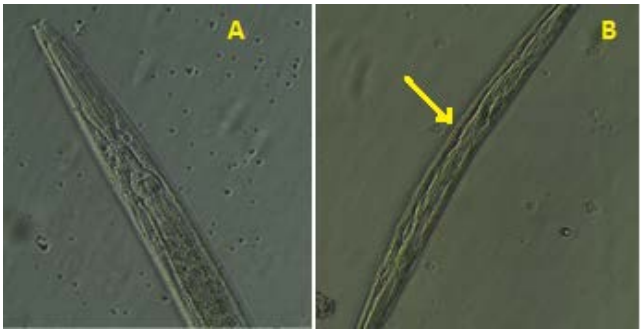

Fig. 2. Morphological changes caused by the thyme EO. **A)** Control and **B)** treated with 1.5% of thyme EO. Arrow indicates the wrinkled surface.

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4. Conclusions

This study evaluated the nematicidal effect of rosemary and thyme essential oils against phytoparasitic nematodes. The strong nematicidal activity of thyme EO was observed at 1.5% (v/v) which indicates that it has potential as a biological nematode control. The structural damages caused by essential oils in nematodes were evident and the nematicidal effect in the greenhouse and field is being studied.

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Ramírez, F., Grijalva, R., Navarrete, X., & Guerrero, R. (2016). NEMATODOS FITOPARÁSITOS ASOCIADOS CON TOMATE DE ÁRBOL (Solanum betaceum Cav.) EN LAS PROVINCIAS DE IMBABURA, PICHINCHA Y TUNGURAHUA, ECUADOR. *ECUADOR ES CALIDAD-Revista Científica Ecuatoriana, 2*(1).

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Ligand-based virtual screening of a benzylisoquinoline alkaloids dataset with anti-inflammatory potential activity.

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Abstract:

Inhibitor of nuclear factor kappa B kinase beta subunit (IKK-B) and extracellular signal-regulated kinase 1 (ERK1) are two proteins involved in cytokine intracellular signaling pathways, which have a great importance due to their anti-inflammatory role. In this work, from the ChemBL database were obtained 775 and 48 structures with activity against IKK-B (CHEMBL1991) and ERK1 (CHEMBL3385) respectively. The compounds were classified using values of pIC_{50}, presenting a range of 4.29 (from 5.01 to 9.30) for IKK-B and 3.10 (From 5.05 to 8.15) for ERK-1. From SMILES codes, two-dimensional (2D) structures were generated in Standardizer and after calculated 1064 two-dimensional molecular descriptors in Dragon 7 software. Obtained results were imported to Knime 3.1.0 software. 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%. (Q)SAR models was performed using a Random Forest (RF) algorithm. Models were evaluated through cross validation (leave-one-out), Q^2_{LOO} = 0.69 and 0.66 as well as external test, Q^2_{ext} = 0.74 and 0.58 for IKK-B and ERK1 respectively. Finally, pIC50 value of 179 benzylisoquinoline alkaloids were predicted in the (Q)SAR models found 4 compound with the highest activity for each one protein studied.

Keywords: benzylisoquinoline alkaloids, IKK-B, ERK1, anti-inflammatory activity, Virtual screening

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YouTube link: *please, paste here the link to your personal YouTube video, if any.*

1. Introduction

The inflammatory process is a nonspecific complex, stereotype, coordinated response of tissues to injury [1]. Several proteins with specific roles are present in the signaling pathways involved in these processes.

Nuclear factor-κB (NF-κB), is a transcriptional factor, which plays a key role in numerous physiological, these include inflammatory processes [2]. NF-κB activation is stimulated by a kinase complex, IκB kinase (IKK), which is composed of three core proteins: IKK1/ IKK-α, IKK2/IKK-β and NEMO/IKK-γ [2,3], being IKK-α and IKK-β two catalytic subunits which are structurally related kinases.

IKK-β, is an interesting target due to its role in the inflammation-induced tumour growth and progression, as well as an important modulator of tumour surveillance and rejection [4].

In turn, extracellular signal-regulated kinase 1 (ERK1) being one of the two isoforms of ERK described. It is present in the Ras/Raf/MEK/ERK signaling pathway, which has vital importance, since many essential cell processes are involved. In ~30 % of cancers and cognitive disorders, exists an abnormally activation [5].

ERK1 is a serine/threonine kinase of the GMGC group that plays a critical role in the regulation of cell growth and differentiation [6].

Benzylisoquinoline alkaloids (BIA) are metabolites which present a great diversity (~2,500 BIAs are known today) and several pharmacological activities such as antimicrobial agent, muscle relaxant, and potential anticancer drug, among others [7,8].

In this work, through of a random forest model using 2D molecular descriptors was performed to in order to predict the anti-inflammatory activity 179 BIAs structures.

2. Results and Discussion

Ligand-based virtual screening

(Q)SAR models was performed using a Random Forest algorithm. Models were evaluated through cross validation (leave-one-out), Q^2_{LOO} = 0.69 and 0.66 as well as external test, Q^2_{ext} = 0.74 and 0.58 for IKK-B and ERK1 respectively (see Figure 1 and 2)

After, pIC50 value of 179 benzylisoquinoline alkaloids were predicted in the (Q)SAR models. For IKK-B and ERK1, four structures presented the highest anti-inflammatory activity for each target (Table 2).

The two BIAs with highest anti-inflammatory activity for IKK-B present a structural similarity regarding to the presence of methoxyl and hydroxyl groups at the positions 9 and 10 respectively (Table 2a).

Meanwhile for ERK1, protoberberine skeleton presents the highest predicted value of pIC50, therefore, none structural modification regarding the core of this structure present in our database seem to increase the activity (Table 2b).

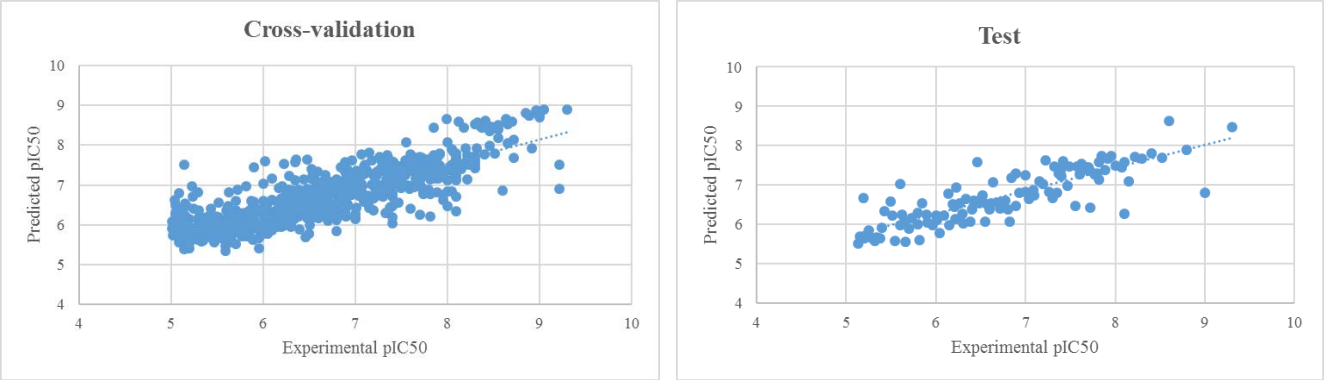


Figure 1. Plot of experimental vs predicted values of pIC50 for cross-validation and test set generated by IKK-B QSAR model

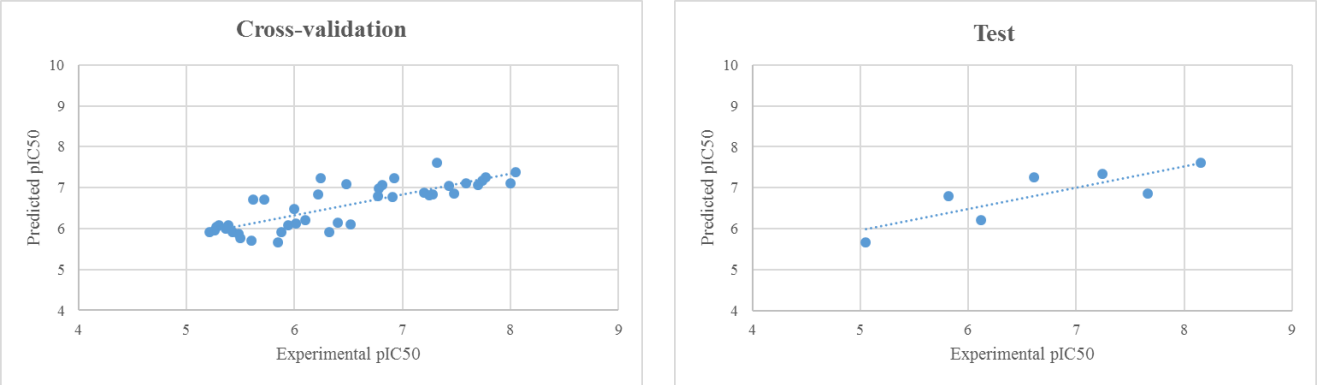
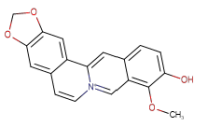
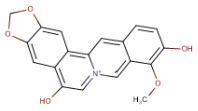
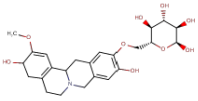
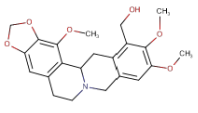
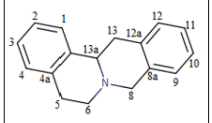
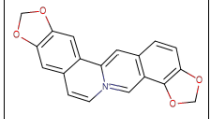
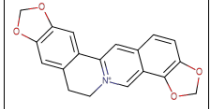
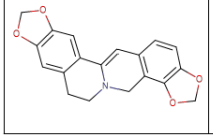


Figure 2. Plot of experimental vs predicted values of pIC50 for cross-validation and test set generated by ERK1 QSAR model

Table 1. benzylisoquinoline alkaloids with the highest anti-inflammatory activities for a) IKK-B and b) ERK1

Structure	Predicted pIC50
	6.39
	6.38
	6.36
	6.33

a)

Structure	Predicted pIC50
	6.17
	6.13
	6.12
	6.11

b)

3. Materials and Methods

From the ChemBL database were obtained 775 and 48 structures with activity against IKK-B (CHEMBL1991) and ERK1 (CHEMBL3385) respectively (https://www.ebi.ac.uk/chembl/). The compounds were classified using values of pIC_{50} (-log IC_{50}), presenting a range of 4.29 (from 5.01 to 9.30) for IKK-B and 3.10 (From 5.05 to 8.15) for ERK1. In this case, IC_{50} represents the concentration required for 50% inhibition of enzymatic activity. From SMILES codes, two-dimensional (2D) structures were generated in Standardizer software that canonized structures, added hydrogens, performed aromatic form conversions [JChem 14.9.1.0, 2014; ChemAxon (http://www.chemaxon.com)]. After were calculated 1064 two-dimensional molecular descriptors in Dragon 7 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%. (Q)SAR models was performed using a Random Forest algorithm. Models were evaluated through cross validation (leave-one-out), $Q^2_{LOO,}$ as well as external test, Q^2_{ext}

4. Conclusions

The Ligand-based model using Random Forest and 2D molecular descriptors selected protoberberine skeleton with methoxyl and hydroxyl groups at the positions 9 and 10 as the most potential activity structures against IKK-B from an in-house database of benzylisoquinoline alkaloids. For ERK1 the RF model selected the same core without any substitution as the most potential active compound.

Acknowledgments

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Author Contributions

LS, MFA built database; CHA performed all calculus; and CHA, MTS and MFFMD 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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Immunohistochemestry vs. Immunofluorescence: Comparative analysis via software of total colorimetric reaction of GPR43 protein in adipose tissue

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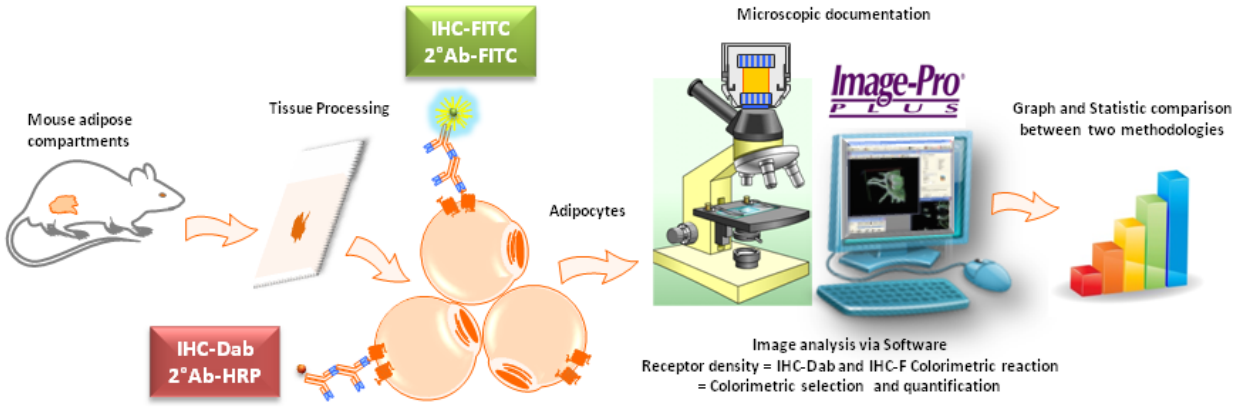
Abstract: Immunohistochemistry revealed by diaminobenzidine (IHC-DAB) or fluorescence (IHC-F) are two of the most common techniques used in histopathology. In order to evaluate the similarity between these methodologies that share the same antigen-antibody reaction principle, using Image-Pro Plus software we performed total colorimetry quantification and comparison of immunohistochemical reaction; revealed with diaminobenzidine (IHC-DAB) and FITC fluorescence (IHC-F). As biological model, GPR43 protein was assessed by immunohistochemistry in inguinal, mesenteric and gonadal (WAT) cd1 mice adipose tissue. In predefined areas of digital images, we evaluated the reaction obtained following the same processing conditions. Results: The GPR43 protein in both IHC-DAB and IHC-F was evidenced in adipocytes membrane and infiltrated immune cells. The average of positive reaction in μ^2 (total colorimetry x μ^2) on a predefined area or region of interest (ROI) of 25x104 μm^2, showed no significant differences in the distinct tissues between the performed methods. This colorimetric similarity between the two techniques, besides supporting the quantitative analysis of

IHC-DAB, indicates that the latter could be comparable to the IHC-F, with the advantage of being used in retrospective studies. Conclusions: In the total colorimetric analysis between the two methodologies IHC-DAB and IHC-F, the resulting quantitative values were similar in all three compartments of adipose tissue studied. Subsequent studies could validate the IHC-DAB respect to IHC-F.

Keywords: *Immunohistochemistry, immunofluorescence, colorimetric-analysis, GPR43, adipocytes.*



Graphical Abstract : The figure shows the methodological sequence to compare colorimetric reaction between two indirect immunoreactions (IHC-DAB and IHC-F) via Software

Introduction: Pathology is a multidisciplinary science that besides studying structural and functional disease abnormalities from development to its senescent stage[1], it is based on morphofunctional areas of comparative biology and medicine. Its clinical components are: epidemiological, etiologic and physiopathogenic factors[2]. Contemporary pathology continues using microscopy for describing abnormal processes using a wide range of dyes and chromogens to demonstrate biological responses[3]. Pathognomonic disease findings were subsequently supported by the incursion of molecular pathology, where the use of biomarkers (antibodies) is and will remain as an essential tool for diagnosis[4]. In addition to morphological aspects evidenced by dyes, immunohistochemistry revealed by diaminobenzidine (IHC-DAB) or fluorescence (IHC-F) are two of the most common methodologies used in histopathology[5,6]. In IHC-DAB various chromogens determine the enzymatic reaction on conjugated antibodies (Avidin-Biotin Complex Peroxidase ABC or alternate labeling method) and the resulting color (e.g. brown color intensity) indicates the presence of the antigen of interest. Although several studies quantify IHC-DAB from digital images, it still prevails as a semiquantitative technique, where the score assigned depends on the observer appreciation. In immunofluorescence, when a specific wavelength excites the antibody coupled fluorochrome, physical changes occur in the

molecule. The electronic excitation results in fluorescence emission. The antigen-antibody reactions can have variable distribution therefore fluorescence emitted corresponds to a particular antigen density. In this context, the mean fluorescence intensity is conditioned by the sample quality, magnitude-time of exposure to light source, the filters quality and the maximum fluorescence emitted. However in a scanned image, you can see the total, maximum, minimum, mean and average fluorescence.

Nowadays, the integration of technological developments on optical instruments, continue to support biomedical research but also allow us to consolidate new pathology disciplines, such as computational pathology and telepathology[7,8]. In abnormality patterns on histopathologic image, new software tools allow accurate and automated analysis that may be applied in "omics" studies, databases and mathematical prediction. It is noteworthy referring that the appreciation limitations, interpretation and analysis of a digital image are subjected to variables, such as; the reagents quality and quantity, tissue fixation, tissue section thickness, dye/antibodies/chromogen incubation time. For immunofluorescence also counts the time between fluorochrome incubation and the sample exposure to natural/laser/halogen light sources. For histopathology using digital image, there is a growing number of software e.g. Aperio, Lucia, MetaView, Metamorph, ImageJ, Scion, Adobe Photoshop, Image Pro Plus[9]. In turn, these are provided with multiple applications to optimize the analysis and automated processes (macros, filters, extracting regions of interest (ROI), colocalization, interposing images, measuring and cell count).

On the other hand, GPR43 protein is a molecule expressed on the intestinal epithelium surface, lymphocytes, muscle, platelets and adipose tissue. It is a short-chain fatty acids receptor produced by intestinal microbiota; involved in energy metabolism, adipose tissue differentiation and obesity development[10]. Given the growing demand in obesity studies, the GPR43 protein is considered as a potential therapeutic target[11]. In this context a few qualitative reports do exist on GPR43protein detection via IHC-DAB or IHC-F[12,13]. For the IHC reactions colorimetric quantification, adipose tissue was selected due to the adipocytes large size and less cell interposition. For the aim of this study, inguinal, mesenteric and gonadal adipose tissue samples (WAT) were processed with IHC-DAB and IHC-F to evaluate the GPR43 protein density on adipocytes. Comparative analysis of the total colorimetry IHC-DAB and IHC-F on the documented images was performed.

Materials and Methods: The procedures were performed in cd1 healthy male mice (24 weeks-age, 35-40g. n=6), in compliance with the Mexican NOM-062-ZOO-1999 and international bioethical standards. Mice were euthanized by CO_2 chamber. Visceral, inguinal and gonadal adipose tissue were dissected, fixated in 10% buffered formaldehyde and embedded in paraffin; 4µm sections were obtained and

indirect immunohistochemistry was performed using anti-FFAR2/GPR43 (Santa Cruz-LS-A1578, dilution 1:50) primary antibody, anti-rabbit HRP IgG (Biolegend-406401, dilution 1:100) secondary antibody and DAB staining. The same primary antibody was used for immunofluorescence, replacing the peroxidated secondary antibody with anti-donkey FITC IgG (Biolegend-406403, 1:100 dilution). In both, indirect IHC-DAB and IHC- F, the primary antibody incubation lasted 12h, and 1.5h for the secondary antibodies. Fresh IHC-DAB slides were evaluated in Axiostar Carl-Zeiss microscope. IHC-F (FITC) reactions were analyzed with a microscope coupled Epi-IV FI fluorescence condenser, filter-487710 (blue excitation) with 50W halogen source. Digital photos (Tiff) were taken with the same white balance by IScapture® software; the images were captured at 400X magnification with Cooled-CCD Tucsen camera (5MP). In a predefined area or region of interest (ROI 25x104 μm2), the colorimetric reactions IHC-DAB and IHC-F were manually selected, using Image-Pro Plus 5.1 software (Media-Cybernetics, Silver Spring, MD). Subsequently a range of luminance was delimit (85-255 units) in the red histogram (RGB format), it was recorded as template and applied in subsequent images. In IHC-F, the total fluorescence emitted was quantified. The colorimetric reactions were compared with Man-Whitney U test.

Results and Discussion: Regarding quantitative analysis, there was no significant difference comparing the GPR43 protein colorimetric results for aforementioned methodologies (IHC-DAB vs. IHC-F). GPR43 immunoreactivity was documented in both adipocyte membrane and in immune cells infiltrates which also express this protein (Figure 1). Total colorimetry expressed in μm^2 and quantified on a ROI of $25x10^4 \mu m^2$, in inguinal tissue was 2429.1 ± 985.3 (IHC-DAB) vs. 2362 ± 882.7 (IHC-F) in visceral tissue 2532.8 ± 361.6 vs. 371 ± 2614.1 and in gonadal tissue 3737.7 ± 309.8 vs. 4013.6 ± 554.8. Therefore a similarity was observed between the two methods (Figure 2). The equivalence between both methods indicates similar antigen-antibody reactions. Hence IHC-DAB further than support quantification and comparison with IHC-F may be used in retrospective studies.

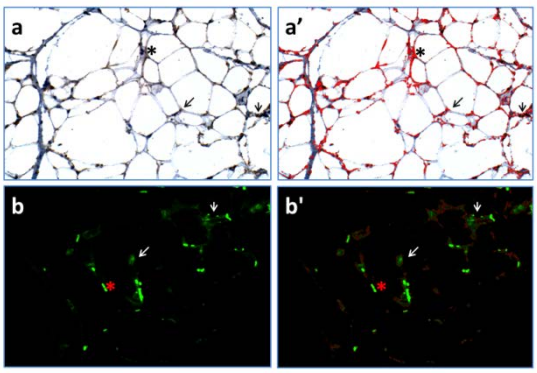


Figure 1. Colorimetric comparison of gonadal adipose tissue photomicrographs by Immnohistochemistry. a) Reaction revealed by DAB and; a') Corresponding colorimetric selection in red (dark arrows); b) Immunohistochemistry reaction by immunofluorescence (FITC), and; b') Colorimetric selection of immunofluorescence reaction outlined in red (light arrows). Star (*); representation of lymphocytic infiltrate. Paraffin sections of 4μm, 400x magnification.

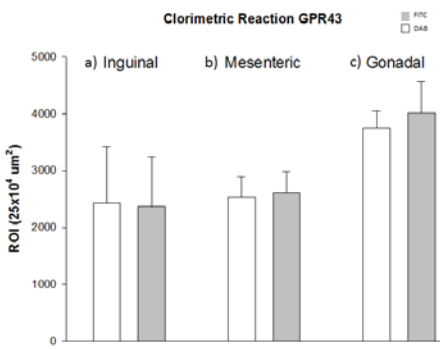


Figure 2. Statistical comparison of data obtained by immunohistochemistry. a) Comparison between DAB and FITC in inguinal adipose tissue b) Comparison between DAB and FITC in mesenteric adipose tissue c) Comparison between DAB and FITC in gonadal adipose tissue. Mann Whitney U, confidence level of 95%

Nowadays, histopathological software tools keep improving[9]; however it remains a lack of terms that unifies the criteria for quantitative analysis between different software and the comparison of IHC-DAB with other methodologies. Moreover few studies in individual methodologies perform a colorimetric quantification of an IHC reaction [14] and in most of them, only a score is assigned to distribution and quantification of cell immunoreactiviy.

Some studies used these tools in order to automate, assist and increase efficiency in pathology diagnosis and research purposes. For example in a pathologist-assisted scoring based study, using software tools (Metamorph, Aperio, Definiens Image Analysis), both methodologies were applied to different samples; the apoptotic index was quantified in IHC-F and the positive reaction expressed in pixels within a predefined area was quantified in IHC-DAB[15]. In the present study, the previous focal calibration of microscopic lenses within Image-Pro Plus software, allows the conversion of pixels into μm^2. Studies that compare quantitatively IHC-DAB vs. IHC-F on the same tissue were not yet found.

Conclusions: The equivalence between both methods indicates similar antigen-antibody reactions. Therefore IHC-DAB further than support quantification and comparison with IHC-F may be used in retrospective studies.

Author Contributions: QFB SGVD, M in Sc DRM, and M.D EYPM drafting the article and analysis and interpretation of data, Ph.D. ETG, and JLDlRA substantial contributions to conception and design, acquisition of data; Ph.D. ROP, final approval of the version to be published.

Conflicts of Interest: The authors declare no conflict of interest

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Computational Study of Natural Phenolic Acid Solubility and Their Interactions with Chitosan

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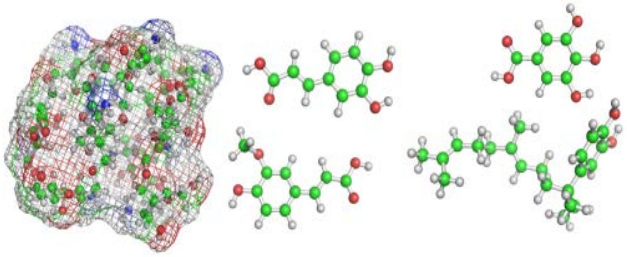
Abstract: *Natural phenolic acids such as gallic, caffeic, ferulic and sinapic acids, have received great attention due to their biological activities, like antioxidant, anti-inflammatory and others. These properties put them as good candidates for the new controlled drug release systems. Among the various types of polymeric materials used in the development of controlled release systems for active drugs and films, chitosan is highlighted because it has many favorable characteristics, such as biocompatibility and biodegradability. To verify the behavior of such phenolic acids in hydrophilic biological fluids and hydrophobic biological barriers aimed at the production of new systems of modified drug release, in the present work it was conducted in silico simulation of solubility in water and in 1-octanol by molecular dynamics. The interaction of these phenolic compounds with chitosan was also investigated by molecular docking. The results showed that all investigated phenolic acids showed adequate solubility and good interaction with chitosan. The results show that the methodology applied in the present work can be well used for the development of pharmacologically active compounds and can aid the understanding of the interaction of such compounds with polymers, saving time and resources.*

Keywords: *phenolic acids, chitosan, molecular dynamics, molecular docking*

Graphical Abstract:



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Introduction:

Natural phenolic acids, such as ferulic, gallic, caffeic and sinapic acids have received great attention due to their biological properties, such as antioxidant action [1,2], antithrombotic antitumor and anti-inflammatory [3,4].

The biological properties of phenolic acids make them good drug candidates for new systems of modified drug release, and the antioxidant property make them good candidates for additives to the new active films development for food packaging, because the antioxidants, in addition to being known to remove or inhibit free radicals production in the body [5], also play an important role in preventing oxidative damage to food during the processing and storage [6].

There is a growing interest in the new and effective modified drug delivery systems development, which are driven by the need to increase the therapeutic effect and minimize the drugs side effects [7]. In simplified form, drugs modified release may be defined as the process of releasing a bioactive substance in a specific quantity at a specific site [8]. These modified drug delivery systems, usually, are constituted by the drug addiction in a polymer base, forming a drug-polymer complex [9]. In addition to the interest in new modified drug delivery systems, there is also the new materials development interest that can be used as active packaging in the food industry, maintaining food integrity for a long time [10, 11]. As with modified drug delivery systems, much of the active packaging is also characterized by the addition of some additive to a polymer base. Among the materials used in the new systems development of active drugs and films modified release, chitosan has gained prominence. Chitosan is a polysaccharide with favorable characteristics and properties for novel modified drug delivery systems synthesis and active films for food packaging, because it is a natural polymer, renewable, biodegradable, biofunctional, biocompatible and non-toxic [12], besides having a cationic polymeric character and gel properties and film forming [13-15].

Natural compounds isolated from plant sources have an extensive use from the past and continues being well used today in several areas, because besides serving as interest compounds by their natural form, can also serve as models for the analogues synthesis with higher activity and lower toxicity [16].

Molecular modeling studies (*in silico*) have been increasingly used to elucidate the natural compounds physicochemical properties, such as solubility, a highly important aspect for the active substances bioavailability [17] and for predicting the such compounds interaction with a polymer base, providing better characteristics understanding of the compounds and the drug-polymer complex formation [18,19].

Motivated by the chitosan features and the phenolic acids biological properties, the present study sought to investigate in silico, by molecular dynamics and machine learning algorithm, the water and 1-octanol solubility of the ferulic acid compounds, Gallic acid, caffeic acid and sinapic acid, besides observing how these compounds interaction occurs with chitosan, through molecular docking, aiming at a better compounds behavior understanding in biological fluids (hydrophilic) and cell membrane (hydrophobic) and in interaction with chitosan (drug-polymer).

Materials and Methods:

Quantum Mechanic Optimization

The geometric optimization of gallic, ferulic, caffeic and sinapic acid molecules (**Figure 1**) was performed using the Density Functional Theory method (DFT), which is a theory based on electronic density, with B3LYP [20, 21] and 6-31G basis set. For the ab initio calculation, the GAMESS-US software was used [22] with the GABEDIT graphical interface aid 2.4.8 [23].

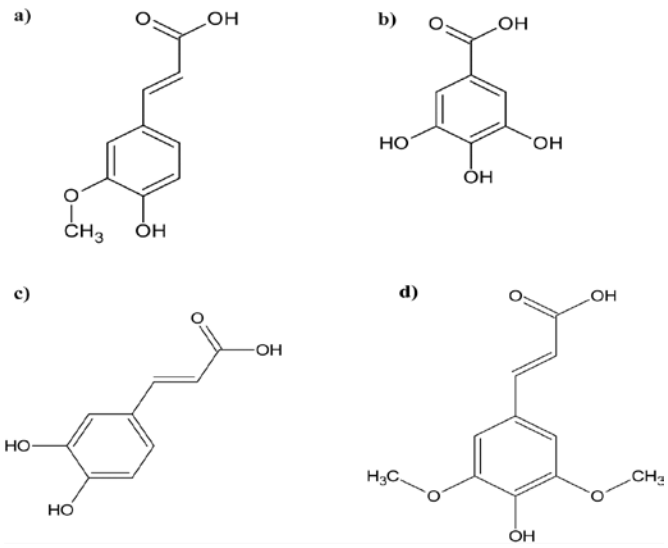

Figure 1. Chemical structure of phenolic compounds a) ferulic acid b) gallic acid c) caffeic acid and d) sinapic acid

Binders solubility study

Based on the compounds solubility importance for their pharmacological activity, the present study investigated the phenolic acids solubility in aqueous and lipophilic medium, as well as the behavior after being solubilized. The ALOGPS 2.1 software was used for partition coefficient (log P) and water solubility (log S) calculations [24]. The ALOGPS was built on the Associative Neural Network (ASNN), which is a machine learning algorithm that combines neural network with k-neighbors [25].

The software GROMACS 4.5.5 [26] was used to simulate the phenolic acids in solvated water and 1-octanol, by molecular dynamics calculations. For the organic compounds energy minimization and simulation in solvated medium the field of force OPLS-AA [27]. The set temperature for simulation was constant at 300 K and the pressure was kept constant at 1 bar. The free energy of solvation in water (ΔG water) and in 1-octanol (ΔG oct) was obtained by the Bennet Acceptance Ratio (BAR) method. The solvation free energy was calculated by creating 21 points: 0; 0.05; 0.1; 0.15; 0.2; 0.25; 0.3; 0.35; 0.4; 0.45; 0.5; 0.55; 0.6; 0.65; 0.7; 0.75; 0.8; 0.85; 0.9; 0.95 and 1.00. The system was balanced over time in 2 ns, while the points simulation occurred in 20 ns. The electrostatic interactions were obtained by PME with cutoff space at 1.3 nm. The details on solvation simulation setup details are given on **Table 1**. The coulombic energies and Lennard-Jones interactions, solvent accessible surface, solvation free energy and root mean square fluctuation (RMSF) of phenolic acids were calculated using the g_energy, g_sas, G_bar and g_rmsf, respectively. Both present in the GROMACS package.

Docking

For the molecular docking simulations, two chitosan molecules were used, one with nine and one with 12-meres acquired in the PDB format through the "Human Metabolome Data Base" banks [28] and "PolySac3DB" [29] respectively. After download, the structures were submitted to molecular mechanical optimization (MM), with the AMBER force field [30], present in GABEDIT.

The software Autodock 4.2 [31] was used as the choice to perform the molecular docking study.

In Autodock 4.2, Gasteiger partial loads and hydrogens needed for the calculation were added in the chitosan molecules. The rotational bonds of the binders have been automatically defined and their nonpolar hydrogens suppressed. Autogrid 4.2 software was used to generate the pre-calculated three-dimensional map around the chitosan molecule. For the 12-meres structure, the grid was positioned around the entire molecule with dimensions of 46 Å on the X-axis, 126 Å on the Y-axis and 28 Å on the Z- axis, In 0.503 Å spacing. For the nine-meter structure, the grid was positioned around any molecule with 126 Å dimensions in the X-axis, 40 Å in the Y-axis and 40 Å in the Z-axis, spaced 0.375 Å.

To find the most stable conformations of the ligands, we used the Lamarckian genetic algorithm (LGA). 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, just as the number of elitism was kept at 1. The genetic mutation and crossover rates 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 (Root Mean Square deviation) values, according to the *AutoDock* default. During the search process, chitosan was kept rigid and the binders flexible.

Table 1. The solvation simulation system description

System	Components (number of molecules in parentheses)	Box size (Å)
A	(1) Gallic acid + (4134) Water	5 x 5 x 5
B	(1) Ferulic acid + (4134) Water	5 x 5 x 5
C	(1) Ácido cafeico + (4132) Water	5 x 5 x 5
D	(1) Sinapic acid + (4131) Water	5 x 5 x 5
E	(1) Gallic acid + (512) 1-Octanol	5 x 5 x 5
F	(1) Ferulic acid + (512) 1-Octanol	5 x 5 x 5
G	(1) Caffeic acid + (512) 1-Octanol	5 x 5 x 5
H	(1) Sinapic acid + (512) 1-Octanol	5 x 5 x 5

Results and Discussion:

In relation to the octanol/water partition coefficient (LogP) calculated by ALOGPS 2.1, the values found for sinapic acid, caffeic acid and ferulic acid were very close (**Table 2**). Gallic acid was the compound that presented the lowest logP value, representing greater solubility in water when compared to the other compounds. In relation to the water solubility (LogS) calculated by the software ALOGPS 2.1, the sinapic acid was the one that presented the lowest value (less soluble in water), whereas gallic acid had the highest value (greater solubility in water).

LogS values above -1 are related to very polar molecules and have difficulty permeabilizing on hydrophobic surfaces. Empirically, 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 [32].

Table 2. LogP and logS the compounds values calculated by ALOGPS 2.1 and their experimental values.

Compound	Log P	Log S	Log P_{exp}	Log S_{exp}
Ferulic acid	1.58	-2.33	1.42 [32]	-
Gallic acid	1.17	-1.54	0.89 [33]	-
Sinapic acid	1.63	-2.55	-	-
Caffeic acid	1.67	-2.05	1.24 [34]	-

Drugs or drug candidates must have hydrophilicity and lipophilicity to interact with biological fluids and cross some biological barriers, such as the plasma membrane that is extremely lipophilic. Based on this assumption, all phenolic acids included in the study have adequate solubility for the pharmacokinetic

requirements, as they have sufficient hydrophilicity for aqueous solubility and sufficient lipophilicity to interact with hydrophobic surfaces.

It is observed that the logP and logS values do not present linearity, despite having a connection in their results. This is because logP shows compounds solubility relation in inorganic (water) and organic (1-octanol) solvents, whereas logS is related only to the compounds solubility in water.

In the free energy calculation of solvation performed by GROMACS 4.5.5, it was observed that gallic acid presented the best solubility in water and in 1-octanol (**Table 3**).

Table 3. Solvation free energy in water and 1-octanol

Compound	Solvation free energy in water	Solvation free energy in 1-octanol
Gallic acid	-6.16 +/- 0.22	-6.45 +/- 1.14
Caffeic acid	- 0.36 +/- 0.17	-3.10 +/- 0.57
Ferulic acid	3.67 +/- 0.04	-3.34 +/- 0.54
Sinapic acid	5.87 +/- 0.02	-4.05 +/- 0.21

The free energy obtained for ferulic and sinapic acids in water indicates that the compounds are practically insoluble in water. The high gallic acid solubility in both solvents may be due to the high polar interactions density formed between the phenolic and gallic acid carboxylic hydroxyls with the water and the hydroxyls of 1-octanol.

It can also be observed that all compounds were more soluble in 1-octanol than in water. In **Table 4** the solvent accessible area of the phenolic compounds studied is presented. The solvent accessible surface area is divided into hydrophilic and hydrophobic based on the atomic partial charges.

Figure 2 shows the hydrogen bonds formed between phenolic acids and water. From the figure, it can be observed that the oxygens of the phenolic hydroxyls of all compounds tend to form hydrogen bonding with water. In all cases, too, the carboxylic group oxygens promote hydrogen bonding with water. It can also be observed that ferulic, sinapic and caffeic acids undergo a twist close to the carboxylic group when binding to the solvent.

Table 4. Solvent Accessible Surface Area of phenolic compounds

Compound	Hydrophilic area (nm^2)	Hydrophobic area (nm^2)	Total (nm^2)
Gallic acid	1.82	1.02	2.84
Caffeic acid	1.43	1.77	3.20
Ferulic acid	1.28	2.09	3.37
Sinapic acid	1.22	3.13	4.35

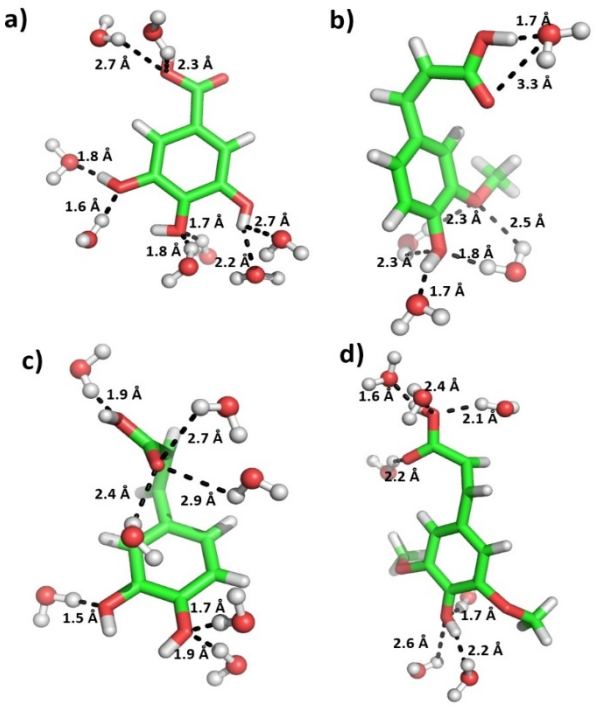

Figure 2. Hydrogen bonds of phenolic compounds in water. a) Gallic acid, b) Ferulic acid, c) Caffeic acid d) Sinapic acid

In **Figure 3** the hydrogen interactions formed

between the phenolic compounds and the 1-octanol solvent can be observed. Note that even promoting less hydrogen bonding, caffeic, ferulic and sinapic acids suffer the same twists as when they are in aqueous medium. To verify the behavior of the compounds in solvated medium, specifically their structural stability in solvated medium, the quadratic mean root fluctuation (RMSF) per atom of the phenolic acid molecules was calculated during the simulation of the compounds in water solvent and 1-octanol.

In **Figure 4** it can be observed that gallic acid has greater fluctuation in the -COOH group in both solvents, however, it is also observed, small fluctuations in the hydrogens of the phenolic hydroxyls when the compounds are in water. It is also observed that caffeic, ferulic and sinapic acids undergo fluctuations in the -COOH groups and the phenolic -OH and -OCH_3 groups, in addition to a considerable fluctuation in the aliphatic chain linking the -COOH group in the phenolic ring, in both solvents. As expected, fluctuations occur more strongly when phenolic acids are in the presence of water.

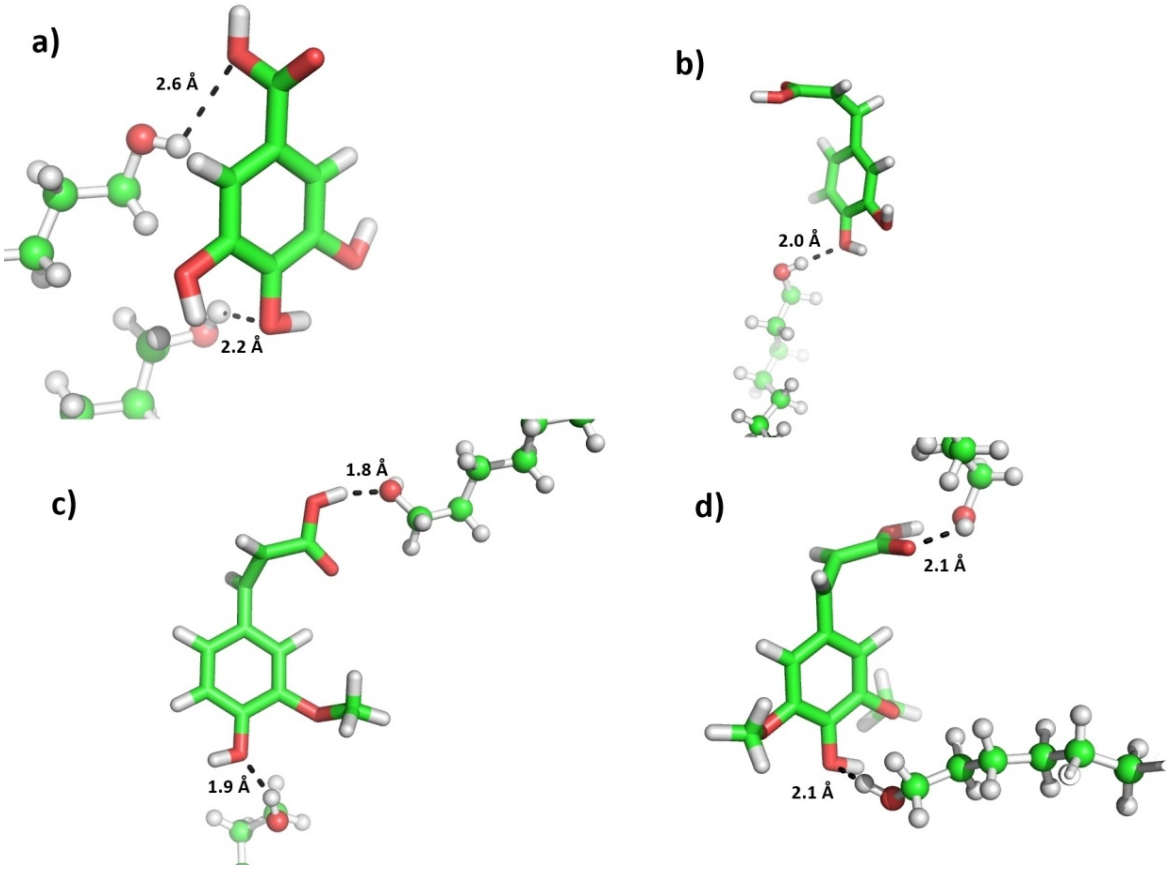

Figure 3. Hydrogen bonds of phenolic compounds to 1-octanol. a) Gallic acid, b) Caffeic acid, c) Ferulic acid, d) Sinapic acid.

By evaluating pharmacokinetic aspects, RMSF calculations show that the compounds although more soluble in organic solvents, promote denser polar interactions with water, adding to the notion that compounds interact in a stable way with biological fluids without compromising the absorption of these substances. The solubility study of the compounds shows that even though the compounds exhibit better solubility in contact with hydrophobic surfaces, they can interact with aqueous fluids through the polar interactions between phenolic hydroxyls, carboxyl and methoxyl groups with water, which is of great significance for the biological effect of these compounds, since they act in an aqueous

medium from the beginning to the end of their pharmacokinetic cycle.

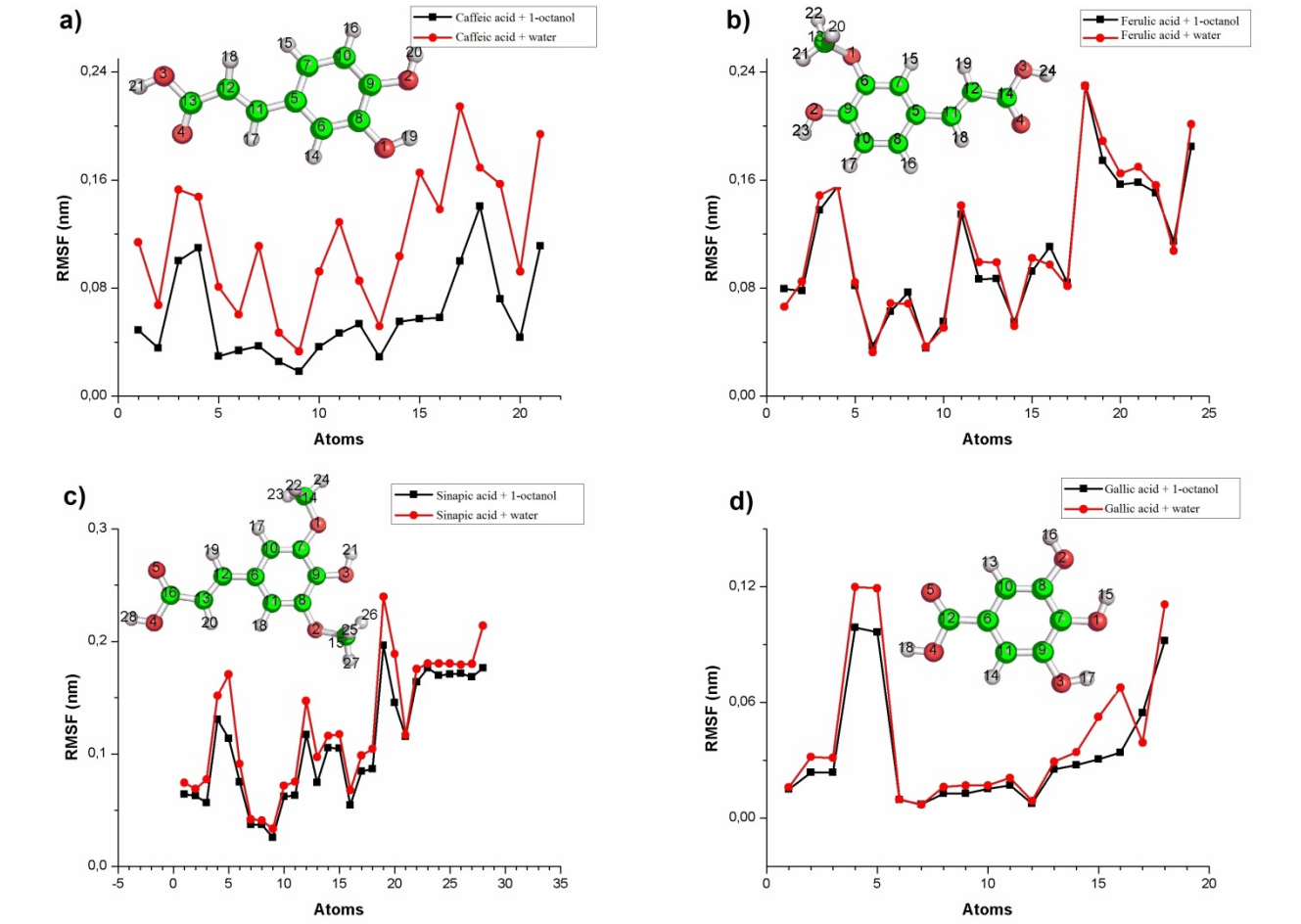

Figure 4. Quadratic mean root fluctuation (RMSF) per atom of the phenolic acid molecules. a) Caffeic acid; b) ferulic acid; c) gallic acid; d) sinapic acid.

Docking Study

In **Table 4** are shown the results of the interaction free energy of phenolic compounds with chitosan (12 meres) obtained through the molecular docking study, besides the torsional energy of the ligands and energy of the electrostatic interactions. Lee et al. [33] investigated the antioxidant activity of sinapic, gallic and ferulic acids alone and conjugated with chitosan. The results proved that the antioxidant capacity of the conjugates was superior to the antioxidant activity of the chitosan alone. The study published by Lee and co-workers also showed that the conjugates have good antimicrobial activity when tested in food pathogens, as well as good cytocompatibility in hepatic cells of mice. In another study conducted by Panwar et al. [34] the antifungal activity of chitosan microcapsules with conjugated ferulic acid was tested. In the Panwar study it was observed that the microcapsules have good activity against *Candida albicans*. Among the phenolic compounds targeted by this study, caffeic and gallic acids were the ones that obtained lower binding energy, being more stable in complexes with chitosan, but the interaction energies of sinapic acid and ferulic acid were also strong.

In **Figure 5** it can be observed five hydrogen bonds between caffeic acid and chitosan through the -COOH and -OH group of caffeic acid and the -OH and -O- groups of chitosan. It can also be observed that the interaction between the hydroxyl of the carboxylic acid and the chitosan occurs with the shortest distance. It can also be observed that the

formation of two hydrogen bonds between sinapic acid and chitosan occurs, one through the -COOH group with -OH group of chitosan and another through the -OH group of sinapic acid with a -O- of chitosan. It is also observed that two repulsive polar interactions occur between the oxygen of the carbonyl of the sinapic acid with -OH and -O- groups of the chitosan. Gallic acid promotes five hydrogen bonds with chitosan, one through the -COOH group of gallic acid with the -OH group of chitosan and another four through the phenolic -OH groups of gallic acid with a -OH and -O- of chitosan.

Table 4. Result of the study of docking of phenolic compounds with chitosan of 12 mer (kcal/mol)

Complexes	Interaction free energy (ΔG interaction)	Vdw_hb_desolv energy (ΔG vdw+hb+desolv)	Electrostatic Energy	Torsional Energy
Ferulic acid + Chitosan	-2.11	-3.66	0.06	1.49
Caffeic acid + Chitosan	-2.95	-4.10	-0.34	1.49
Sinapic + Chitosan	-2.52	-4.06	-0.24	1.79
Gallic acid + Chitosan	-2.88	-3.87	-0.05	1.49

The present study is close to the experimental study carried out by Rosa et al. [35] which, when characterizing microcapsules with conjugated gallic acid through FTIR and NMR spectra, identified possible hydrogen bonds between the phenolic hydroxyls of gallic acid and chitosan. Ferulic acid promotes two hydrogen bonds with chitosan, one through the -COOH group of ferulic acid with -O- group of chitosan and another through the -OH group of ferulic acid with a -O- group of chitosan. It can also be observed that the two hydrogen bonds have the same distance. In the study published by Panwar and collaborators [34], where microcapsules of chitosan with conjugated ferulic acid were developed, the authors propose the possible electrostatic interactions between the carboxylic group of ferulic acid and chitosan amines. The results obtained by molecular docking show that there are polar interactions between the carboxyl group with polar groups of chitosan.

Another important detail, presented in **Figure 6**, is that the caffeic, ferulic and sinapic acids bond in the same site of the chitosan. Gallic acid, however, was bond up somewhere else. In the docking simulation, it was observed that caffeic and gallic acids presented greater stability when complexed with chitosan, due to lower binding energy, but it should be taken into account that sinapic and ferulic acids also have attractive interaction. It was also observed that in all cases they had hydrophilic interactions, which shows the importance of the polar groups of phenolic compounds for interaction with chitosan.

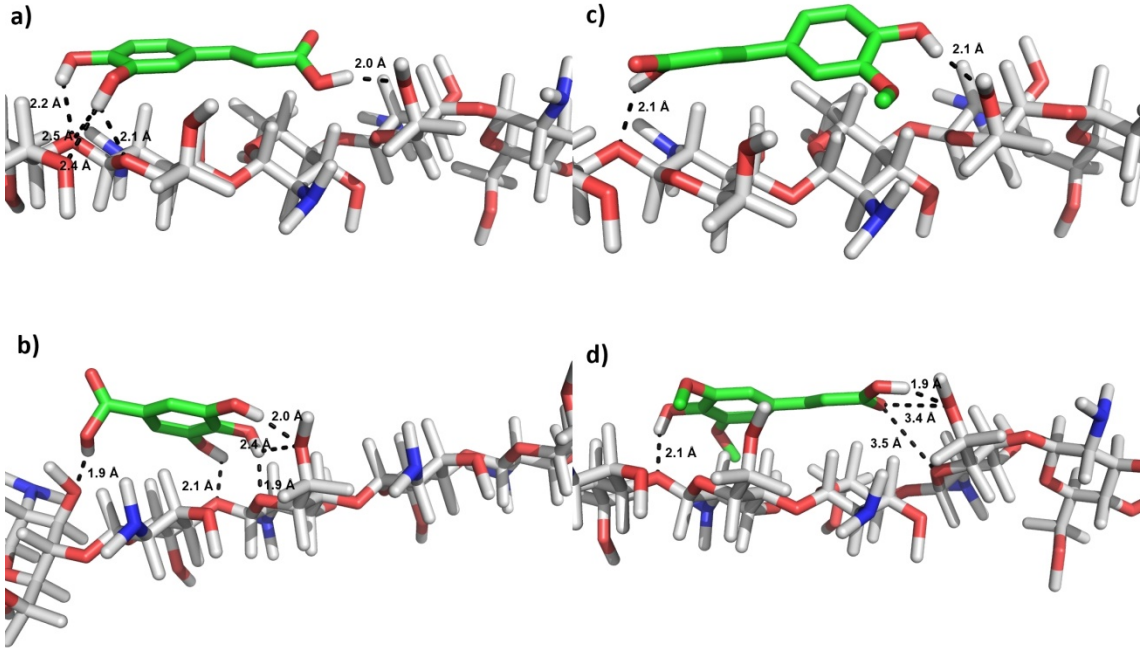

Figure 5. More stable conformation of phenolic acids in complex with 12 mers chitosan.a) caffeic acid; b) gallic acid; c) ferulic acid; d) sinapic acid.

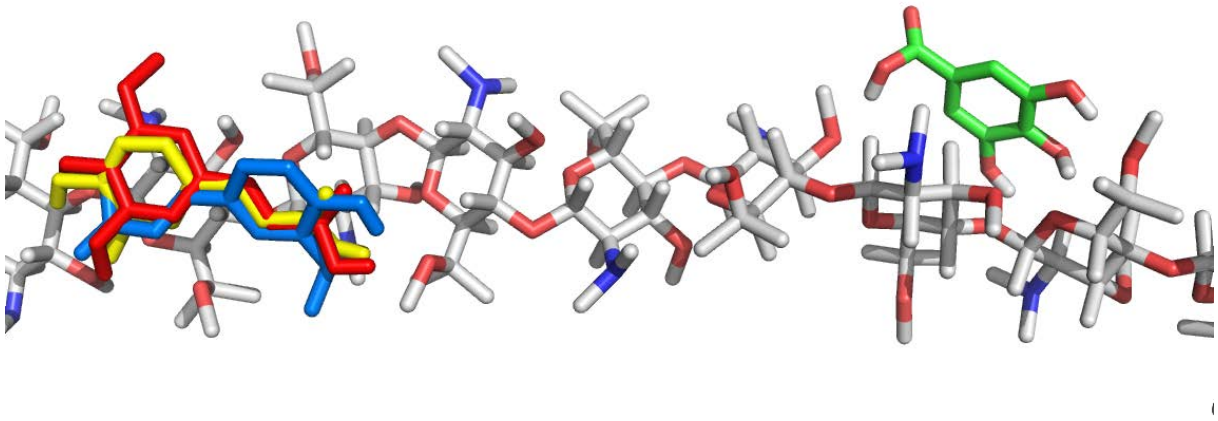

Figure 6. Overlap of sinapic acid, ferulic acid and caffeic acid complexed with chitosan. The caffeic acid is represented in yellow; Ferulic acid in blue and sinapic acid in red.

In **Table 5** are shown the results of the interaction free energy of phenolic compounds with chitosan (nine meres) obtained through the molecular docking study, as well as the torsional energy of the binders and energy of the electrostatic interactions obtained in the docking study. As can be seen in **Table 5**, all the compounds interacted with the chitosan in an attractive manner, with caffeic and ferulic acids having the lowest energies. It is noteworthy that gallic and sinapic acids also presented negative energies, which represents the attractive interaction between the compounds and the chitosan of nine meres.

Figure 7 shows the most stable conformation of the interaction of phenolic acids with chitosan. It is observed that caffeic acid interacts by hydrogen bonds through the phenolic hydroxyl and carboxylic hydroxyl with chitosan. No interaction of hydrogen between ferulic acid and chitosan was observed through the docking study, however, it is observed that the hydrogens of hydroxyls are directed to the oxygen of chitosan, characterizing polar interactions between ferulic acid and chitosan. As with chitosan of 12 meres,

sinapic acid also promotes polar interactions with chitosan nine meres. As in the docking study between gallic acid and chitosan of 12 meres, gallic acid also promoted hydrogen bonding between the phenolic hydroxyls and chitosan, with distances varying from 2.1 to 2.8 Å.

Table 5. Result of the docking study of the phenolic compounds with nine mers chitosan (kcal/mol)

Complexes	Interaction free energy (ΔG interaction)	Vdw_hb_desolv energy (ΔG vdw+hb+desolv)	Electrostatic Energy	Torsional Energy
Ferulic acid + Chitosan	-2.94	-4.28	-0.15	1.49
Caffeic acid + Chitosan	-3.05	-4.45	-0.09	1.49
Sinapic + Chitosan	-2.83	-4.45	-0.16	1.79
Gallic acid + Chitosan	-2.85	-4.16	-0.19	1.49

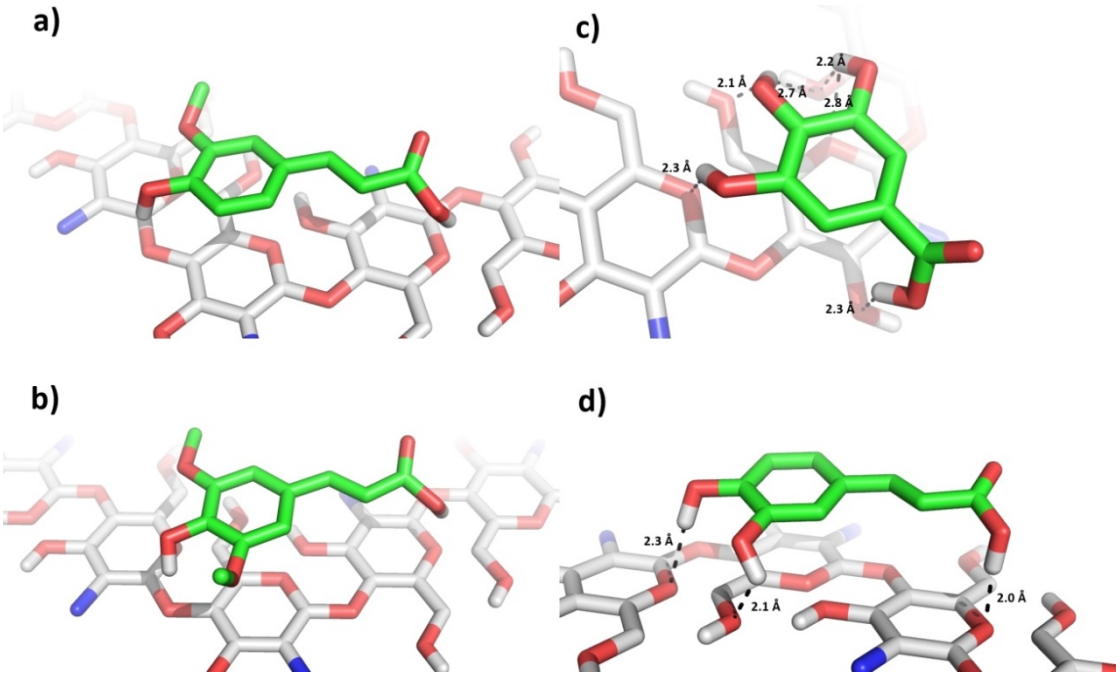

Figure 7. More stable conformation of phenolic acids in complex with nine meres chitosan. a) ferulic acid; b) sinapic acid; c) gallic acid; d) caffeic acid.

Conclusions:

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. The present study investigated the solubility of gallic, ferulic, caffeic and sinapic acids, in order to verify the behavior of these compounds in contact with aqueous biological fluids and with hydrophobic barriers, such as the plasma membrane, for example. This work also investigated the interaction of these compounds with chitosan, to understand how these substances complex with the polymer in the formation of a controlled release system of drugs (film, microcapsule and others) and active films.

It concluded from the *in silico* simulations that caffeic, ferulic, sinapic and gallic acids have adequate solubility to cross hydrophobic

biological barriers and to interact with hydrophilic biological fluids. It was also verified that the phenolic acids involved in the study are good candidates to the development of controlled release systems for active drugs, in the form of microcapsules or films, since they presented strong interaction energy when complexed with chitosan.

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Conflicts of Interest:
The authors declare no conflict of interest

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Influence of the variability of the operational parameters in obtaining cane syrup in sensorial attributes

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Abstract: Problems of variability in the design of equipment, the availability of equipment, the changes in the environment and in future changes which have been extensively investigated in order to determine the chances of success in environmental, technological and economic matters. This research considers the variability of the operational parameters in the quality of the final product, as an element in the process design, this approach is not an usual activity. Some previous studies on the production of sugarcane syrup were related to sensory attributes (viscosity, flavor and presence of crystals) with operational parameters (pH and °Brix).
It was generated the pH and °Brix using different probability distributions and the results were plotted by control charts. It was determined the influence of the variability of the °Brix in the sensorial acceptance of the final product.

Keywords: process design; quality control; uncertainly

1. Introduction

Uncertainty plays a very important role when it comes to whether or not a product meets certain specifications. For this, it must be verified whether the analytical result is within or without a "tolerance" or range of values defined in the specifications [2]. In this case, the first step will be the characterization of the probability distributions of the values of the variables and, the second is the study of the spread of uncertainties of the values of the variables, through the calculation process, using analytical methods (First-order Taylor series) or by numerical methods (Monte Carlo simulation)[5]. These models to optimization resulting will follow a stochastic system, which reflects the initial conditions plus the generated noise [6]. The Monte Carlo method is one of the many methods for the analysis of propagation of uncertainty, where the objective is to determine how a random variation in the amount of input or error affects the sensitivity, performance or reliability of the system being modeled [7].

The mathematical models proposed by [10] allow to-predict the viscosity, flavor and presence of crystals in cane syrup. These models correlate three pH levels (3.5, 4.0 y 4.5) y °Brix (74, 76 y 78) with the above quality attributes. The experimental data were obtained by sensory evaluation.

Six Sigma methodology is based on the normal distribution curve to know the level of variation of any activity. The drivers of this tool define Six Sigma as an applied quality methodology to offer a better product or service, faster and at lower cost, focusing its focus on the elimination of defects and customer satisfaction [14].

The sensorial evaluation of a goat milk yogurt with pineapple semi-fluid jelly, for which they used a hedonic scale of 5 points, (From 5: "I like it very much", going through 3: "I do not like or dislike me", Until 1: "I dislike much") was performed by [15].The sensorial analysis is one of the most important activities in the different stages of the process of manufacture of a product, development, maintenance, improvement and optimization, as well as the potential market evaluation [16] and is a scientific discipline that is used to measure, analyze, evoke and interpret reactions to some characteristics of food and materials, which are perceived by the senses of sight, smell, flavor, touch and hearing [17].

In the case of cane syrup, the viscosity, flavor, and possible presence of crystals, considered as defect, have a significant importance in the parameters of quality perceived by the consumer, at the same time as they identify and personalize the cane syrup. Determining these sensorial parameters could be subjective, so it is considered a certain degree of uncertainty. To calculate uncertainty with the approximation of ISO has the advantage that, as it has had to identify and quantify all sources of uncertainty of the analytical method, it can reduce the uncertainty of the results improve those parts of the method that contribute more to the final uncertainty of the result [19]. The objective of the present work was to determine the variability of the operating variables as an element of control of the organoleptic quality perceived by consumers.

2. Results and Discussion

The methodology was applied to the cane syrup processing process, and the following results: Were obtained: Reference was made to the publication of [10], which correlated the operational parameters °Brix and pH (coded values) with the response parameters viscosity, flavor and presence of crystals in sugarcane syrup.

The experiments performed by [10] were performed with the help of experimental design 3^2, taking as operational parameters the pH and concentration of sugars, In which obtained 3 mathematical models were for the response parameters as viscosity, flavor and presence of crystals that were measured by a sensorial analysis that is the emotional response of the consumer that is preceded by the cognitive evaluation that the user performs from what he perceives [16].

These models could be applied only using the encoding established by the author. With the experimental data reported we used the statistical tool RSM (response surface methodology). With the STATISTICA 8.0 program and with this statistical technique three statistical models were obtained (Table 1) for each of the response parameters mentioned above.

Tabla 1. Statistical models of response parameters - pH (X_1), °Brix (X_2) -

Response Parameters	Statistical Models
Viscosity	$-10806.86-37.56*X_1+0.33*X_1^2+287.14*X_2-1.90*X_2^2+0.45*X_1*X_2$
Flavor	$144.68+115.69*X_1-13.13*X_1^2-9.98*X_2+0.06*X_2^2-0.04*X_1*X_2$
Presence of crystal	$-4092.39+22.46*X_1-3.79*X_1^2+108.38*X_2-0.72*X_2^2+0.07*X_1*X_2$

With the software Arena 7.01 the probability distribution of the operational parameters was established, it was obtained that they are better adjusted distribution to the uniform probability This analysis revealed that the parameters also fit the beta distribution. The Monte Carlo method is classified as a sampling method because the quantities of inputs are randomly generated from a probability distribution in order to simulate the sampling process of a real population [7]. This method was used to generate random values of the operational parameters with the two probability distributions mentioned above. According to [19], the scalar control test in the sensory evaluation panels establishes a value of 6 for "I like very little", and 10 for "I like it very much", thus establishing the upper and lower limits of the control chart.

The beta and uniform probability distribution was applied in the Monte Carlo method to generate a thousand combinations of pH and ° Brix within the ranges 3.5 to 4.5 and 76 to 78 as proposed by [10].

With this procedure was predicted behavior of the variables response viscosity, Flavor and presence of crystals and the percentage of defects was quantified and a 30% (Fig. 1A), 48 % (Fig. 1B) y 30 % (Fig. 1C) respectively in the values generated with the distribution to probability beta.

In calculating the six sigma quality of the process we considered the simulation of the response parameters under the conditions set by [10] (Table 2) for each of the response parameters mentioned above.

Tabla 2. Sigma of the process

Response Parameters	Value
Viscosity	1.13
Flavor	1.44
Presence of crystal	1.51

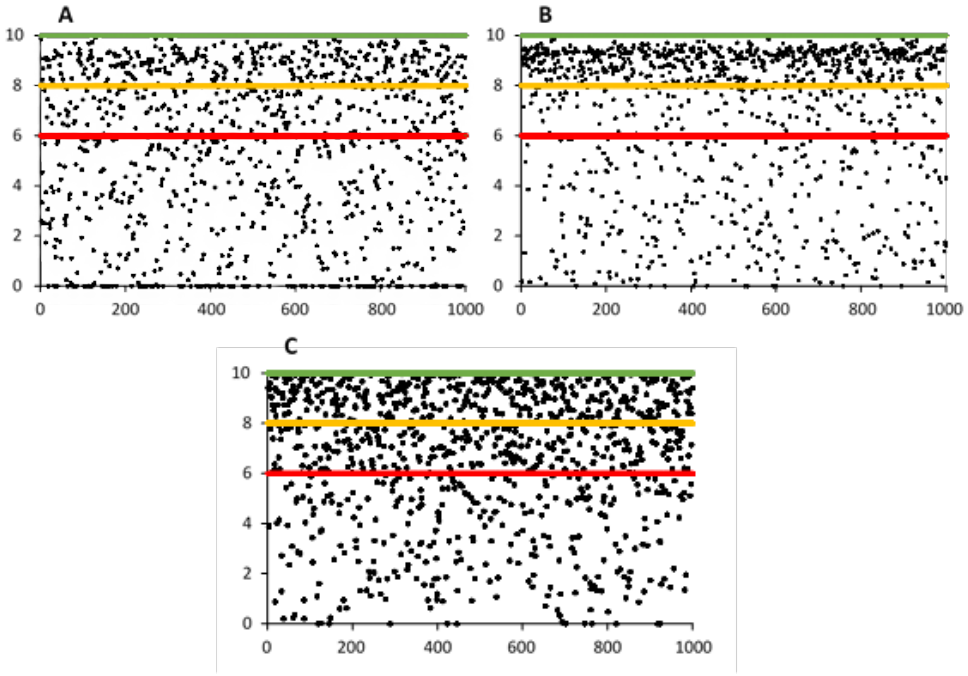

Figure 1. Control Chart of Sensory Attributes of Response Parameters. Random data generated with beta probability distribution. The data of the graphs on the left were generated with the limits of the parameters proposed by the author [10] .The data of the right graphs were generated from the limits obtained after the calculation of the uncertainty. A: Viscosity, B: Flavor, C: Presence of crystals. Hedonic scale of acceptance of the product. Maximum limit, midpoint Lower limit.

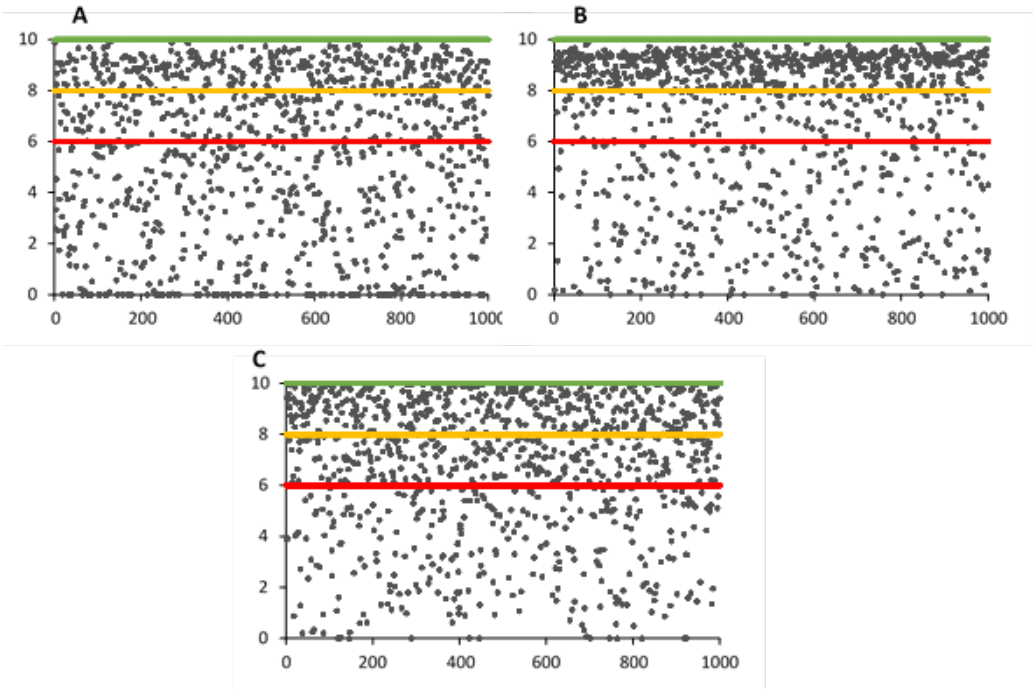

Figure 2. Control Chart of Sensory Attributes of Response Parameters. Random data generated with Uniform Probability Distribution. The data of the graphs on the left were generated with the limits of the parameters proposed by the author **[10]**. The data of the right graphs were generated from the limits obtained after the calculation of the uncertainty. A: Viscosity, B: Flavor, C: Presence of crystals. Hedonic scale of acceptance of the product. Maximum limit, midpoint Lower limit.

3. Materials and Methods

The experimental results obtained by [10] are used in the present work as starting point for the working procedure shown in Figure 3

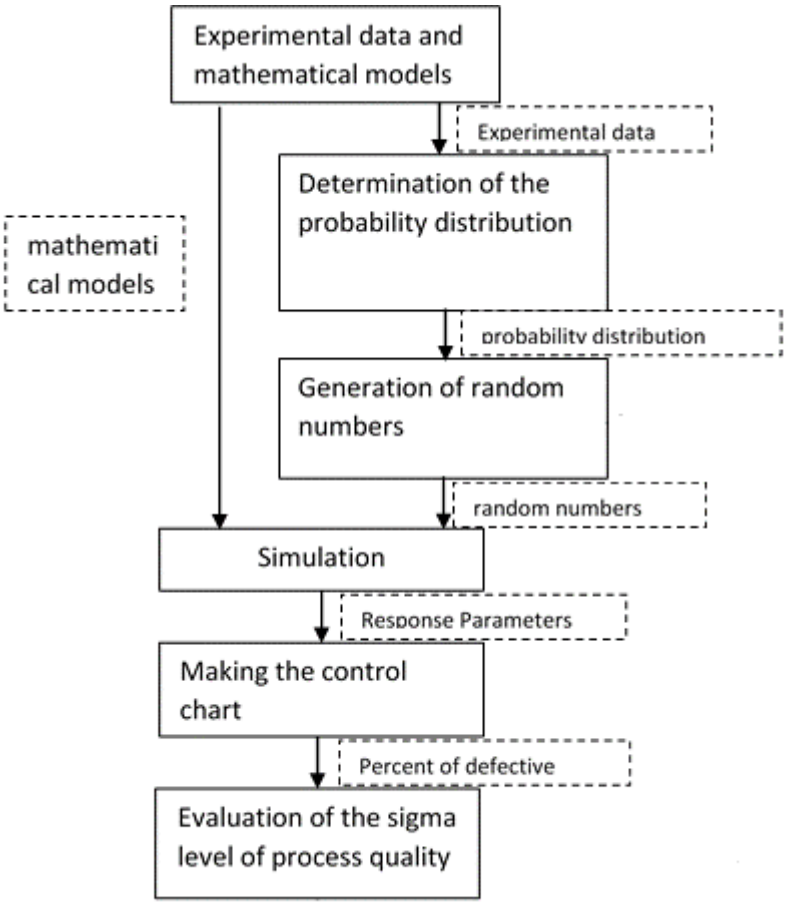

Figure 3. Scheme of applied methodology.

4. Conclusions

- On average 31% of the sensory attributes are outside the values defined as acceptable in the hedonic scale (From 6 to 10). This shows that a small variation in the operational parameters significantly affects the response parameters.
- The percentage of rejections when generating random values with Uniform distribution within the upper and lower limits of an average of (31%).
- The process of obtaining sugarcane syrup under the conditions defined in the operational parameters of pH between 3.5 to 4.5 and ° Brix between 74 to 78 establishes a sigma of the process less than 2, which causes a quantity of defective greater than 30%.

Author Contributions

All the authors contributed equally for the execution of the work and the writing of the manuscript

Conflicts of Interest

The authors declare no conflict of interest.

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Molecular Docking Studies Of Natural Phenolic Compound and Derivates With Phospholipase A2

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Abstract: The enzym phospholipase A2 (PLA2) catalyzes the conversion of membrane phospholipids in the inflammatory mediators, such as prostaglandins and leukotrienes. Because of this role, substances with inhibitory activity of PLA2 enzyme, has gained prominence in the scientific community like possible anti-inflammatory. Several studies have shown that phenolic compounds such as flavonoids, phenolic acids and other, has, among various biological activities, anti-inflammatory activity by inhibition of the enzyme PLA2. Based on this context, this study aimed to conduct a molecular docking study of various natural phenolic compounds and some of their derivatives forward to the enzyme PLA2. The crystallographic structure of PLA 2 was obtained from Target Database Protein Data Bank [PDB ID: 1KPM] and the ligands were obtained from PubChem Database. The docking was performed using the AutoDock 4.0 software. It was observed that among the phenolic compounds included in the study, those with better interaction with the enzyme were rosmarinic acid 3'-O-beta-glucoside, 4-nerolidylcatechol, rosmarinic acid methyl ester, quercetin 3-O-malonylglucoside, quercetin pentaacetate and rosmarinic acid, respectively. The present study provides a better understanding of the inhibition of PLA2 by phenolic compounds, which may contribute to the development of new anti-inflammatories.

Keywords: Phospholipase A_2, phenolic compound, molecular docking, anti-inflammatory.

1. Introduction

Phospholipase A2 (PLA2) are enzymes that hydrolyze the bond in the sn-2 position of cell membrane phospholipids and lipoprotein, in a Ca2+ dependent reaction [1,2], producing free fatty acids, precursors of various pro-inflammatory lipid mediators such as leukotrienes, prostaglandins and platelet activating factor [3,4]. The mammalian PLA2 are subdivided into two main families: the secretory enzymes with low molecular weight (sPLA2) consisting of four types (I, II, V and X), and cytosolic PLA2 high molecular mass existing as two types (IV or VI or cPLA2 and iPLA2)[5-7].

PLA2 is extremely important in the inflammation cascade by catalyzing the conversion of membrane phospholipids in free arachidonic acid by hydrolysis [8,9], Then is converted by cyclooxygenases to prostaglandins and leukotrienes, increasing the inflammatory process [10].

Due the role of PLA2 in the inflammatory process, inhibition of this enzyme has won major therapeutic interest1. Many natural compounds, such as aristolochic acid, indol derivatives, atropine, eugenol and berberine bind to the active site of the enzyme mainly by hydrophobic interactions [11].

The importance of natural products in modern medicine has been well recognized. Natural Compounds isolated from plant sources has an extensive use since the past and is still used until now in the treating of various pathologies. In addition to serving like compounds of interest in its natural form, these compounds can also serve as templates for the synthesis of derivatives with higher activity and lower toxicity [12]. Thus, molecular modeling studies PLA2 as the target can be useful in designing new and more potent anti-inflammatory drugs [11].

Among the natural substances, phenolic compounds such as flavonoids, phenolic acids and others have become very prominent due to its diverse biological activities, among them, anti-inflammatory activity by inhibiting the enzyme phospholipase A2 [1,13–15]. These compounds are secondary metabolites of plants that are involved in defense against ultraviolet radiation and pathogens [16]. These compounds have aromatic rings with one or more hydroxyl groups or methoxyl [17].

Currently, the anti-inflammatory non-steroid drugs present on the pharmaceutical market, reduces the conversion of arachidonic acid into prostaglandins by inhibiting the cyclooxygenase enzyme [18–23], however, does not inhibit 5-lipoxygenase enzyme. Thus, the excess of arachidonic acid produced is converted to leukotrienes by the 5-lipoxygenase, which in high levels are directly related to adverse effects on the gastric and renal pathways, as observed in patients who use non-steroidal anti-inflammatory drugs. Then, for an anti-inflammatory drug be effective and with less side effects, the substance may selectively inhibit the PLA2 and avoid not only prostaglandin, but also the formation of leukotrienes [24].

This study objective was to compare interaction's affinity of natural phenolic compounds with the PLA2 enzyme, because the interaction mode and other structural details of the complex formed by the phenolics compounds and PLA2 enzyme may contribute to the new and more potent anti-inflammatory development.

2. Results and Discussion

2.1 Docking Study

Based on the results the compounds that showed better interaction development with the PLA2 enzyme were the rosmarinic acid 3'-O-beta-glucoside, 4-nerolidylcatechol, rosmarinic acid methyl ester, quercetin 3-O-malonylglucoside , quercetin pentaacetate and rosmarinic acid with binding free energy of -9.68, -9.44, -8.87, -8.81, -8.79 and -8.13, respectively (**Table 1**).

The **Figure 1** shows that, with the exception of rosmarinic acid methyl ester and quercetin acetate, compounds that had better binding energy were interacting with the ASP 49 amino acid by H-bond. It can also be observed that the compounds rosmarinic acid methyl ester and quercetin pentaacetate, although it does not interact with ASP 49 amino acid, it interacts with the HIS 48 amino acid by H-bond. These amino acids interactions are important for the compounds pharmacological activity, since the ASP 49 and HIS 48 amino acids are involved in the water molecule activation, and means of

basic catalysis, hydrolyze phospholipids at the sn-2 position [24]. Since amino acids TYR 28, GLY 30, GLY 32 and ASP 49, are involved in the coordination of calcium ion are responsible for polarization the correct positioning of the phospholipid ester carbonyl providing a region suitable for water molecule nucleophilic attack [15,24]. As can be seen, the rosmarinic acid 3'-O-beta-glucoside promotes interaction with the amino acids GLY 30 and ASP 49, the 4-nerolidylcatechol in the ASP 49, quercetin 3-O-malonylglucoside with GLY 32 and ASP 49, quercetin pentaacetate with GLY 30 and the rosmarinic acid with the ASP 49. Only the compound rosmarinic and acid methyl ester does not promote any polar interaction with an amino acid involved in the calcium ions coordination.

The compounds that presented binding energy more favorable were interacted to the amino acids essential for the enzyme catalytic activity, those are responsible for the water molecule activation (HIS 48 and ASP 49) or responsible for coordinating ion Ca2 + (TYR 28, GLY 30, GLY 32 and ASP 49) they can be considered as PLA2 inhibitors[11].

Table 1. Docking energies of phenolic compounds against the enzyme phospholipase A2

Compound	**ΔG Binding (Kcal/mol)[a]**	**ΔG vdw_hb_desolv**	**ΔG Eletrostatic**	**ΔG Total Internal**	**ΔG Unbound energy**	**ΔG Energy torsional**
Quercetin	-7.06	-8.42	-0.42	-1.12	-1.12	1.79
4-Nerolidylcatechol	-9.44	-11.96	-0.46	-1.09	-1.09	2.98
Caffeic Acid	-5.75	-6.95	-0.29	-0.47	-0.47	1.49
Ferulic Acid	-5.54	-5.82	-1.21	-0.48	-0.48	1.49
Sinapic Acid	-5.95	-6.48	-1.26	-0.85	-0.85	1.79
Rosmarinic Acid	-8.13	-10.92	-0.79	-1.24	-1.24	3.58
rosmarinic acid 3'-O-beta-glucoside	-9.68	-14.17	-0.58	-4.17	-4.17	5.07
Rosmarinic Acid Methyl Ester	-8.87	-12.12	-0.03	-0.92	-0.92	3.28
Rosmarinyl glucoside	-7.62	-12.74	-0.25	-1.97	-1.97	5.37
Quercetin 3-Methil Ether	-7.30	-8.6	-0.49	-0.88	-0.88	1.79
Rutin	-2.87	-7.21	-0.42	-2.01	-2.01	4.77
Retusin	-7.71	-9.08	-0.42	-0.65	-0.65	1.79
Amentoflavone	-5.62	-7.9	-0.4	-1.63	-1.63	2.68
Gallic Acid	-4.43	-5.18	-0.74	-0.74	-0.74	1.49
Quercimeritrin	-7.76	-10.97	-0.37	-0.48	-0.48	3.58
Quercetin pentaacetate	-8.79	-11.9	-0.17	-0.24	-0.24	3.28
Isoquercetin	-7.24	-10.49	-0.33	-1.14	-1.14	3.58
Quercetin 3-O-malonylglucoside	-8.81	-13.23	-0.35	-0.67	-0.67	4.77

[a] ΔG binding = ΔG vdW+hb+desolv + ΔG elec + ΔG total + ΔG tor - ΔG unb.

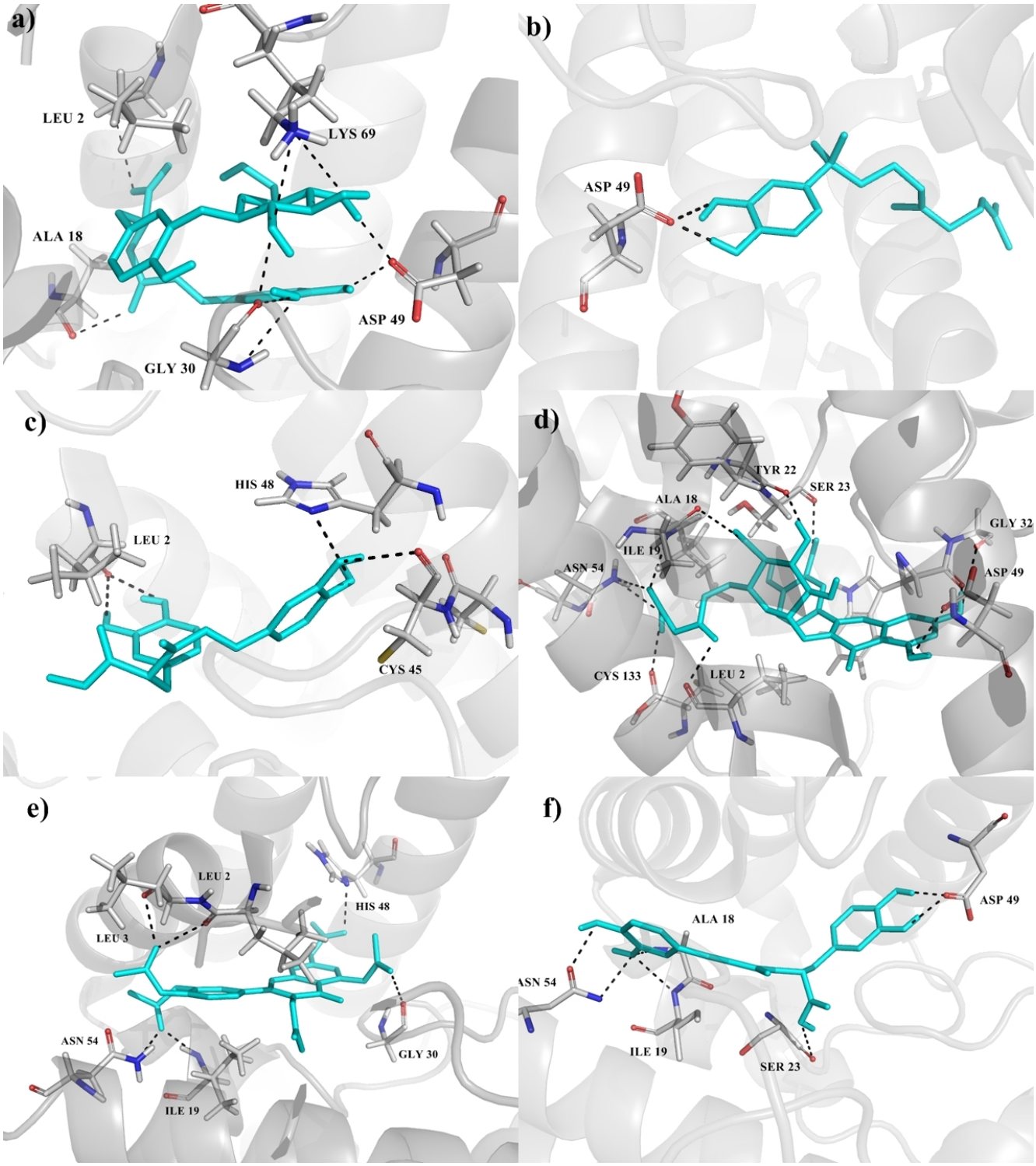

Figure 1 Hydrogen bonds of phenolic compounds **a)** rosmarinic acid 3'-O-beta-glucoside, **b)** 4-nerolidylcatechol, **c)** rosmarinic acid methyl ester, **d)** quercetin 3-O-malonylglucoside, **e)** quercetin pentaacetate e **f)** rosmarinic acid with amino acids at the active site of PLA2

Figure 2 shows that all the ligands that are more favorable with docking energy, fits perfectly in active site of the PLA2 enzyme, which also promotes hydrophobic interactions, as well as hydrophilic interactions.

It can be noted that the phenolic hydroxyls have a great importance to the interaction of

compounds with the active site of PLA2, however, was not observed any relation in the amount of hydroxyl with the interaction energy. It was also observed that replacement of the phenolic hydroxyls by other polar groups, as in the quercetin's case pentaacetate, can maintain or improve the interaction efficiency of the compound with the enzyme active site. The quercetin pentaacetate is a semisynthetic quercetin derivative that were obtained by acetylation [31,32] and presented a better interaction with the active site of PLA2 than its prototype, quercetin. But unfortunately it was not found in the literature about their inhibitory activity of PLA2.

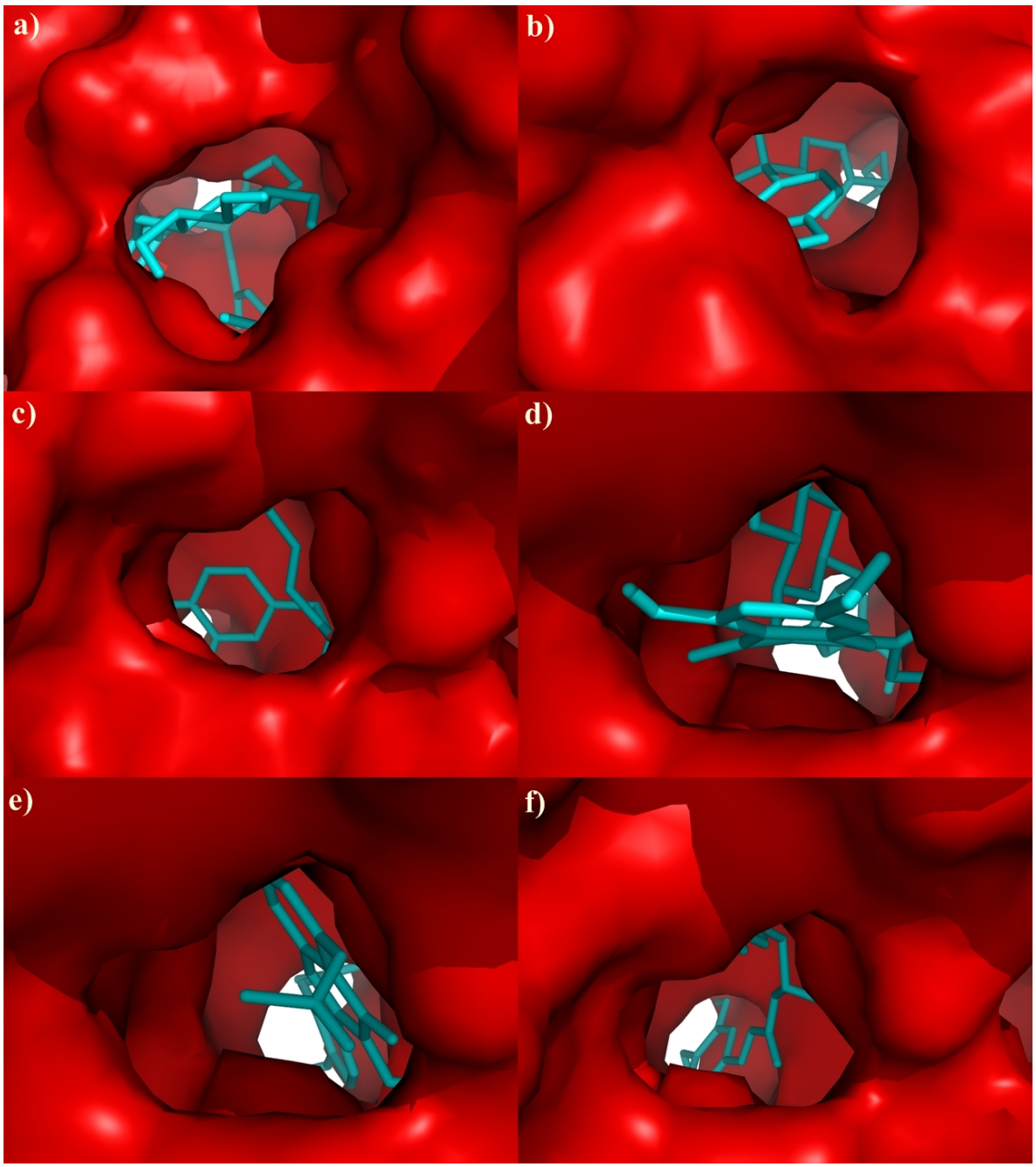

Figure 2. Compounds **a)** rosmarinic acid 3'-O-beta-glucoside, **b)** 4-nerolidylcatechol, **c)** rosmarinic acid methyl ester, **d)** quercetin 3-O-malonylglucoside, **e)** quercetin pentaacetate e **f)** rosmarinic acid in active site of PLA2.

2.2 Validation of the Methodology

The redocking presented value of RMSD = 1.89 Å, considering the pose most stable of the

most populous cluster. This result is considered satisfactory when the RMSD (which measures the deviation) between the best pose and the ligand complexed crystallographic is less than 2.0 Å [33]. Thus, the value displayed at this step validates the conditions used for the present docking study.

3. Materials and Methods

3.1 Preparation of target protein

The crystallographic structure of the target enzymatic PLA2 was obtained from the Protein Data Bank database [PDB ID: 1KPM] [25]. This structure was chosen because it has been used in other molecular docking studies [9,26]. The enzyme was elucidated by X-ray crystallography, with a resolution of 1.80 Å. Gasteiger charge and polar hydrogens required for the potential calculations were added considering the target structure, and the water molecules removed.

3.2 Preparation of ligand molecules

All the structures of ligands were obtained by PubChem Database [27] (https://pubchem.ncbi.nlm.nih.gov/). They were included in the study 18 structures of natural phenolic compounds and some of its derivatives (**Table 2** and **Figure 3**). The molecules of the binders were optimized by quantum mechanical, semi-empirical method PM6 [28], with software MOPAC7 [29]. The ligands non-polar hydrogens were suppressed and torsional links of each binder were set automatically.

Table 2. Natural phenolic compounds included in the study

Compound	IUPAC Name	PubChem CID
Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	5280343
4-Nerolidylcatechol	4-[(6E)-3,7,11-trimethyldodeca-1,6,10-trien-3-yl]benzene-1,2-diol	5352089
Caffeic Acid	(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid	689043
Ferulic Acid	(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid	445858
Sinapic Acid	(E)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid	637775
Rosmarinic Acid	(2R)-3-(3,4-dihydroxyphenyl)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxypropanoic acid	5281792
rosmarinic acid 3'-O-beta-glucoside	(2R)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-[4-hydroxy-3-[(2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]propanoate	25245848
Rosmarinic Acid Methyl Ester	methyl (2R)-3-(3,4-dihydroxyphenyl)-2-[3-(3,4-	3012090

	dihydroxyphenyl)prop-2-enoyloxy]propanoate	
Rosmarinyl glucoside	(2R)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-[3-hydroxy-4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]propanoic acid	11606086
Quercetin 3-Methyl Ether	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one	5280681
Rutin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	5280805
Retusin	2-(3,4-dimethoxyphenyl)-5-hydroxy-3,7-dimethoxychromen-4-one	5352005
Amentoflavone	8-[5-(5,7-dihydroxy-4-oxochromen-2-yl)-2-hydroxyphenyl]-5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one	5281600
Gallic Acid	3,4,5-trihydroxybenzoic acid	370
Quercimeritrin	2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	5282160
Quercetin pentaacetate	[2-acetyloxy-4-(3,5,7-triacetyloxy-4-oxochromen-2-yl) phenyl] acetate	14005
Isoquercetin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	5280804
Quercetin 3-O-malonylglucoside	3-[[(2R,3S,6S)-6-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxochromen-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-3-oxopropanoic acid	44259188

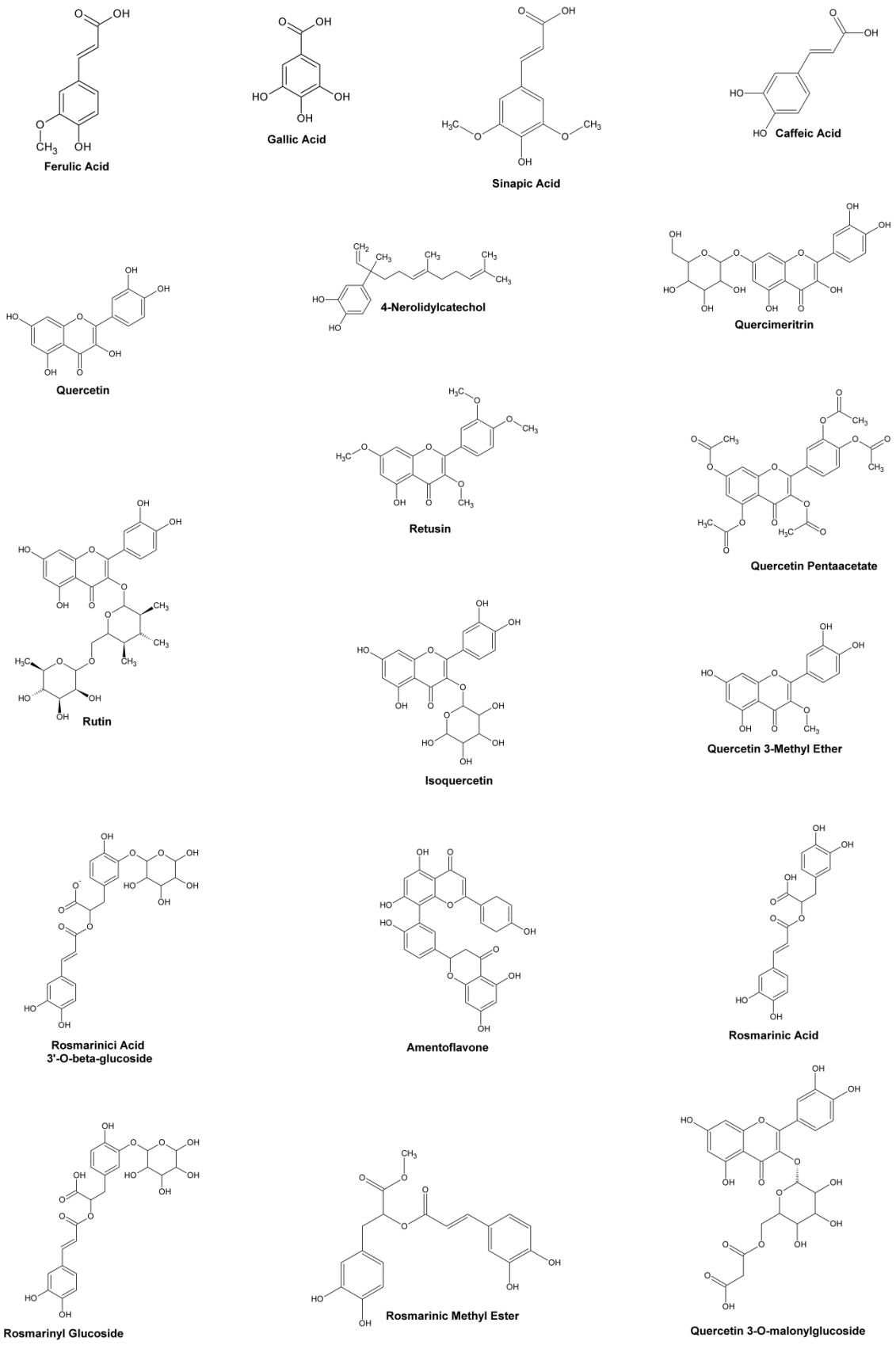

Figure 3. Structure of phenolic compounds involved in the study

3.3 Docking Study

The *AutoDock 4.0* [30] software The AutoDock 4.0 software it was used like choice for conducting studies on PLA2 target. The AutoDock Tools module was used to prepare and analyze the computer simulations. The AutoDock require three-dimensional maps pre-calculated, arranged in a housing composed of a three-dimensional grid points (grid maps) in a defined region in the macromolecule (target site). The AutoGrid 4.0 program was used to generate the maps for the ligands. The box was placed in the catalytic region of the enzyme. The dimensions of the box in the X-, Y- and Z-axis were respectively 52 Å 60 Å and 48 Å spacing of 0.375 Å. The Lamarckian Genetic algorithm (GA-LS) was chosen to search for the best conformations 100 runs for each ligand (genetic algorithm with local search). During the search process, the enzyme was kept 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 power ratings chosen, was 25,000,000. The maximum number of generations was 27,000. The number of elitism chosen was 1. The gene mutation and crossover rates were defined respectively as 0.02 and 0.80. At the end of the calculations, 100 different poses were obtained and grouped into different clusters defined by proximity and energy RMSD values ("root mean square deviation") in accordance with the default of AutoDock.

3.4 Validation of the Methodology

The validation of the method was performed by technique of redocking, using the same target protein and its complexed ligand ("native"): vitamin E.

4. Conclusions

This study aimed to investigate, through molecular docking, affinity interaction of various natural phenolic compounds and some of their derivatives with phospholipase A2, and see how this interaction occurs in order to contribute to the development of new drugs with anti-inflammatory potential.

It was observed that compounds which showed the best energy interaction with the active site of the enzyme interacted with the amino acids essential for the enzyme catalytic activity, and even if the phenolic hydroxyl groups are essential for interaction of compounds, there was no relationship between the amount hydroxyls with the energy of interaction. Another important observation was regarding the phenolic hydroxyls replacement of the flavonoid quercetin with other polar groups, such as the quercetin pentaacetate case, which showed better interaction energy when compared with quercetin.

Acknowledgments

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Author Contributions

Main text paragraph.

Conflicts of Interest

The authors declare no conflict of interest.

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COENUROSIS AN EMERGING DISEASE IN WILD RABBITS

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Abstract: The emerging diseases in the different animal species require the necessary attention, to carry out actions that diminish their diffusion in animal populations. The aim of this study is to provide a current reference on coenurosis a wild rabbit (hare), and determinants of its presentation on animal health, which can put at risk man´s health. A sample of muscle tissue was evaluated and a hydatid cyst (3 x 6 cm) was obtained from a wild rabbit. The tissue was punctured, extracting transparent aqueous liquid discarding an abscess or hematoma. Content analysis showed the presence of many scolices, corresponding to coenuro *Taenia serialis*. In the histological preparation it was observed: muscle tissue, connective tissue fibroblasts and an inflammatory surrounding area composed of eosinophils and lymphocytes which in turn limited the parasite scolices. This finding representes an important condition to evaluate the natural environment of wild animals in the area, due to the risk of dissemination and contamination of plants and animals of the área, even though, may not be of of public health importance, unless meat of wild rabbits gets eaten.

Keywords: *Taenia serialis;* coenurosis; wild rabbit; emerging disease.

1. Introduction

Domestic and wild carnivores are important reservoirs of tapeworms that affect hunting animals and other wildlife animals, it is well recognized that directly or indirectly carnivores play an important role as ecological routes of transmission of tapeworms to humans and pets, therefore, tapeworms contained in carnivores are important not only for parasitologists but also for veterinarians, biologists and physicians.

Ecological biology, pathogenicity, prevention and other aspects of the tapeworm, is very dispersed, knowing more about its phylogenetic position, hosts classes and geographic distribution.

Tapeworms of carnivores require two or more different species of hosts to complete their life cycles. The coenurosis is caused by consumption of food contaminated by dogs´ feces or other feces of hosts carriers of tapeworm. The rabbit´s coenurosis is a metacestodosis localized in muscle and subcutaneous tissue, produced by *Coenurus serialis,* a small intestine parasite of dogs and other canids. It measures 20 to 72 cm long. The larval stage of the tapeworm serialis has a prepatent period of 7 to 14 days, which is acquired by eating raw viscera containing coenuros.

The aim of this study is to provide a current reference about the pathology of coenurosis in hares (wild rabbit), and determinants of its presentation on animal health, which can be of risk to human health, as well.

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2. Materials and Methods

Case review.

According to the person submitting the sample of muscle tissue - hydatid cyst to study; this sample was obtained from a wild rabbit near the ecological park area: Nevado de Toluca, which at that time was trapped and caught in a trap; apparently the animal did not show any clinical signs, and was sacrificed in order to take advantage of his flesh for feeding the family, however, at the time of eviceration no alteration was seen, except an alteration located on the right thigh level of the animal, which measured 3 x 6 cm long, surrounded by soft connective and muscular tissue (Figures 1 and 2). The sample preparation, was done with 10% buffered formalin, for further histopathology, clear liquid was obtained containing soft mass and lots of

scolices, the preparation ended with hematoxylin and eosin staining.

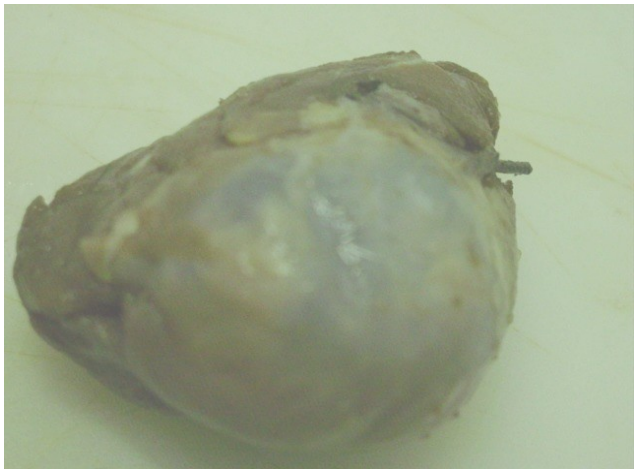

Figure 1. Hydatid cyst surrounded by muscle tissue of soft consistency

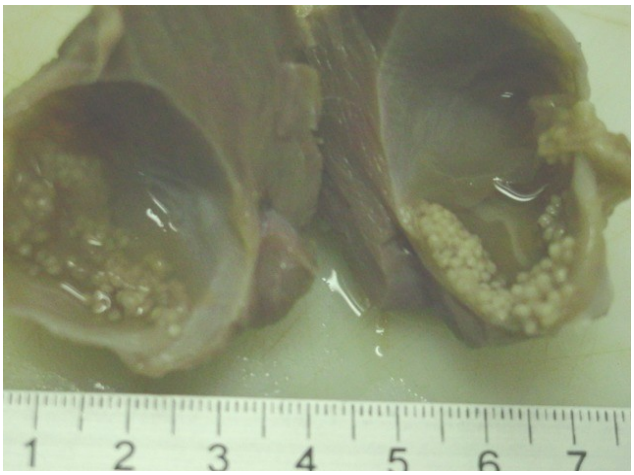

Figure 2. Sagittal section of hydatid cyst containing scolex of Taenia

Histopathology

In the prepared tissue, it was observed: muscle tissue, connective tissue and fibroblasts surrounding an inflammatory area composed of eosinophils and lymphoctes limiting to scolices of the *Tapeworm serialis* parasite (Figures 3 and 4).

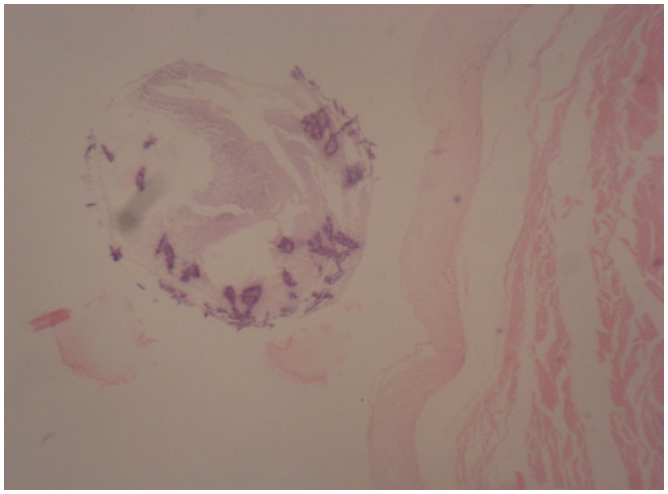

Figure 3. Histological section of muscle mass - cyst. Presence of capsule: fibroblasts and connective tissue surrounding scolex. H & E. 10X.

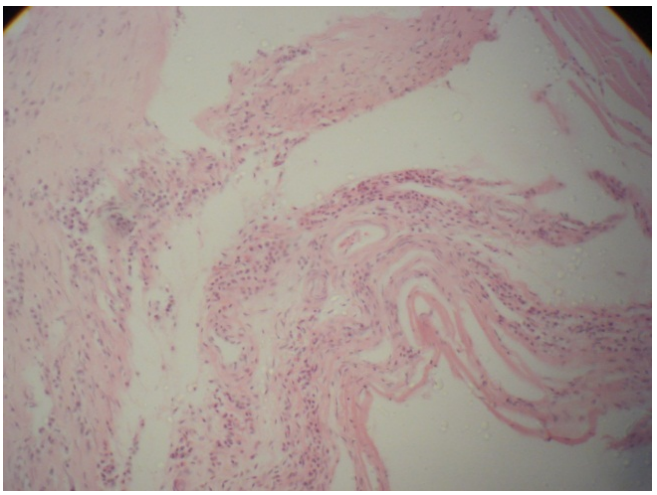

Figure 4. Histological section muscle mass - cyst. Inflammatory reaction (eosinophils - lymphocytes). H & E. 10X.

3. Discussion and Conclusion

A case of a rounded sample of muscular tissue, with an increased volume of 10 cm diameter was localized in the right thight area of a wild rabbit, a soft mass was felted with complete delimitation of all edges.

For the diagnosis, it is usually examined the content of the nodules, once the diagnosis is confirmed coenuros can be removed surgically. At the beginig of diagnostic, a puncture was performed on the tissue, extracting transparent aqueous liquid, discarding an abscess or hematoma. Content analysis was observed and revealed the presence of many scolices, corresponding to coenuro *Tenia serialis*.

Coenurosis in connective tissue of leparopidae is caused by the larval stage of *Taenia serialis. C serialis* is similar to *C. multiceps*, but can be distinguished by the arrangement of the cephalic invaginations in linear position. The scolex measures from 900 microns to 1.3 mm, tends to form daughter internal and external vesicles. This cestodosis has a cosmopolitan distribution, lagomorphs are used as intermediate hosts, as well as certain rodents and weasels, as well as goats and rarely man, where coenuro develops. It has also been described in cats as an accidental host.

Coenurosis is caused by the consumption of food (fodder), proceeding from meadow contaminated by proglotides of dogs or other hosts of tapeworm carriers of *Taenia serialis.* The transmission is similar to that of cysticercosis, however the endogenous pathway migration does not occur by the hepatic parenchyma, so oncosphere spread through the liver by the general circulation linfohemática for distribution throughout the body. Coenuros tissue as the transparent vesicles very variable dimensions from 2 to 15 cm in diameter develop, according to their location and numerous scolex inside. They reach their infective capacity within 2 to 3 months post ingestion and their longevity is at least two years. Cysts develop in the connective tissue and muscle fascias of hares and rabbits, especially in the neck, back, chest, as well as in kidneys, peritoneum and occasionally in spinal cord and brain.

Clinical signs are due to comprehensive action of coenuros and vary according to their location. Coenuros located in muscle can be compressed and elongated, while subcutaneous can reach large dimensions and could be clinically diagnosed by palpation, where they can be appreciated as cold and easily movable nodules.

This tapeworm infection takes place when the eggs of *Taenia serialis* contained in the small intestine of dogs are ingested, the cenuro reaches its development in three months, the dogs are infected by eating infected rabbits with *C. serialis.* Clinically coenurosis of subcutaneous connective tissue manifests with a soft, cold, painless, mobile rounded swelling, reaching different areas of the body such as chest wall, back, thighs and head. When it occurs in the head region, causes mandibular deviation, other cenuros are transformed into abscesses causing hot and painful injurier when rubbed; when they appear in the eyes clinically ophthalmia occurs. There may also be paresis or paraplegia when infestations occur in subarachnoid, causing spinal cord compression. When the parasite is presented in subpleural tissue, there are

respiratory problems such as shortness of breath. The general health is unaffected except in heavy infestations.

The parasitic infection due to *Coenurus serialis* in subcutaneous tissue of rabbits and hares, clinically presentes subcutaneous swelling with the size of a lemon. At the necropsy vesicles are in various locations, wrapped in a membrane perivascular by host reaction. Sometimes there are lesion tissue necrosis due to the action of the vesicular fluid which diffuses and has cytotoxic action are observed.

O'Reilly *et al.* (2002) reported a case of ocular location which originated exophthalmia from the right eye in a rabbit; literature report cases by these authors and this case are limited, therefore, such diseases should be considered a risk factor not only for animal health but also to humans.

Usually, the diagnosis is made at necropsy by observing and identifying characteristic vesicles. Specific treatment is not known; praziquantel could have an effect, it could be useful for preventing this pathology to avoid green fodder contaminated with feces of dogs, or throw dead rabbits outdoors.

Because there is no pharmacological treatment for coenurosis it is important to take preventive measures in dogs, avoid feeding raw viscera containing cysts, as well as periodic and timely treatment against taenia. In domestic animals and in free-living (wildlife), there is a risk of contamination of various diseases. Parasitic diseases originate in animals as long as the parasite is maintained in any of the natural hosts, either intermediate or final hosts, which should be a precedent for the attention and care of wildlife specialists.

This present finding detected in hares, is an important condition to evaluate the natural environment of animals in the area, it represents an important case study because of the risk it represents for the animals of that area, contagion and contamination of the vegetables consumed by animals that coexist in it, until now considered as not of public health importance.

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An approach to Trypanosoma cruzi Vaccine through the epitope prediction from proteins surface with IEDB

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ABSTRACT

Chagas disease is one of the most important in America transmitted by Trypanosoma cruzi diseases with approximately 7 million people at risk, most of them from Latin American. Due the non-availability of an ideal drug or treatment, development of an effective, and affordable vaccine could be a solution for control and prevention of this disease. In this study, use an bioinformatic approach to predict possible epitopes of the candidates with help of MHC-II Binding Predictor from IEDB,using the prediction method of recommended in IEDB and the set from Allele Class II from DbMHC and allelefrequencies.net with maximal population coverage, we analyze 10 sequence of surface protein expressed in *Trypanosoma cruzi* in its three different stages present in the human body ,and keep the only ones with allotypes referring to the Latin American. A prediction of 70,000 epitopes per protein was obtained which were classified into three groups according to the shared epitopes, where the cruzipain belongs to a single group as it does not present similar epitopes with the other proteins. The first group contains the proteins Asp-3, Asp-2, Gp85, Gp90, Tc85, Sa85 with 17 shared epitopes and a population coverage of 87.89%. The second group Asp-3, Gp82, Gp83 with 31 shared epitopes and 87.89% population coverage. Because Cruzipain is not sharing any epitope, was selected the largest number of replicates contained in the same protein with a coverage above of 80 %. The selected epitopes are going to be synthesized to evaluate their potential as a possible vaccine against *Trypanosoma cruzi.*

INTRODUCTION

Trypanosoma cruzi is the causative agent of Chagas disease an endemic pathology in Latin America and a huge public health problem, it is transmitted by insects from the family Triatoma, Panstrongylus y Rhodnius. This protozoan is a hemoflagellate parasite that develops in three different cellular forms: amastigote, trypomastigote and epimastigote. Approx 7 to 8 million peoples are infected and 40 million are in risk of take the Chagas disease (Coura, 2007; Yun et al, 2009; WHO,2014) *T.cruz*i is one of the most successful pathogens due to its capacity for infection, survival and persistence in mammalian hosts. A key step for *T. cruzi* persistence is the stage of invasion express different adhesion molecules on its surface, such as mucins, trans-sialidases and other glycoproteins that allow it to enter the host cell.

These molecules are expressed in the different cellular forms of the parasite and are essential for the host-parasite interaction. *T.cruzi* have in their different stages changes the molecules in their surface this characteristic allows the parasite infect a differences types of cells and brings protection from the immune system of the host (Buscaglia, Campo et al. 2006). In the first stage *T.cruzi* enter to the host in the form of trypomastigote Metacyclic rising the Ca+ surrounding. In this form penetrate the tissue through surface glycoproteins with a negative charge (Scharfstein, Schmitz et al. 2000); while the invasion of the host cell is realized the

trypomastigote create a vesicle becoming in amastigote form and replicate in the cytoplasm. The cell invasion can be classified in three stages: adhesion and recognize - signaling, and invasion (Málaga and Yoshida 2001).

They are currently known different glycoproteins that are expressed in the surface and have adhesion properties expressed in trypomastigote metacyclic like gp90, gp82, gp30 and gp83/50(Yoshida 2006) this type of proteins represents 1% of genome of *T.cruzi* and can be found in other stages of the life cycle of this parasite, also this protein can be classified like trans-sialidases. Trans-sialidases are expressed by trypomastigote and are anchored by glycosylphosphatidylinositol (GPI) to the parasite plasma membrane.

In the form of epimastigote the gp85 is expressed in the membrane this superfamily gp85/TS, have a subgroup of glycoproteins that have a role in the process of adhesion and invasion (VALENZUELA, SEPÚLVEDA et al.).

Stage	Protein	Entry
Trypomastigote	Gp90	Q8M369
	Tc85	O77209
	Gp85	Q03877
	Gp82	A9XBF2
	Gp83	P90604
Metacyclic trypomastigote	SA85	P18269
Amastigote	ASP-2	P90605
	ASP-3	B5U6T8
	ASP-4	B5U6T9

Epitope prediction MHC II

The innate immune system reacts quickly against several compounds supposed to be foreign or very rare in a healthy and uninfected individual is able to very specifically react against proteins and peptides specific for pathogenic cells and foreign organisms.

Epitopes were originally defined as the part of an antigen that defines the binding to an immunoglobulin (Huang and Honda 2006). Antigen is generally a processed part of a protein in complex with an MHC protein.is which part of a protein (peptide) is responsible for an immune response. Thus, often this part is referred to as the epitope and the native protein from which the epitope originated as the antigen.

MHC binding prediction methods are today of a very high quality and can predict MHC binding peptides with high accuracy. This is possible for a large range of MHC alleles and relevant length of binding peptides (Lundegaard, Lund et al. 2012).

There are several programs for the prediction of affinity of the epitopes with the MHC but the Immune Epitope Database is considered the most complete. The Immune Epitope Database (IEDB) incorporates more than 120,000 curated epitopes, most of which are extracted from scientific publications and, in contrast to SYFPEITHI, includes also a lot of data on synthetic peptides (Vita, Overton et al. 2015). The identification of specific epitopes can define the most important fragments of sequence in a protein to lead new treatments. The accuracy of this tools must do with the increase in data volume in the past years that improve the machine learning methods. To improve predictions in machine learning, multiple predictors can be combined to perform a consensus prediction. The most frequently used consensus methods are CONSENSUS, which is hosted on the IEDB website (Moutaftsi, Peters et al. 2006).

The MHC class II binding groove has special pockets that will fit defined amino acids of the binding peptide, and have a major influence on the binding energy(Bordner 2010). HLA class II ligand prediction is more difficult than class I prediction owing to the unknown position of the binding core within the generally longer peptides. This turns out to be an interesting combinatorial optimization problem: select the minimal set of epitopes maximizing the coverage on the whole world population (represented by its global allele frequencies) (Toussaint, Maman et al. 2011). As epitopes are a true subset of what can bind the MHCs of a given individual, the high degree of polymorphism imposes a big challenge on epitope discovery. Fortunately, there are allele frequency databases in web, like allelefrequencies.net (Gonzalez-Galarza, Christmas et al. 2011).

The knowledge of which strong epitopes a protein contains has further importance when considering the use of proteins and peptides as therapeutic drugs. For MHC class II binding, it is inherently harder to go from peptide binding data to a defined motif of the binding core as this is a continuous stretch of nine amino acid residues placed somewhere in a larger peptide usually in the range of 12–20 residues in length. In human's MHC class II chains are encoded by genes in the HLA-DR, -DQ and -DP loci. Knowledge of the allotypes is thus essential for predicting HLA-presented peptides.

Material and Methods

Understanding the different stages of *T. cruzi* inside the host and the different proteins involved in the process, we chose the proteins according to literature, those has a key function in the process of adhesion and invasion, 10 proteins fit with this requirement to cover a big spectrum of the parasite surface proteins.
Adhesion Molecules in Metacyclic trypomastigote gp90, gp82, gp30 and gp35/50 (Yoshida 2006) and mucins that correspond to 1% of the whole genome of *T. cruzi* and has a function in relation parasite - host (Freitas-Junior, Briones et al. 1998).
Trans - sialidases are hardly related with the invasion process (Mattos, Tonelli et al. 2014) gp82, gp80, gp35/50 y gp85 (Barrias, de Carvalho et al. 2013) use for transportation of sialic acid to parasite mucins, as *T. cruzi* cannot synthesize (Osorio, Ríos et al. 2012). The Superfamily Tc-85 are found in the trypomastigote membrane but is not present in the epimastigote form, are known as Gp85 (Mattos, Tonelli et al. 2014).
Cruzipain or gp57/51 is a lysosomal cysteine protease from *T.cruzi* that can also be found to a lower quantity in the parasitic plasma membrane(Alvarez, Niemirowicz et al. 2012). It is the best characterized cysteine protease and plays a fundamental role in the progression of

Chagas disease (Gea, Guinazu et al. 2006). This is expressed mainly in trypomastigote and amastigotes. In trypomastigote is in the flagellar pocket while in amastigotes is located on the cell surface, probably to interact with the host cell cytoplasm (Gea, Guinazu et al. 2006).

IEDB (MHC II Binding Predictor)

A prediction that identifies the share epitopes could help to develop a better treatment and give us information to understand how *T. cruzi* protects against the immune system. In IEDB MHC II we found possible epitopes that can activated a response immune system. The MHC II set was obtained from the allelefrequencies.net with maximus global coverage to the initial process(Greenbaum, Sidney et al. 2011). Every protein was submitted to the same parameters, and filter according the consensus score, in this case we eliminate all epitopes with a score above 25. This guarantees the high affinity to the MHC II. We obtain a matrix with 700000 approx. epitopes for each protein sorted by consensus score.

Share (core) epitopes

Using the previous matrix sorted by consensus score, we search for the core of each epitope per the SMM method. The SMM-align method was shown to outperform other state of the art MHC class II prediction methods. The method predicts quantitative peptide :MHC binding affinity values, making it ideally suited for rational epitope discovery. The method has been trained and evaluated on the, to our knowledge, largest benchmark data set publicly available and covers the nine HLA-DR super types suggested (Nielsen, Lundegaard et al. 2007).

We search the repeats of each core in their respective protein with a length between 8 and 10 amino acids. This method allowed to find the most expressed epitopes in each protein and discard the epitopes with low frequency. We assumed that a core with a high expression ensures a best chance to MHC II to detect this region in the protein and trigger the immune response.

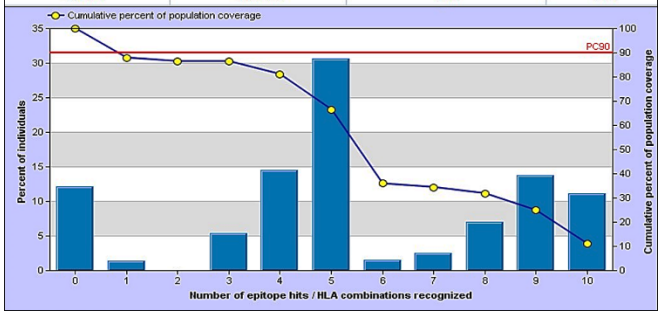
A cluster analysis was performed to group the core of the epitopes of the different proteins to discard those with a low frequency. to make sure this epitope has a significant population coverage in Latin America we use Population Coverage Calculation from IEDB Analysis Resource(Bui, Sidney et al. 2006).

Results

We obtain 68 epitopes divided in three groups, the first group contains the proteins Asp-3, Asp-2, Gp85, Gp90, Tc85, Sa85 with 17 shared epitopes and a population coverage of 87.89%.

Graph. 1 Group A Protein Asp-3, Asp-2, Gp85, Gp90, Tc85, Sa85

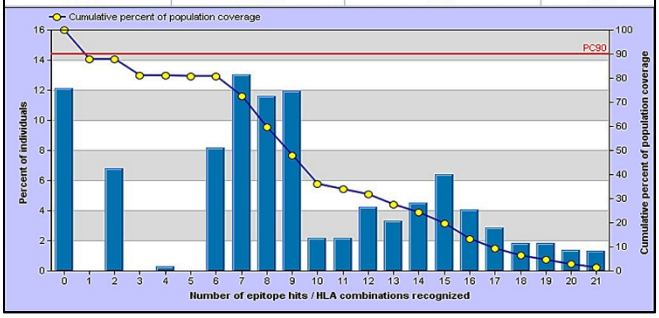
MHC Class	Coverage [a]	Average hit [b]	PC90 [c]
Class II	87.89%	5.47	0.83



The second group Asp-3, Gp82, Gp83 with 31 shared epitopes and 87.89% population coverage.

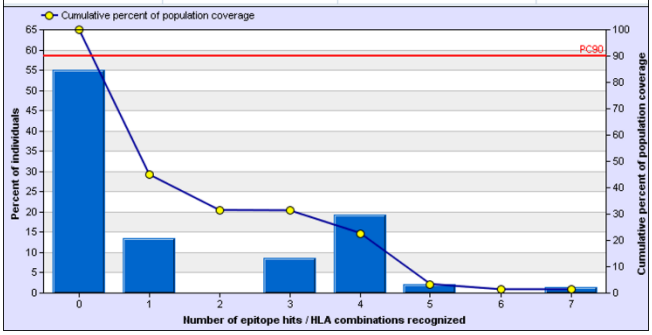
Graph. 2 Group B Protein Asp-3, Gp82, Gp83

MHC Class	Coverage [a]	Average hit [b]	PC90 [c]
Class II	87.89%	8.90	1.65



The groups obtained and the protein integrate them correspond to the type of proteins in case of the group 1 all proteins are glycoproteins and the second group all are trans-lidase, this verify the well conserved in the superfamily's and must take in consideration in the future of develop vaccine. Cruzipain is not sharing any epitope, that was reflect in the coverage obtain of 44.91%.

MHC Class	Coverage [a]	Average hit [b]	PC90 [c]
Class II	44.91%	1.36	0.18



Conclusion

The disease of Chagas is one of the main parasitic diseases in Latin America and at present does not have a vaccine.
Thanks to the new amount of data, the methods of machine learning and to databases such as allelefrequencies.net, the predictions are each more reliable with the capacity to cover a larger population and reduce the time in the development of new vaccines. The results obtained show that it is possible to find epitopes that fulfill these qualities, despite the different stages of *T. cruzi*. Subsequently the epitopes will be synthesized to evaluate their tripanolititic activity against the disease of Chagas.

Conflicts of Interest

The authors declare no conflict of interest.

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QUANTIFICATION AND CHARACTERIZATION OF NATIVE MICROORGANISMS UNDER CONTRASTING RAINFOREST ENVIRONMENT IN ECUADORIAN AMAZON

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Abstract

Ecuadorian Amazon Region is known as one of the richest biodiversity environment worldwide. However, it is a fact that microorganisms biodiversity have been poorly studied. In order to contribute to unravel microbe biodiversity and applications, this research aimed to quantify and characterize native microorganisms associated with cocoa (*Theobroma cacao*) plantations under high cadmium levels in two contrasting Ecuadorian Amazon conditions (CIPCA and Ahuano). Soils samples were collected from two depth levels (0-10 cm and 10-30 cm) to compare the number of bacteria and fungi in both environments assessed. For microorganisms quantification, decimal dilution methods was performed and most probable number was calculated. Bacteria biodiversity was assessed by isolation of every different single colony and morpho-cultural characterization was performed measuring: colour, growth, shape, elevation, edges, Gram stains and morphology. Fungi biodiversity was evaluated by mycelia shape, colour and radial growth. Results showed the increase in bacteria and fungi under CIPCA environment, where the rainy range was not so high. However, in both ecosystems from 10-30 cm depth, the number of microorganisms were remarkable as compared with 0-10 cm depth. Bacteria characterization highlighted a huge diversity, with 22 different isolates in CIPCA and 16 isolates in Ahuano. For fungi, the differences in morpho-cultural characteristics within both ecosystems were not wide, but also CIPCA had a high diversity with 25 different isolates as compared with 22 from Ahuano.

These results are the base for further researches related with microbe applications, such as cadmium bioremediation.

Keywords: bacteria, fungi, isolation, identification, bioremediation

1. Introduction

Before the advent of the Omics era, microorganisms were generally identified and characterized based on their morphological, physiological and cultural characteristics. Biotyping, serotyping, bacteriocin typing, phage typing, antimicrobial susceptibility patterns, and other protein-based methods are all examples of commonly employed phenotypic methods (Fakruddin et al., 2013). Although molecular techniques have revolutionized the microbiological studies, nowadays these traditional methods are still proper and used for microbe typing where the molecular analyses are unreachable.

Ecuadorian Amazon biodiversity is being increasingly investigated. New accession of plants, amphibians, birds, fishes, reptiles and other species are often reported (Lessmann et al., 2016). However, microorganism communities are still under limit knowledge.

Microbe identification is crucial for further application in any biotechnological process (Sanguinetti and Posteraro, 2016), much more in those related with environmental processes such as bioremediation. Unravel efficient microorganism upon specific environment to reduce contaminants, could become a cornerstone for restoring ecosystems (Liu et al., 2017). In this regards our research aims to quantify and characterize native bacteria and fungi associated with cocoa (*Theobroma cacao*) plantations under high cadmium levels in two contrasting Ecuadorian Amazon conditions.

2. Results and Discussions

The total bacteria and fungi were quantified at seven days after plating. Figure 1 shows the comparison between both depths levels measured (0-10 and 10-30 cm) for bacteria (panel A) and fungi (panel B) at the two sampled sites. In Ahuano, the bacteria colony-forming unit (CFU) were statistically higher at 10-30 cm depth (3.08E+07) as compared with 0-10 cm (6.73E+06); while in CIPCA no significant differences among depths levels were observed.

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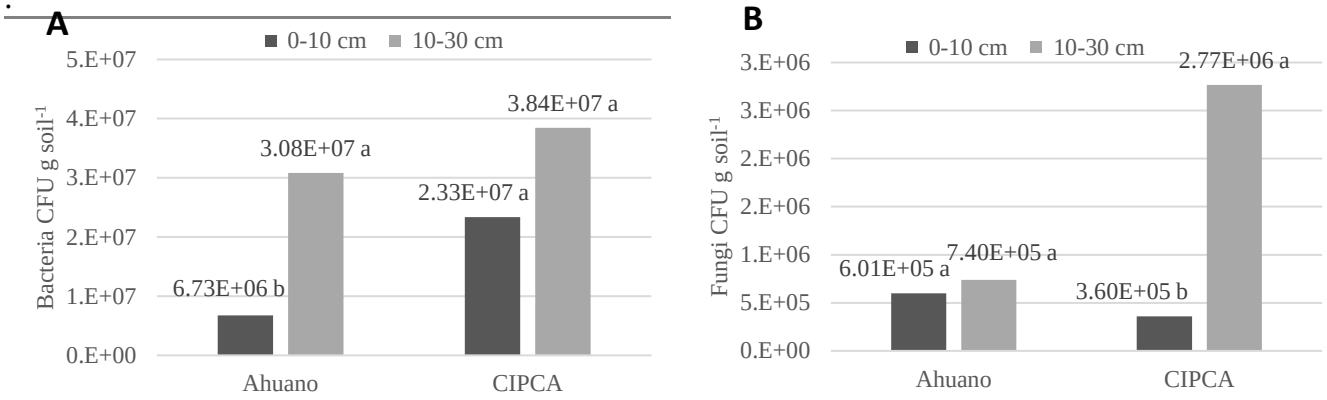

Figure 1. Quantification of bacteria (Panel A) and fungi (panel B) communities at two levels depth (0-10 and 10-30 cm) in Ahuano and CIPCA. Values are given in colony-forming unit (CFU) per each gram of soil. Different letters in columns differs statistically (p<0.05, Tukey HSD).

For fungi, the results were reverse than for bacteria regarding sampling sites, being the CIPCA the site with more fungal abundance. However, as for bacteria, the depth level 10-30 cm in CIPCA turned out the best significant values. In Ahuano no significant differences were shown.

Although for bacteria and fungi the assessed site varied the quantification of microbial communities (Figure 1 A and B), these results clarify the influence of depth levels in the abundance of each microbe group. As well as for Ahuano and for CIPCA, a distribution pattern of the microbial communities was presented, favouring bacteria

and fungi from 10 to 30 cm depth. It might be due to the edaphoclimatic conditions that are presented in this Amazon region, mainly the rainy regime, allowing the leaching of organic matter and the microbe community with it.

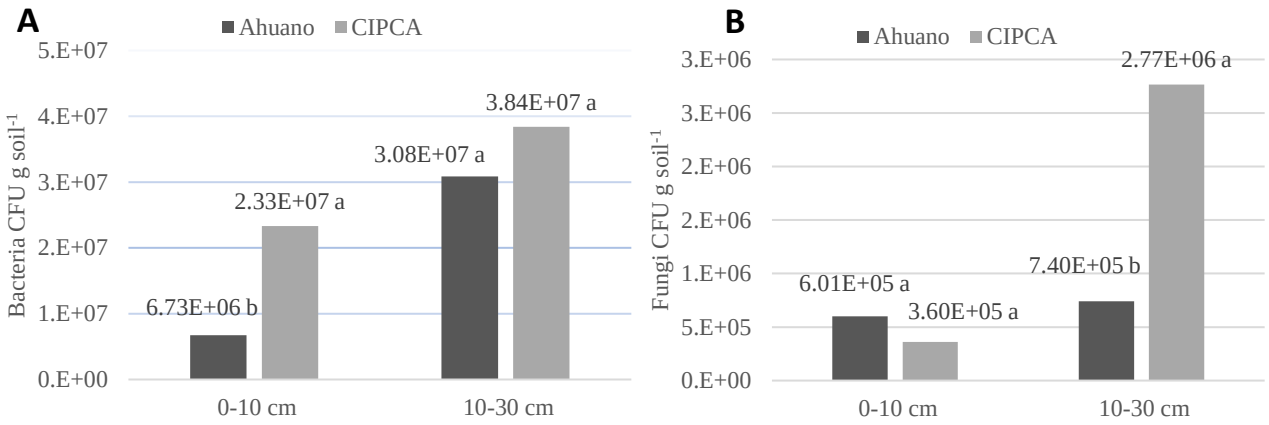

Figure 2. Comparison of bacteria (Panel A) and fungi (panel B) quantification in Ahuano and CIPCA according to depth levels. Values are given in colony-forming unit (CFU) per each gram of soil. Different letters in columns differs statistically (p<0.05, Tukey HSD).

The comparison of bacteria and fungi quantification among sampling sites can be seen clearly in Figure 2. In panel A is evident the increase of bacterial community in CIPCA at both depth levels, although only at 0-10 cm significant difference were observed. Despite the number of bacteria at this depth level was less than from 10-30 cm, there was a difference of 28.9% of CIPCA bacteria compared to the those found in Ahuano. As seen for previous results (Figure 1), the quantification of fungi colonies was remarkable in CIPCA as compared with Ahuano, having an increase of 26.7%.

Several studies have reported the close link of microbial communities with organic matter in soils. Schnecker et al. (2014) focus the effect of organic matter properties and microbial community composition even in Artic soils. Authors found microbial community composition (estimated by phospholipid fatty acid analysis), was similar in cryoturbated material and in surrounding subsoil, although carbon and nitrogen contents were similar in cryoturbated material and topsoils. Our work is concomitant with this finding, suggesting that as the organic matter progresses in the soil, the microbial communities move with it.

Table 1 represents the morpho-cultural characterization of all different bacteria isolates in both contrasting sampling sites. From all the colonies grown in nutrient agar media, a total of 22 different isolates for CIPCA and 16 for Ahuano were released. All colonies differed at least in one of the parameters assessed. Within the most significant results in this table, stands out the 20% of bacteria with moderate and abundant growth in CIPCA and 34% in Ahuano, as well as 32% of Gram negative stain bacteria in CIPCA against 42% in Ahuano. Similar results are reported by Sánchez et al. (2014), which highlighted the wide range of bacteria in tropical soils and its biodiversity.

Table 1. Morpho-cultural characterization of different bacteria isolates in contrasting sampling sites.

Location	Apar.	Shape	Elevation	Edges	Growth	Colour	Gram stain
CIPCA	70%- B 20%- C 10%- E	80%- D 10%- B 10%- E	90%- A 10%- B	90%- B 10%- E	80% + 10% ++ 10% +++	70% - B 30% - A	68% (+) 32% (-)
Ahuano	92%- B 8%- C	67%- D 33%- B	50%- B 42%- A 8%- C	67%- B 33%- A	66% + 17% ++ 17% +++	43%- A 33%- B 8%- C 8%- D	48% (+) 42% (-)

	8%- E

Legend: Appearance: equinulade (B), barbade (C), arborescent (E). Shape: circular (B), irregular (D), rhizoid (E). Elevación: flat (A), elevated (B), convex (C). Edges: continuous (A), wavy (B), prickly(E). Growth: light (+), moderate (++), abundant (+++). Colour: white(A), beige (B), yellow(C), orange (D), red(E). Gram: negative (-), positive (+).

3. Conclusions

The amounts of bacteria in the Amazonian ecosystems evaluated are high, especially in CIPCA soils (6.2E + 07) compared to Ahuano (1.9E + 07), with a predominance of bacterial communities in the horizon of 10-30 cm in soil.

There is a broad biodiversity of bacterial communities, expressed in the differentiation of morpho-cultural characteristics. Twenty-two different isolates were determined in CIPCA and 16 in Ahuano.

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Comparative Modeling of the Three-Dimensional Structure of Protein Kinase D from Mycobacterium Tuberculosis.

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Abstract: Among the main aims of the United Nations is to eradicate communicable diseases such as tuberculosis, which affect millions of people worldwide producing more critical problems in countries with low- and middle-income. *Mycobacterium tuberculosis*, it is the causative agent of tuberculosis; it is one of the most lethal human pathogens further characterized by being strongly resistant. Despite more than 100 years of research developed until today, some two million people die every year around the world due to tuberculosis. For this reason in this study are postulates and analyzed the homology modeled to the protein kinase D from *mycobacterium* tuberculosis. Homology modeling aimed at constructing three-dimensional structure of a protein model using experimentally determined structure of related family members as the template. Protein kinase homology or comparative protein structure modeling was performed with the help of *UniProt* (de universalt protein) and SWISS-MODEL workspace, which is an integrated web-based modeling expert system and by selecting suitable template solved by experimental methods and stored at Protein Data Bank (PDB) database. On the basis of a sequence alignment between the target proteins Pks D and the template structure PKn B, a three-dimensional model for the target proteins were generated. SWISS-MODEL workspace derived the restraints automatically from related known structure (template) present in the database. Three-dimensional structure or model was generated by optimizing the molecular probable density function. The generated model with the highest score was validated by the probable density functions. The validated model was chosen for further studies and refinements. The rough model generated was subjected to energy minimization using the steepest descent technique to eliminate bad contacts between protein atoms. Computations were carried out in vacuo by the GROMOS96 force field set, implemented through Gromacs 4.1, and steriochemical quality of minimized model was performed using Ramachandran Plot, PROCHECK tool, WHAT_CHECK , ERRAT, VERIFY_3D and ProSA-web.

Keywords: mycobacterium tuberculosis, protein Kinase D, homology models, topological analysis, secondary and tertiary structures.

Introduction: Historically, one of the main aims of the United Nations is to eradicate communicable diseases such as tuberculosis, which affect millions of people worldwide producing more critical problems in countries with low- and middle-income. *Mycobacterium tuberculosis*, it is the causative agent of tuberculosis; it is one of the most lethal human pathogens characterized by be strongly resistant. Despite more than 100 years of research developed until today, some two million people die every year around the world due to tuberculosis. According to the World Health Organization (WHO), a third of the world's population carries the infection in an inactive form known as latency **[1]** and a hallmark of the disease is the ability to persist in the host for years and to reactivate under conditions of immune suppression. The situation is worsened by the increasing incidence of multi-drug-resistant strains. In this sense, our current inability to control the spread of this disease can be explained by the lack of an effective vaccine, lack of multidrug resistant **[2-4]** and the great adaptability of the *mycobacterium tuberculosis* in different environments (e.i.; high mutation) **[5]**. It is therefore imperative to identify novel *mycobacterium tuberculosis* antigens/targets for the development of new effective anti-tubercular drugs and vaccines. The Protein Kanases (Pks) plays an important role in controlling proliferation and differentiation in eukaryotic cells in living organisms, are enzymes that catalyze the protein phosphorylation process. One reason to investigate the protein phosphorylation is due to that its rationalization represents an attractive drug target in a variety of diseases such as cancer **[6]**, alzheimer **[7]**, chronic inflammations **[8]**, etc. PKs present in the human body have been widely studied by the interest in their use as therapeutic targets in these diseases; however, not much is known about the PKs involved in tuberculosis for this reason propose new models using the homology modeling can help to get new insights in tuberculosis treatment today.

The determination of the 3D structure also provides valuable information about the function of such proteins whose functions are otherwise unknown. X-ray crystallography is a powerful tool for determining protein 3D structures but it is time consuming and expensive, and not all proteins can be successfully crystallized. This is especially the case with most membrane proteins like kinase that are difficult to crystallize and that do not dissolve in commonly used solvents. Therefore, very few membrane protein structures from *mycobacterium tuberculosis* have been determined by X-ray crystallography so far.

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Materials and Methods: Homology modeling aimed at constructing three-dimensional structure of a protein model using experimentally determined structure of related family members as the template. Protein kinase homology or comparative protein structure modeling was performed with the help of *UniProt* (de universalt protein) and SWISS-MODEL workspace, which is an integrated web-based modeling expert system and by selecting suitable template solved by experimental methods and stored at Protein Data Bank (PDB) database. On the basis of a sequence alignment between the target protein Pkn D and the template structures Pkn E (homology: 65.7% with PKn D), a three-dimensional model for the target protein was generated. SWISS-MODEL workspace derived the restraints automatically from related known structure (template) present in the database. Three-dimensional structure or model was generated by optimizing the molecular probable density function. The generated model with the highest score was validated by the probable density functions. The validated model was chosen for further studies and refinements. The rough model generated was subjected to energy minimization using the steepest descent technique to eliminate bad contacts between protein atoms.

Computations were carried out in vacuo by the GROMOS96 force field set, implemented through Gromacs 4.1, and steriochemical quality of minimized model was performed using Ramachandran Plot, PROCHECK tool (checks the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry), WHAT_CHECK (derived from a subset of protein verification tools from the WHATIF program, this does extensive checking of many sterochemical parameters of the residues in the model), ERRAT (analyzes the statistics of non-bonded interactions between different atom types and plots the value of the error function versus position of a 9-residue sliding window, calculated by a comparison with statistics from highly refined structures), VERIFY_3D (determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc) and comparing the results to good structures), PROVE (calculates the volumes of atoms in macromolecules using an algorithm which treats the atoms like hard spheres and calculates a statistical Z-score deviation for the model from highly resolved (2.0 Å or better) and refined (R-factor of 0.2 or better) PDB-deposited structures). Finally, was used ProSA-web, this program calculates an overall quality score. If this score is outside a range characteristic for native proteins the structure probably contains errors. A plot of local quality scores points to problematic parts of the model which are also highlighted.

Results and Discussion: In the model predicted to Pkn D the Ramachandran Plot analysis showed that amino acids of model (**Figure 1B**) and template in the most favorable region were 76.4% and in the additional allowed region were 22.7% amino acids, respectively. This stipulates that protein backbone dihedral angles φ-ψ occupied reasonably accurate positions in the 3D model.

The template was selected based on amino acid sequence similarity and crystal resolution, 100 models were generated and the model showing the least RMSD with respect to trace (C α atoms) of the crystal structure of the template was saved for further modification and validation (**Figure 1**). The modification was performed to obtain the best conformation of modeled Pkn D. Among the available potential templates, crystal structure of Pkn E with 2.8 Å resolution was selected as the model quality assessment template structure to build molecular model of the Pkn D.

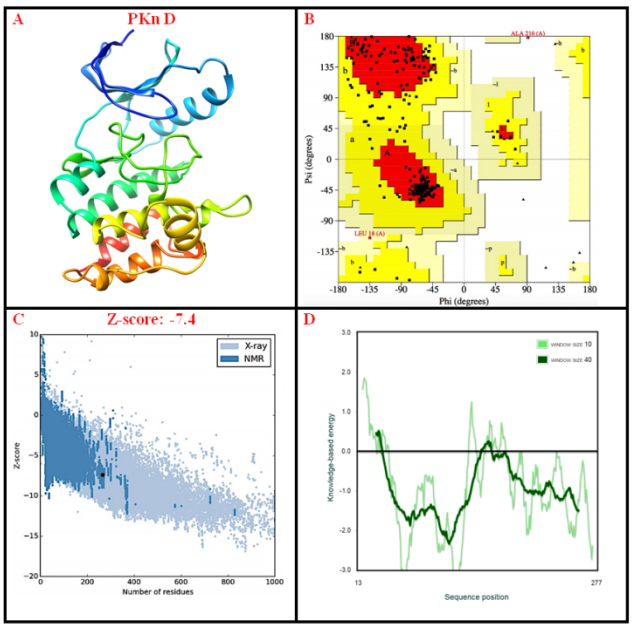

Figure 1. **(A)** three-dimensional model predicted to Pkn D. **(B)** Ramachandran plot for the predicted model of Pkn D. Residues in most favoured regions [A,B,L] 172 (76.4%), residues in additional allowed regions [a,b,l,p] 51 (22.7%), residues in generously allowed regions [~a, ~b, ~l, ~p] 1 (0.4%),

residues in disallowed regions 1 (0.4%), number of non-glycine and non-proline residues 225 (100%), number of end-residues (excl. Gly and Pro) 1, number of glycine residues (shown as triangles) 21, number of proline residues 18, total number of residues of 265. **(C)** the *z*-score indicates overall model quality. Its value is displayed in a plot that contains the *z*-scores of all experimentally determined protein chains in current PDB. In this plot, groups of structures from different sources (X-ray, NMR) are distinguished by different colors. **(D)** this plot shows local model quality by plotting energies as a function of amino acid sequence position *i*. In general, positive values correspond to problematic or erroneous parts of the input structure.

The ProSA-web study, the interaction energy per residue was calculated by PROSA 2003 program. The PROSA Z-score indicates overall model quality (acceptable values are below 0.5). The overall model quality showed the Z-score of −7.4 in **Figure 1C**. The score achieved by our model is within the range of scores found in native-protein structures of similar size. The overall quality parameters (stereochemical, geometrical as well as energetic) achieved by our Pkn D model suggested that it can be trusted. Another test for quality assessment is to examine the ProSA profile which describes the energy of residues as a function of sequence position; in which positive values indicate a problematic or erroneous part of the structure and vice versa. The energy analysis for our model (**Figure 1D**) demonstrated favorable residues energy as indicated by the negative values achieved along the amino acids sequence. In **Figures 2A** and **2B** is shows the ERRAT and VERYFY_3D plots to this Pkn D.

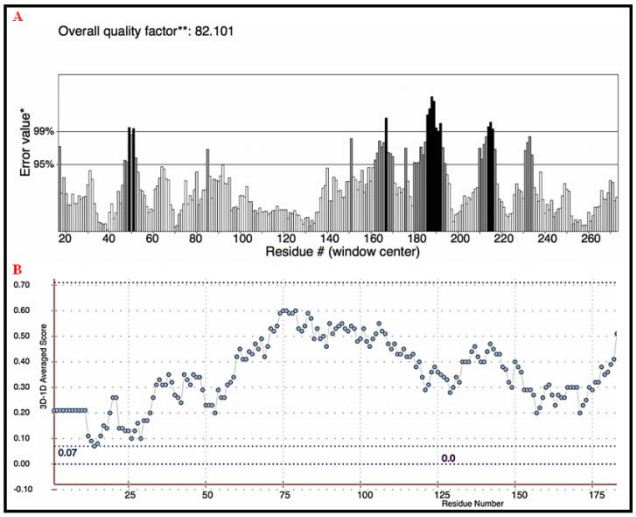

Figure 2. ERRAT and **VERIFY_3D** plots to the Pkn D model. **(A) ERRAT** plot analyzes the statistics of non-bonded interactions between different atom types and plots the value of the error function versus position of a 9-residue sliding window, calculated by a comparison with statistics from highly refined structures. **(B) VERIFY_3D** determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc) and comparing the results to good structures.

In **Figure 2**. ERRAT for non-bonded atomic interactions and higher scores means better quality. The normal received range for a high quality model is >50. The ERRAT score of Pkn D model was shown in **Figure 1A**. The above validation suggests that the backbone conformation and non-bonded interactions of Pkn D homology model was all reasonable within a normal range. The prediction of the modeled Pkn D structure was checked by VERIFY_3D, **Figure 2B**. The VERIFY 3D analysis indicated a reasonably good sequence-to-structure agreement because none of the amino acids had a negative score and it is 0.61 Pkn D. It is to be noted that compatibility scores above zero correspond to acceptable side chain environment. Therefore, the validation used shown that our homology model can be an alternative to the Pkn D from tuberculosis unknown today.

Conclusions: the homology modeling has been used to propose the first 3D structures for Protein kinases D from *mycobacterium tuberculosis*. With the assistance of the well-defined features associated to protein kinases involved the "gatekeeper door", hinge zone, C Helix, Asp-Phe-Gly (DFG), C-terminal and N-terminal, we can predict functional and binding sites, which can help in understanding what biological role it fulfills and desing new inibithors to the tuberculosis treatment. The validation used shown that our homology model can be an alternative to the protein kinase B from tuberculosis unknown today.

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

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Chemometrical analysis of structure-structure and structure-activity trends of cycloartane-based saponins in *Astragalus* genus

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Abstract: *Astragalus* genus represents the widest terrestrial plant taxon with more than 2200 species of herbs or shrubs. Under phytochemical aspect, this genus was characterized by a high structural diversity of saponins essentially based on cycloartane. The high number of saponins offers a strong basis for analysis of structural properties and metabolic trends governing molecular synthesis and diversity. Such trends can be highlighted from significant correlations between chemical substitution types/positions and aglycone forms. Beside the high number of chemical structures, pharmacological activities of saponins provide another variation aspect which was less invested because of not systematic evaluations of elucidated molecules. Despite this disproportion constraint between pharmacological evaluations and structural elucidations, preliminary significant structure-activity (SA) trends can be highlighted using appropriate statistical tools. This work focused on statistical analysis of structure-structure (SS) and structure-activity (SA) trends in *Astragalus* saponins by a sequential way including detections, significance evaluations and predictions. Dataset concerned 193 cycloartane-based saponins including 35 evaluated ones shared between cytotoxic and immunomodulatory activities (the most published activities in *Astragalus* saponins). SS and SA trends were initially highlighted by correspondence analysis and their significances were evaluated by Fisher's exact test. Results revealed significant affinities between aglycone forms and glycosylation positions. Moreover, both cycloartane forms and glucosylation positions showed significant effects on considered pharmacological activities. Finally, using the significantly influent structural variables, SAR models were developed by logistic regressions. Obtained models showed high sensitivity and specificity in favor of good predictability and distinctness of each separated activity. These results remain preliminary and need more confirmation from more pharmacological data that could be cumulated in the future.

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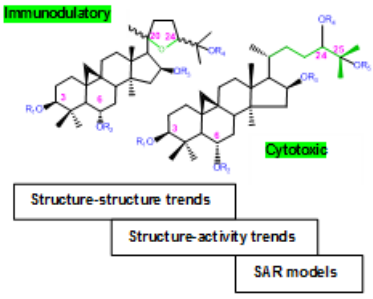
Keywords: *Cytotoxic activity, immunomodulatory activity, structure-activity trends, correspondence analysis, Fisher's exact test, logistic regression*

Graphical Abstract:



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Introduction: Saponins represent a wide family of secondary metabolites showing high structural diversity. Although new structures are subject to continuous elucidations, pharmacological activities are not systematically evaluated leading to much more not evaluated molecules than evaluated ones. Such a state can be represented by two separated subsets concerning saponins with known (evaluated) and unknown (not evaluated) activities, respectively. Despite such a disproportion between pharmacologically evaluated and not evaluated saponins, wide phytochemical data cumulated in literature offer high structural variability that can be statistically analyzed to highlight preliminary significant associations between structural traits and activities.

This work concerned statistical analysis of a wide dataset of *Astragalus* saponins (cumulated in literature) by focusing on link analysis between structural traits and cytotoxic and immunomodulatory activities [1].

Materials and Methods: The aim of the current work was based on the following question: how the small set of known active saponins can be structurally distinguished from the wide set of not evaluated ones? This question found responses through three sequential statistical analyses including (1) structure-activity (SA) trends detections, (2) significance evaluations and (3) SA-predictive models (**Figure 1**).

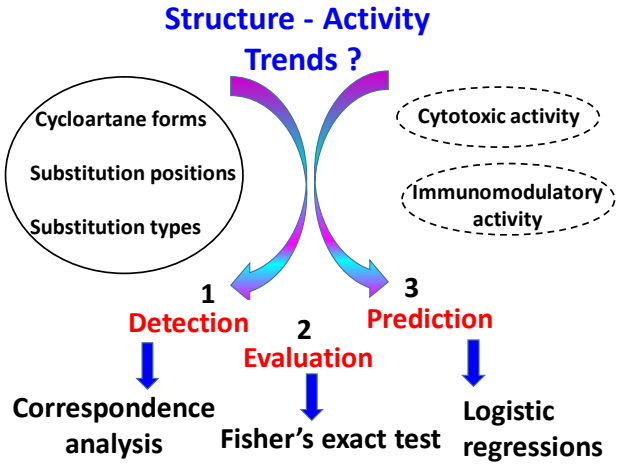

Figure 1. Three methodological steps for statistical highlighting (1), significance evaluations (2) and prediction (3) of structure-activity trends applied for cycloartane-based saponins in *Astragalus* genus.

Detection of SA trends was carried out by correspondence analysis (CA) applied on a dataset containing 178 cycloartane-based saponins in rows and chemical substitutions of carbons in columns [2]:

For rows, saponins were initially identified by their cycloartane forms including 20,24-epoxyxyloartane (*Ep1*), 20,25-epoxcycloartane (*Ep2*) and cycloartane with aliphatic lateral chain (*LCh*) (**Figure 2**).

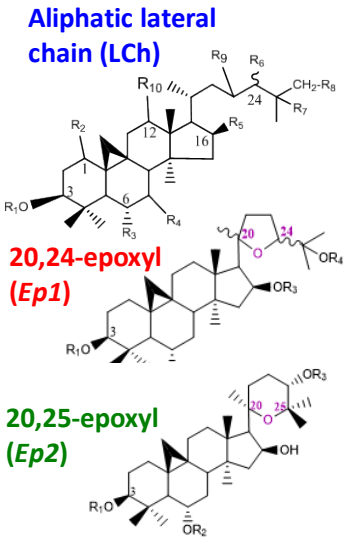

Figure 2. Chemical structures of different cycloartane forms in *Astragalus* saponins.

Also, saponins were characterized by two indicative variables concerning evaluated cytotoxic (*Cyt*) and immunomodulatory (*Imn*) activities, respectively. In all, *Cyt* and *Imn* were represented by 35 molecules among the 178 ones. Chemical substitution concerned carbons C3, C6, C16, C24, C25 susceptible to attach hydroxyl, glycosyl and/or acetyl groups. Glycosyls included glucosyl, xylosyl, rhamnosyl, arabinosyl, apiosyl and glucuronic acid. Other carbons were not considered because of their rare chemical substitutions leading to outlier cases.
SA trends highlighted from factorial plots of CA were statistically evaluated by means of Fisher's exact test (*FET*) [3]. In this link test, well-known evaluated saponins were considered as a target set the characteristics of which were confronted to the global state of random set containing all the not evaluated molecules (**Figure 3**). Randomness was attributed to this second set because it can include active and not active molecules the pharmacology of which remains unknown by waiting confirmative evaluations.

Finally, a synthesis of significant SA trends was carried out by applying two logistic models on the subset of 35 *Cyt* or *Imn* molecules to predict each activity in relation to the most discriminant structures given by *FET* (**Figure 1**) [3].
CA was carried out by ADE statistical software [4]. *FET* and logistic regressions were applied using JMP statistical software [5].

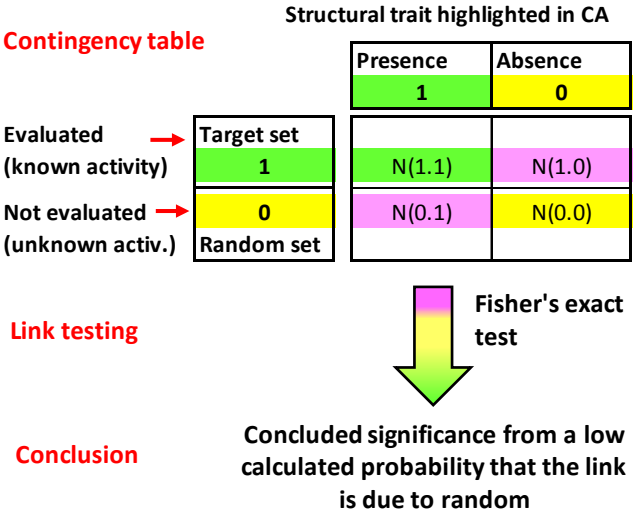

Figure 3. Principle of Fisher's exact test used for evaluating significances of SA trends highlighted in CA.

Results and Discussion: Strong structure-structure (SS) associations in *Astragalus* saponins were highlighted by the second factorial plan (*F3F4*) concerning both *LCh* and *Ep1*: massive *LCh* points projected in the same subspace ($F3^+F4^+$) than the 16-*Glc* variable (**Figures 4b, 4c**); this positive association was confirmed by a low *p*-value (=0.002) in *FET*. Concerning *Ep1*, it was relatively more characterized by 6-xylosylation (6-*Xyl*) than *LCh* form as shown in the upper left quadrant $F3^-F4^+$ **Figures 4b, 4c**; such a metabolic affinity between *Ep1* and *6-Xyl* was confirmed by low *p*-value in *FET* ($p = 0.01$). Apart from SS associations, different SA trends were highlighted by CA. Concerning cytotoxicity, CA highlighted topological proximity between *Cyt* and C3-glycosylation (3-*Glc*) points (plan *F1F2*) (**Figure 4a**) and superimposition between *Cyt* and *LCh* (plot *F7F8*) (**Figure 4d, e**). This indicated some positive trends between *Cyt* and both 3-*Glc* and *LCh*. Along the eighth principal component (*F8*), *Cyt-LCh* association showed topological opposition to *Imn-Ep1* one; this later indicated some positive trend between immunomodulatory activity and the 20,24-epoxycycloartane (**Figure 4d, e**). This was also confirmed by projections of *Imn* and *Ep1* points in a same subspace in *F3F4* plot (**Figure 4b, c**). Moreover, *Imn* projected close to 6-*Glc* in *F3F4* indicating some association between C6-glucosylation and this activity (**Figure 4b**).

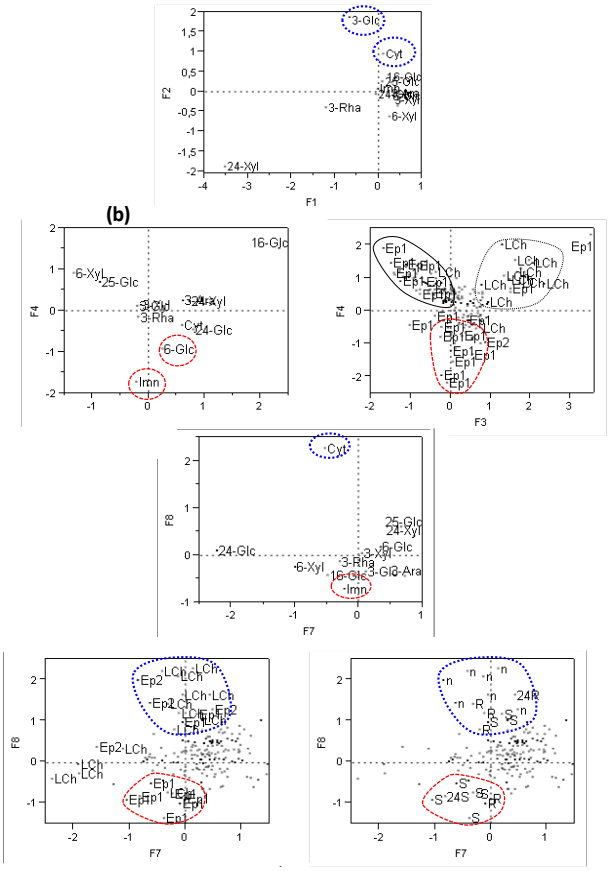

Figure 4. Factorial plots given by correspondence analysis and highlighting trends between *Cyt* or *Imn* activities and structural traits of *Astragalus* saponins.

Apart from cycloartane forms and glucosylation positions, spatial configuration *R* and *S* of cycloartane showed opposite projections in subspaces occupied by *Cyt* and *Imn*, respectively (**Figure 4f**). This could indicate some implication of aglycone configuration in pharmacological activity.

For synthesis, relative occurrences of different structural traits were calculated for the subsets of *Cyt* and *Imn* by reference the whole set of all the saponins: structural profiles of *Cyt* and *Imn* showed well-distinct even opposite aspects (**Figure 5**).

After FET application on all SA trends highlighted in CA, the lowest *p*-values of positive effects on *Cyt* concerned interaction between *LCh* and 3-*Glc* ($p = 5.10^{-4}$) (**Table 1**). Concerning *Imn*, the most significant positive effect resulted from interaction between *Ep1* and 6-*Glc* ($p = 6.10^{-4}$) (**Table 1**).

Using these four most significant and interactive variables given by *FET* (*LCh*, *Ep1*, 3-*Glc*, 6-*Glc*), logistic regressions were applied to develop SA models predicting *Cyt* and *Imn* activities. Both models showed high sensitivity (*Ss*) and specificity (*Sp*) (**Figure 6**): *Ss* = 85.7% for *Cyt* vs 90.0% for *Imn*; *Sp* = 76.5% for *Cyt* vs 81.8% for *Imn*. These results were in favor of good predictive and distinctive ability of both models of different structure-activity subsets.

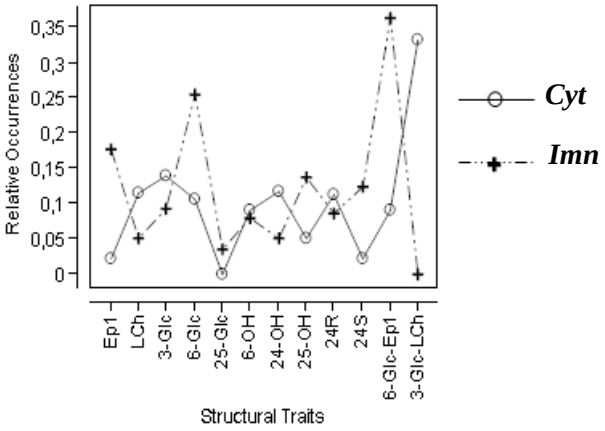

Figure 5. Two profiles showing relative occurrences of different chemical substitutions in saponins showing *Cyt* and *Imn* activities.

The synthesis of these preliminary results highlighted effects of aglycone form and glycosylation position on *Cyt* and *Imn*. For *Cyt*, Verotta et al. (2001) [6] evoked not significant cytotoxic activity in 20,24-epoxyxloartane (*Ep1*). Interaction effect of *LCh* and 3-*Glc* was in agreement with other works on saponins of not *Astragalus* species revealing key roles of aglycone and glycosylation in cytotoxic activity [7]. For *Imn*, previous works on some *Astragalus* saponins evoked positive implications of 20,24-epoxycycloartane and *6-Glc* in *Imn* activity compared to *LCh* and not glucosylated C6, respectively [8, 9].

Conclusions: This work concerned a preliminary analysis of SA trends from updated data of *Astragalus* cycloartane-based saponins cumulated in literature. Although, the current results remain preliminary because of the limited number of evaluated molecules, the method provided a sequential statistical way to extract significant information on SA trends despite sparse states of phytochemical-pharmacological data. Interaction

between aglycone (cycloartane) form and glycosylation (glucosylation) position seemed to be crucial for saponins' activities (**Figure 7**).

This three steps-method can be applied to larger datasets (with more available pharmacological evaluations) to confirm and/or improve knowledge on SA links of saponins and other metabolic families.

Table 1. Results of Fisher's exact test concerning significance evaluations of structure-activity trends previously highlighted by correspondence analysis. Legend: - effect, + effect: negative and positive effects, respectively.

Activity	Influencing structural traits				*p* -values	
					- effect	+ effect
Cytotoxic	*LCh*					0.002
	Ep1				0.01	
		3-Glc				0.063
			24-OH			0.094
				24R		0.033
				24S	0.033	
	LCh	***3-Glc***				**5.10^{-4}**
			24-OH	*24R*		0.004
		3-Glc		*24R*		0.036
	LCh			*24R*		0.013
	LCh	*3-Glc*		*24R*		0.020
Immuno-modulatory	*Ep1*					0.004
		6-Glc				**6.10^{-4}**
	Ep1	***6-Glc***				**6.10^{-4}**
		6-Glc	*25OH*			0.006
	Ep1	*6-Glc*	*25-OH*			0.001

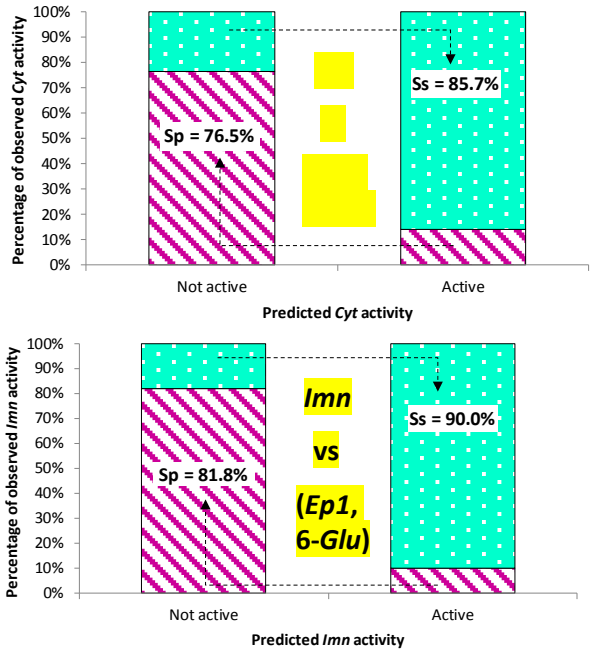

Figure 6. Results given by logistic regression models which were sapplied to predict *Cyt* and *Imn* activities in relation to cycloartane forms (*LCh*, *Ep1*) and glucosylation positions (3-*Glc*, 6-*Glc*). Legend: *Ss*, sensitivity; *Sp*, specificity.

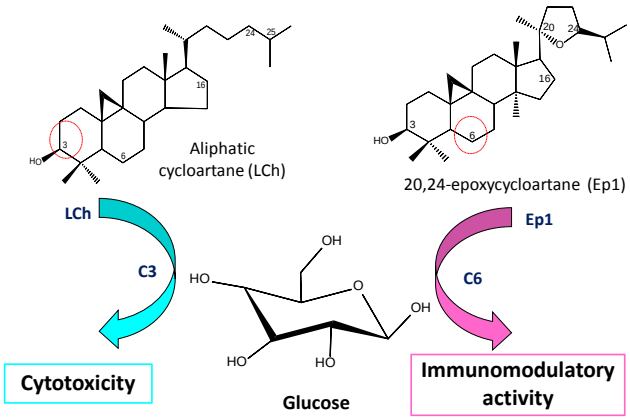

Figure 7. Preliminary results concluded from the three statistical steps-based method consisting of detection, evaluation and prediction of structure-activity trends applied to *Astragalus* saponins.

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Traditional use of plants as antihypertensive in Jipijapa, Manabí. Comparison with reported in the literature.

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Abstract:

The phytotherapy or herbal medicine is the more ancient form of medical attention and refers to the medical use of the plants or their constituents. This work is based on the analysis of the plants referred by the population as useful in the treatment of hypertension, and its chemical composition scientifically proven. Following the line of research defined by Ministry of Public Health of Ecuador on Medications, Supplies, Knowledge and Use of Medicinal Plants, in the area 19 -National System of Health-, this research was conducted on 614 hypertensive adults through interviews. The people had an average of 58 years old, predominance of female (60.09%), with basic level of schooling (29.47%) and an average

of 7.2 years of suffering high blood pressure disease. Approximately 58% of the interviewed population used medicinal plants to treat the hypertension, while 42% did not use or know about medicinal plants. The most widely used plants were *Matricaria recutita* (chamomile), *Plectranthus amboinicus* (oregano), *Cymbopogon citratus* (grass luisa/lemongrass) and *Valeriana officinalis* (valerian). Among these plants, Lemongrass is the only one, which its effectiveness as anti-hypertensive has been proven. The 34.03% of the used plants are purchased at the and only 16.12% grew them in their backyards. The most used part of the plant was the leave and infusion the way of preparation. As part of the antihypertensive treatment, the interviewed mainly consume the drugs of the pharmacological groups ACE and the ARA-II; the major mentioned conditions were visual disorders. Stress, TAG and cholesterol were mentioned as factors of risk. The medicinal use of studied plants is supported by scientific literature that also support them traditional use. Concerning the chemical composition of plant derivatives, oregano contains aromatics and oxygenated compounds, as main constituents. Chamomile contains above all flavonoids, tannins and terpenoids, lemongrass has phenolic compounds and valerian is characterized by flavonoids and terpenoids. The use of the studied plants by the population is attributed to its sedative and relaxing effects, in case of excitation nerve.

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Keywords: Natural products; Medicine herbal; Ethnobotany; Ethnopharmacology.

Mol2Net YouTube channel*:* *http://bit.do/mol2net-tube*

1. Introduction

As part of the acquis cultural of the peoples find the medicine natural and traditional, which is has developed in each country and region of the world with own features, taking the idiosyncrasy of its inhabitants; being the result of an slow evolution, but guaranteed by practical experience [1].

Traditional medicine includes, among others, the use of plants, animals or minerals for the cure of diseases of men in different cultures [3]. The traditional medicine is a reality present in all the world. As its name suggests, it is part of the cultural heritage of each country and practical uses that have been transmitted from one generation to another since hundreds of years prior to the development of our current medicine [4].

Natural drugs have contributed significantly to the progress of modern therapies, and this, while the chemical industrial holds a solid housing drug international, the uses of native herbal persist in different parts of the Earth, where the chemical

drug compounds do not arrive, for various reasons [5].

Medicinal plants are commonly used by the people of virtually every country in the world; According to the World health Organization (WHO), approximately the 80% of the countries in development used them plants with healing purposes, figure that goes in increase to the step of the years [6].

As regards the phytotherapy, many drug used formerly is used in the same way between them medical currently for treat conditions as hypertension, by what the medicinal plants continue being a source extraordinary of material for pharmaceutical industry.

Erroneously, the people believe that consume medicinal plants there is not risk of present adverse reactions. Some patients leave of take conventional medication prescribed by the doctor, appearing complications in the patients. We could say that the activity of medicinal plants is not as powerful or occurs immediately as in the case of synthetic drugs, by what is recommended use it as a first step in the treatment or as adjuvants therapies. Is important highlight that the medicinal plants are drugs and as such can introduce benefits and effects adverse, some of considerable importance, so the benefit/risk balance should be assessed. There are that have present that the active substances of them medicinal plants are chemical and that they may interact with other substances that the patient consumes, including food [7].

For some years, cardiovascular diseases have become the leading cause of death in industrialized countries, is recorded various risk of factors, which influence on the probability of suffering cerebrovascular accidents, coronary disease, heart failure or peripheral artery disease. Among these risk factors is hypertension, which can be modified and it is estimated is one of the biggest problems of public health around the world [8].

The hypertension is a cardiovascular risk of factor very prevalent in the world, that is especially overwhelming in those countries of low and medium income. Recent reports of the WHO highlighted the importance of the chronic diseases such as the hypertension, as obstacle to the achievement of a good state of health. We should be added that, for the majority of low-and middle-income countries, poor primary health care strategies are major obstacles to achieve control of blood pressure. The main causes of premature death and disability in the majority of countries of the America, representing a 60% - 70% of all deaths [9].

In Ecuador, the hypertensive diseases, cerebrovascular disease and ischemic disease of the heart, altogether in the year 2011, were cause of 10 325 deaths, 16.6% of total deaths in the country in all age groups [10].

The causes of morbidity and mortality from this disease include ignorance of the patient about his illness and the management, the non-adherence to the treatment protocols and bad habits and

lifestyles that are risk factors. Our work aims to contribute to promote the use of the medicinal plants as therapeutic option linked to the conventional protocols in the management of hypertension.

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2. Results and Discussion

614 adults with hypertension in the urban zone of the Jipijapa Canton, province Manabí were interviewed, of which 245 (39.90%) were male and 369 (60,09%) females, these constituting the majority. The average age was 57,93 years old and 7,285 years of suffering from hypertension. The universe sample was formed with people that never studied (8.95%) to people of level upper (13,02%), where will include specialists in various areas, being the basic educational level the predominantly (181 persons – 29,47%).

In relation to the variable sex, the majority of the interviewed were of the female sex, similar to it retrieved in another study performed in Colombia [11]. These results are similar to those found in other works. Taveira de Jesus *et. al*., concern that these results may be due to that at the time of the interview (day period), the men were working and women engaged in household chores [12]. Barros *et. al*., study on the community of Jauari, Itacoatiara - AM, Brazil, concerned that women know and most use plants for medicinal purposes than men [13]. From always were given to them women the responsibility on the domestic tasks and of care for those children, many times making the home treatments of the diseases simpler with an herbal tea.

Within the population surveyed, 42% said not to use or learn about medicinal plants, while the 60,09% use plants, which 34,03% are purchased from the market, used and consumed mostly with a frequency of a time to the day by a week in form of tea as preparation therapeutic, calling him so interchangeably to the infusion and to the decoction.

As regards the leaves as it part of the plant more used, them results displayed in other works [14], corroborate the obtained here. On the optics of the conservation of natural resources, the use of the leaves in the medicinal preparations is a positive aspect, because it does not cause the death of the collected specimen, thus contributing to the conservation of flora. Amorozo (2002), He says that the cultivation of plants for therapeutic purposes is a low cost alternative and it is part of the culture of our peoples, Since in the majority of the courtyards we can find these crops, either in urban or rural areas [15].

With regard to the mode of preparation of them medicinal plants, it most are performed as infusion or decoction to what the population indiscriminately called tea. This result coincides with that reported by Paredes *et. al*., in Los Ríos – Ecuador [14], where infusions also proved to be most commonly used form of preparation.

We asked to them interviewed make a list of the plants more used to treat the hypertension, the botanical data relating to them plants cited, as well as them names popular, part of the plant used, number of citations for each plant, form of preparation and frequency of consumption is show in the table 1, where are collected 62 plants used as medicinal belonging to 31 families. The families more represented botanically were Lamiaceae (8 species), Apiaceae, Rutaceae and Rosaceae each with 4 species.

As you can see in table 1 the plants most cited with therapeutic use for hypertension were German chamomile (*Matricaria recutita*), Cuban Oregano (*Plectranthus amboinicus*), Grass luisa (*Cymbopogon citratus*) and Valerian (*Valeriana officinalis*).

German chamomile (*Matricaria recutita*), is found coincidence. With regard to its popularity to treat different medical conditions, related mostly with the gastrointestinal apparatus (pain, slow digestion, diarrhea and nausea), also reported as anti-inflammatory and analgesic use in menstrual period [16]. The essential oil has an antioxidant and antimicrobial activities [17-20]. The plant extract has antidiarrhoeal, antisecretory and antispasmodic activities [21]. The decoction has an antioxidant property, and hepatoprotective effects [22]. The extracts obtained by plant decoction has an antioxidant and antimicrobial activity [23], however, no reports were found that support its popular use and effectiveness as antihypertensive.

Chemical constituents

The chemical constituents of these plant are phenolic compounds (flavonoids) and terpenoids and azulenes (essential oils) [19] and coumarin-like compounds (herniarin, umbelliferone, skimmin, daphnin and daphnetin, the latter is a strong sensitizer, so this compound and its glycosidic derivative can contribute to the allergic potential of chamomile [24].

The species *Plectranthus amboinicus* is used in the popular medicine to treat respiratory diseases, pain of head, fever and diseases of the skin; often eaten raw or used as food seasoning [25]. Other studies show the plant use in diseases of the oral cavity, mainly caused by *Streptococcus mutans* [26]. In addition, found their effectiveness as inhibitor of proteases specific for VIH-1 [27], respiratory, cardiovascular, oral, dermal, digestive and urinary tract diseases. Within its pharmacological properties are described actions such as antimicrobial, anti-inflammatory [28], Antitumor, healing, anti-epileptic, larvicidal, antioxidant and analgesic [25], antioxidant, antibacterial, antimutagenic and anticancer activities [29]. Another study on the leaves of the plant showed to be antimicrobial, also stimulates the growth of bacteria probiotic as is the *Lactobacillus plantarum* [30]. None of the sources cited is about the use or effectiveness as antihypertensive.

Chemical constituents

The chemical constituents of these plant are phenolic compounds (quercetin, rutin, coumaric, caffeic acid and gallic acid) and anthocyanins [29,31], terpenoids (essential oil carvacrol as the

most abundant component, followed by thymol) [32,33].

Cymbopogon citratus, known in Ecuador as grass luisa and in other countries as lemongrass, It is popularly used in different cultures to treat respiratory, gastric and nervous system problems. Is reported as anti - fungal, insecticide, antidiabetic, antiseptic, anti-mutagenic, anti-carcinogenic and anti-inflammatory, in fact, popularly is used primarily to treat inflammation and peptic ulcers. Some ethnopharmacological studies on *C. citratus* in different countries (Argentina, Brazil and Cuba), refer its use as antihypertensive [34].

It has been demonstrated in vitro studies that aqueous extracts and essential oils has a hypotensive effect in rats [2,35,36].

Chemical constituents

The review of the literature shows an extensive list of diverse and varied chemical compounds present in this plant, among them we can mention as main the polyphenols such as flavonoids, phenolic acids, tannins and anthraquinones. Quercetin, kaempferol, apigenin, catechol, chlorogenic acid, caffeic acid and hydroquinone are some one of these compounds. In the review also appear other groups of phytochemicals such as alkaloids, terpenoids (essential oil), steroids and saponins [34,37-45].

Valeriana officinalis is used popularly in Bulgaria as antiseptic, spasmolytic and sedative, also are used to treat edemas, cramps and fainting [46]. Argentina reported its use to treat insomnia and anxiety disorders [6]. The roots of the *V. officinalis* has pharmacological properties, such as anxiolytic, antidepressant, antispasmodic, sedative and anti-HIV [47].

No reports were found in the literature that support the use of this plant as antihypertensive.

Chemical constituents

The chemical constituents of these plants are essential oils, iridoids, flavonoids, alkaloids, amino acids, and lignanoids[48].

Among the phenolics compounds described in the literature for this plant are found olivil, pinoresinol, 8-hydroxypinoresinol, pinorespiol, 8-hydroxy7-epipinoresinol, *trans-p*-hydroxyphenyl- propenoic acid, *cis-p*-hydroxyphenyl-propenoic acid, ferulic acid, isoferulic acid and isovanillin [47].

The terpenoids as valerenic acid and its biosynthetic precursors valerenal and valerenadiene are responsible for the anxiolytic activity and β-caryophyllene for the anti-inflammatory activity [49]. For the above issues raised, we assume that the use of these plants is due first to the popular roots of that nature is beneficial, in second place to the ignorance and lack of guidance about the medicinal plants and third, to relaxing and sedative effect, contribute somehow to reduce the blood pressure values.

Of general way, the population has the false belief that all natural is beneficial, regardless of the amount and way of consumption. Medicinal plants have secondary metabolites, mostly responsible for biological and pharmacological activities, so for its correct use, should dominate the proper dosage, route of administration, the

possible adverse effects, interactions and contraindications. Has been shown in several studies, and our it reaffirms, that people (patients and health workers) have a non-specific knowledge on the use of medicinal plants, and its popularity and use, depends on local culture and above all, of the availability in squares and markets. The use of plants for therapeutic purposes, without proper guidance, is a worrying factor that must be considered by the authorities of the health sector, also by those linked with the education for the health, because the incidence of species with record of toxicity and contraindications of use. We know that the plants are remedies powerful and effective, but the risk of intoxication caused by the use abuse of these must be always led in consideration. Medicinal plants have countless applications and limitations, so knowledge of them is essential, both of the population in general, as of the personal of health, since the same can be used of way complementary to the drug treatment, to improve symptoms and decrease the adverse effects that produce synthetic drugs. Hypertension remains one of the chronic diseases with a high incidence and prevalence worldwide, the Ecuador is no exception to the epidemiological reality of the behavior of these diseases, the national survey of health and nutrition of the year 2011-2012, shows it with figures are alarming, our work of research put of manifest the serious implications of the ignorance of the management of this disease, non-adherence to the treatment protocols as well as the serious consequences that carry to the health of the population suffering from hypertension. The actions aimed to the training as tool fundamental in the promotion and prevention of the chronic diseases, that include modifying habits and styles of life not healthy, will make possible improve the quality of life of the population of the canton Jipijapa.

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Table 1. Medicinal plants used as antihypertensive in Jipijapa, Manabí.

Botanical Family	Botanical name and vernacular name (Ecuador/USA)	Part of the plant used	Preparation	Frequency	Reports
Amaranthaceae	*Chenopodium ambrosioides* L. (Paico/ Mexican Tea)	Leave	Maceration	1	1
			Salad	2	2
			Tea	2	1
Amaryllidaceae	*Allium sativum* (L.) (Ajo/Garlic)	Bulb	Maceration and Tea	3 y 4	19
Anacardiaceae	*Mangifera indica* L. (Mango)	Leave	Tea	1	6

				2	3
Annonaceae	*Annona muricata* L. (Guanábana/ Prickly custard apple, Soursop)	Fruit	Tea	1	1
		Leave	Tea	5	2
Apiaceae	*Apium graveolens* L. (Apio/ Wild Celery)	Leave and stem	Salad and Tea	1	13
	Petroselinum sativum (Perejil/ Parsley)	Leave, stem and whole plant	Tea	1 y 2	13
	Coriandrum sativum L. (Cilantro/ Coriander)	Leave, root and stem	Tea	1	1
	Angelica archangelica L. (Espíritu santo/ Archangel, Angelica)	Leave	Tea	5	2
Araliaceae	*Panax ginseng* C.A.Mey. (Ginseng/ American ginseng)	Leave	Tea	3	1
				4	2
Asteraceae	*Matricaria recutita* (Manzanilla/Chamomila)	Leave	Tea	1	57
		"Seed"	Tea	4	19
	Cynara scolymus (Alcachofa/ Artichoke)	Leave	Tea	5	2
	Ambrosia tenuifolia Spreng. (Altamis/ false ragweed)	Leave	Tea	1	1
Cucurbitaceae	*Cucurbita pepo* (Pepino/Cucumber)	Fruit	Juice	5	3
Cyperaceae	*Cyperus esculentus* L. (Horchata/ Nut grass)	Root	Tea	5	1
Equisetaceae	*Equisetum arvense* L. (Cola de caballo/ Field horsetail)	Leave	Tea	5	3
		Whole plant		5	1
Euphorbiaceae	*Croton lechleri* Müll.Arg. (Sangre de Dragón/ Dragon's blood)	Leave and outbreak	Tea	4	1

Fabaceae	*Medicago sativa* (Alfalfa)	Leave	Tea	2	3
Illiciaceae	*Illicium verum* (Anís/ Star anise)	Seed	Tea	5	10
indet	Carmelita	Leave	Tea	2	1
indet	Germen de trigo	Root	Tea	1	3
indet	Zorrilla	Root, leave and stem	Tea	5	1
indet	Zaragoza	Stem	Tea	2	1
Lamiaceae	*Plectranthus amboinicus* (Lou.) Spreng.	Leave	Tea	1	43
	(Orégano/ Cuban oregano)			5	12
	Melissa officinalis L. (Toronjil/ Lemon balm)	Leave	Tea	1	16
				2	8
	Ocimum basilicum Mill. (Albahaca/ Basil)	Leave	Tea	1 y 2	9
	Mentha piperita L. (Hierbabuena/ Peppermint)	Leave	Tea	5	3
				3	2
				2	2
				1	4
	Lavandula angustifolia Mill. (Lavanda/ Lavender)	Flower	*ad libitum*	4	1
		Oil	oil	2	1
	Salvia scutellarioides Kunth (Mastruante)	Leave	Tea	1	1
	Rosmarinus officinalis L. (Romero/ Rosemary)	Leave	Tea	4	2
Lauraceae	*Cinnamomum zeylanicum* Blume (Canela/ Cinnamon)	Bark	Tea	1	5
	Persea americana Mill. (Aguacate/ Avocado)	Leave	As baths	1	1
Loranthaceae	*Ligaria cuneifolia* Tiegh. (Muérdago/ Mistletoe)	Leave	Tea	3	1

Lythraceae	*Punica granatum* L. (Granada/ Pomegranate)	Fruit	Juice	1	3
			Tea	1	2
Moraceae	*Artocarpus communis* J.R.Forst. & G.Forst. (Fruta del pan/ Breadfruit)	Leave	Tea	5	1
Moringaceae	*Moringa oleifera* Lam. (Moringa)	Leave	Tea	5	1
Myrtaceae	*Psidium guajava* L. (Guayaba/ Guava)	Fruit	Juice	5	1
	Syzygium aromaticum (L.) Merr. & L.M.Perry (Clavo de olor/ Clove)	Seed	Tea	2	2
Oleaceae	*Olea europaea* L. (Olivo/ Common olive)	Leave	Tea	2	3
Passifloraceae	*Passiflora edulis* Sims (Maracuyá/ Passion fruit)	Leave	Tea	1	1
Plantaginaceae	*Plantago major* L. (Llantén/ Common plantain)	Leave	Tea	1	7
Poaceae	*Cymbopogon citratus* (Hierba luisa/Lemongrass)	Leave	Tea	1	31
				2	16
Rosaceae	*Prunus amygdalus* L. var. dulcis (Almendra/ Almond)	Leave	Tea	4	1
	Crataegus monogyna L. (Espina blanca/ May)	Flower	Tea	2	1
	Eucalyptus L'Hér. (Eucalipto/ Eucalyptus)	Leave	Tea and Inhalation	3	9
	Fragaria vesca L. (Fresa/ Strawberry)	Fruit	Tea	1	1
Rubiaceae	*Morinda citrifolia* L. (Noni)	Fruit	Juice	1	2
Rutaceae	*Citrus x aurantium* L. (Lima/ Bitter orange)	Fruit	Whole fruit	1	1
			Juice	5	1
	Citrus x limon (L.) Osbeck. (Limón/ Lemon)	Fruit	Juice	1	5
	Citrus sinensis (L.) Osbeck. (Naranja/ Orange)	Leave	Tea	5	1

	Ruta graveolens L. (Ruda/ Rue)	Leave	Tea	5	3
Solanaceae	*Solanum dulcamara L.* (Dulcamora/ Nightshade)	Whole plant	Tea	3	6
		Leave	Chewed up	1	1
Valerianaceae	*Valeriana officinalis* (Valeriana/ Valerian)	Root	Tea	1	10
		Leave	Tea	5	22
Vitaceae	*Cissus verticillata* (L.) Nicolson & CE Jarvis (bejuco ubí)	Leave	Tea	5	1
		Bark	Tea	3	1
Xanthorrhoeaceae	*Aloe vera* (Sábila/ Aloe)	Leave	Drinkshake	1	3

Indet: *it wasn't possible a botanical identification due to it was impossible to collect the plant material.*

Frequency.

1. Once at day by a week.
2. Twice at the day for a week (in the morning and at night).
3. Twice at the day for a week (in the morning and in the afternoon).
4. Three time at the day for a week.
5. Others (daily or in days alternate) with a frequency more than one week (always).

In addition to the plants mentioned in table 1, in the collection of the data in the polls, four patients referred the liquefied mixture of several medicinal plants to treat high blood pressure, which we then relate: Aloe, cucumber and pepper (Liquefied 1)

Tomato, celery, parsley, beet (Liquefied 2)

Mango, banana, germ of wheat and cinnamon (Liquefied 3)

Corn, bark of pineapple, cloves of smell: Boiling, filtering, add lemon and drink to take fresh (Liquefied 4)

3. Materials and Methods

This research was carried out in the period from May to September 2016 in the canton Jipijapa, Manabí province, includes a territorial surface of 1,420 km2 and has 71.083 inhabitants distributed by sex being the 50.74% men and the 49.26% women, of them 30.851 reside in rural and 40.232 reside in the urban area [50].

The Canton Jipijapa, is located at the South western end of the province of Manabí, To 403 km of capital Quito of the Ecuador. Limits to the North: with the cantons Montecristi, Portoviejo and Santa Ana; South: Pajan canton and the province of Guayas. East: with the cantons 24 de Mayo and Pajan and West: the Pacific Ocean and the Puerto Lopez canton. It is formed by three

urban parishes and seven rural. The weather predominantly is tropical dry, with variations of temperatures average of 24 ° C [50].

Is chose this area because the municipality presents a wealth floristic and cultural, also because the population maintains a form of life where predominates the use of those natural resources (agriculture), as well as the ease of access to these resources. The interviews semi-dealing technique was used for the collection of information and closed questions were formulated partially before going to the field. Two questionnaires were developed, one ethnobotanical and one epidemiological [51] (with modifications).

The interviews are conducted oral e individually in them own homes, being preferentially made with the responsible of the Group family, independent of sex, in order to obtain general information about the interviewees (age, sex, and education).

The data obtained in each questionnaire is analyzed, processed and subsequently is organized in tables where is show plants and parts of these used, origin (cultivated, collected or purchased in the market), form of preparation and number of citations. The sample complied with patients diagnosed with hypertension in the Canton Jipijapa. As already mentioned above, the interviews were conducted in the homes of the population, in the first visit were due presentations, exhibition of the objectives of the study and requesting permission for interviews.

A time completed questionnaires is asked to the interviewee that show it plants (case was possible) for make the record photographic of the same and it collects, for facilitate it identification botanical by consultation and the comparison with the literature specialized (is looked them sites IPNI) (www.ipni.org) and mobot (www.mobot.org)), in other cases the recognition is made by simple observation, as were plants known.

The data obtained were analyzed by calculating absolutes values and percentages. The tables and graphs were conducted using the program Microsoft Excel 2007.

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4. Conclusions

In the six hundred fourteen surveys applied to hypertensive patients in the Canton Jipijapa, were cited a total of sixty-two species of medicinal plants belonging to thirty-one families, of these the most represented botanically were Lamiaceae (eight species), Apiaceae, Rutaceae and Rosaceae each with four species. The plants with the highest number of citations were Chamomile (*Matricaria recutita*), Cuban oregano (*Plectranthus amboinicus*), grass luisa/ lemongrass (*Cymbopogon citratus*) and valerian (*Valeriana officinalis*). Among these plants, Lemongrass is the only one, which its effectiveness as anti-hypertensive has been proven. The 42 % of the population interviewed not consumes or knows about medicinal plants. We believe that it should promote the use of medicinal plants and training the population and health personnel for the proper use of plants for healing purposes, to rescue the wisdom in the community, since it could be seen that this knowledge is being lost in the Canton.

Conflicts of Interest

"The authors declare no conflict of interest".

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Chemometric highlighting of metabolic diversification factors at inter- and intra-molecular scales by a new simplex-based training approach: application to *Astragalus* saponins

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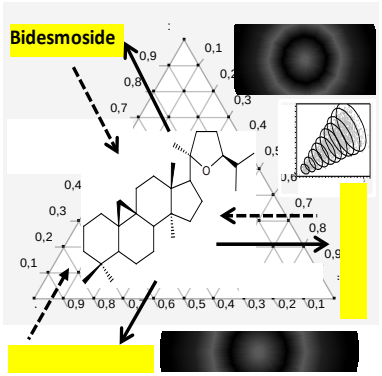
Abstract: *Metabolisms represent highly organized systems characterized by strong regulations obeying to mass conservation law. This makes a whole chemical resource to be competitively shared between several biosynthesis components (ways) at both intra-and inter-molecular scales. Statistically, the whole shared-resource principle can be considered under a constant or unit sum constraint which represents the basis of simplex mixture rule. In this work, a new simplex-based chemometrics approach was developed to extract scaffold information on different biosynthesis regulation factors responsible for the chemical structural diversity at atomic and molecular scales within a large metabolic system. This approach consisted in linearly combining different (q) molecular clusters into a complete set of N mixtures by gradually varying their relative weights. The complete set of the N combinations was given by Scheffé's mixture matrix. In output of Scheffé's design, each molecular combination was represented by a theoretical average (barycentric) molecule which was trained by the characteristics of the different weighted clusters. The mixture design was iterated several (K) times by bootstrap technique to explore chemical variability between and within clusters. Finally, the K response matrices issued from the K iterations were averaged to obtain a smoothed matrix containing scaffold information on different*

regulation factors responsible for molecular diversification at inter- and intra- (atomic) molecular scales. This matrix was used as a backbone for graphical analysis of positive and negative trends between atomic characteristics: the chemical substitutions levels of carbons. This new simplex approach was illustrated by cycloartane-based saponins of Astragalus genus by considering three desmosylation clusters (mono-, bi- and tridesmoside saponins) characterized by relative glycosylation levels of different aglycones' carbons.

Keywords: *Simulation, molecular diversification, glycosylation, desmosylation, simplex mixture design*

Graphical Abstract:



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Introduction: This work presents a new chemometric approach combining structural information contained in a large set of molecular structures to highlight mechanistic order governing molecular diversification via sequential ramifications and elongations occurring at different carbons. The approach was based on Scheffé's mixture design [1].

Simulated results are initially trained by inter- and intra-molecular variability contained in a large set of chemical structures available in literature. The simplex approach was applied to saponins of *Astragalus* genus which is the largest taxon of terrestrial plants with more than 2200 species [2]. Saponins are essentially based on cycloartane aglycone which has several forms including structures with either aliphatic lateral chain or epoxylated cycle(s) [3]. Epoxylated forms consists mostly of 20,24-epoxycycloartane (**Figure 1**).

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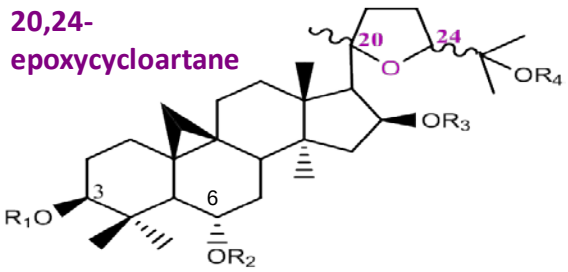

R_1= (OAc)n-Xyl, Rha-(diOAc-Xyl)
R_1= (Ac-Gly), Rha-(OAc-Gly)
Gly= Glc, Xyl
R_1= (Gly2-Gly1); Gly1= Glc, Xyl
Gly2= Api, Ara, Glc, Rha
R_1= Ara-(OAc-Gly); Gly= Ara, Xyl
R_1= H, pi, Xyl, Glc
R_2= H, Ac, Glc, Rha, Xyl, Ac-Rha, pi,
R_3=H, Ac, Glc, COCH2OH, pi
R_4= H, Glc

.

Figure 1. Chemical structure[25] of 20,24-cycloartane with different substitutions implied in saponins synthesis. Xyl, xylose; Rha, rhamnose; Glc, glucose; Ara, arabinose; Api, apiose; Ac, acetyl.

Materials and Methods: Molecular formations obey to mass conservation principle under which the whole mass of a system can be shared between q components (A, B, C, etc.) according to many ways. This leads to many possible multiplets where weights or proportions w_j vary the ones at the expense of the others under constant or unit

sum constraint (**Eq. 1**), i.e. the sum of *q* mass parts equal to the whole initial mass (**Figure 2**):

.

$$\sum_{j=1}^{q} weights \quad w_j = w = Cst \qquad (1)$$

Sharing processes between exclusive and complementary system's components is statistically governed by simplex rule where components' parts vary the one relatively to the other under the constraint of limited total resource available in the whole system.

In molecular systems, the simplex rule find application both between and within molecules (i.e. between carbons of a same molecule) (**Figure 2**):

At inter-molecular level, molecular clusters exclude the one the others by simple biosynthesis (**Figure 2a**). At intra-molecular scale, a molecule can be conceived as a set of carbons competing for chemical substitutions leading the whole molecular substitution level to be shared between carbons (**Figure 2b**).

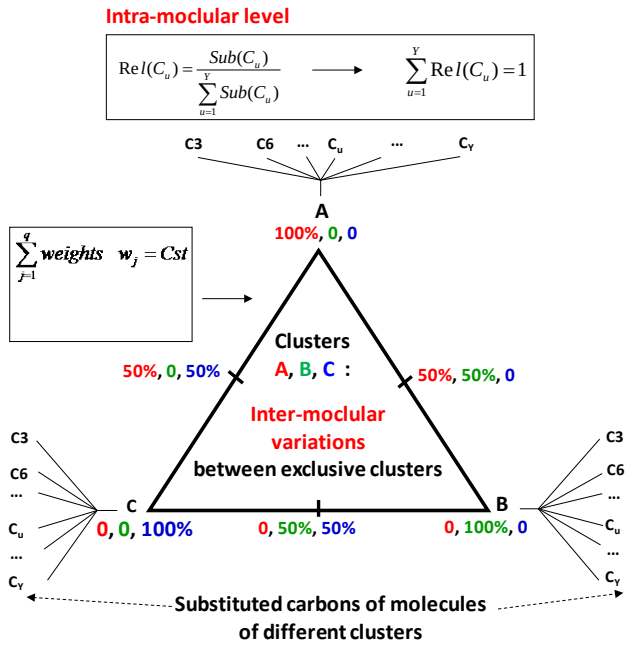

Figure 2. Geometric representation and numeric formulation of simplex rule governing mass conservation between several components at inter- (a) and intra- (b) molecular scales.

In this work, molecular clusters consisted of three desmosylation levels represented by 108 *Astragalus* saponins based on 20,24-epoxycycloartane and characterized by different relative glycosylation levels of carbons. Desmosylation (*D*) and glycosylation (*G*) are responsible for saponins' ramification and elongation, respectively (**Figure 3**). In the current work conceptualization, *D* and *G* represented two metabolic variability factors at inter-molecular and intra-molecular scales, respectively.

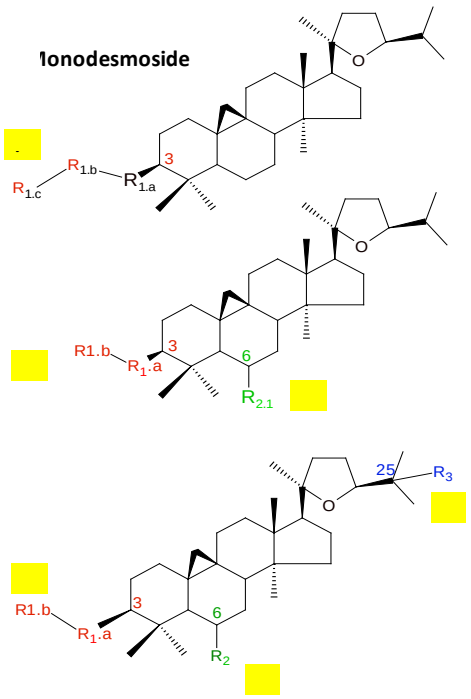

Figure 3. Illustration of three saponin clusters associated to three desmosylation levels and characterized by intra-molecular variation of relative glycosylation levels of different carbons

Molecular diversification mechanisms of *Astragalus* saponins were computztionally approached by a training process based on iterative combinations of structural information characterizing the three desmosylation clusters. The complete combinatorial process between the three clusters was based on Scheffé's mixture design (**Figure 4a**) [1]. With $q = 3$ clusters and by fixing the total weight w to 10 molecules per mixture, combinatorial formula gives a total N of 66 combinations (**Eq. 2**) (**Figure 4b**):

$$N = \frac{(w+q-1)!}{(q-1)!w!} \qquad (2)$$

Application of Scheffé's mixture design consisted in linearly combining the three desmosylation clusters j by varying their weights w_j the ones at

the expense of the others (**Figure 4c**). Each linear combination was applied by randomly sampling a total of w molecules from the three clusters by respecting the three variable weights w_j given by Scheffé's mixture design. In output, the *w* sampled molecules were summarized by a theoretical barycentric molecule calculated by averaging the w glycosylation profiles representing mono-, bi- and tri-desmoside clusters (**Figure 4d**). With 66 combinations, we obtained 66 elementary responses consisting of 66 barycentric molecules characterized by 66 average relative glycosylation profiles (**Figure 4e**).

Scheffé's mixture design was iterated several times in order to explore molecular variability between and within clusters (**Figure 5**): a single mixture design combining w=10 molecules per mixture is insufficient for good training from the whole available molecular data. For that, Scheffé's matrix was iterated several times, i.e. *K* times, leading to *K* response matrices (**Figure 5a**). After K = 30 iterations of mixture design, 30 elementary response matrices were obtained, each one containing N=66 barycentric molecular profiles (**Figure 5b**). Finally, the 30 elementary response matrices were averaged leading to a final matrix containing 66 smoothed molecular profiles integrating high inter- and intra-molecular variability (**Figure 5c**). This final matrix was used for graphical analysis of relationships between substituted carbons (**Figure 5d**).

Relationships between carbons were conditional to each desmosylation clusters. For that, in each plot, the three desmosylation clusters *j* were separately considered by projecting their 11 weights w_j (w_j = 0 to 10) on corresponding points (**Figure 6**). Then, equal weights were statistically grouped by a confidence ellipse. The succession of the eleven ellipses from 0 to w=10 resulted in a trajectory highlighting how the two considered carbons varied the one in relation to the other for the formation of considered desmosylation level.

Results and Discussion: Smoothed relationships highlighted several relationships between carbons depending on the desmosylation level (molecular cluster) (**Figure 6**):

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Global trajectories for monodesmoside formation initially implied increase in glycosylation level of C3 at the expense of C6, C25 (**Figure 6a**). Moreover, at local (intra-molecular) scale, negatively inclined ellipses in the three plots indicated systematic tension between C3, C6, C25 for glycosylation in favor of C3.

For bidesmosylation formation, global relationships between carbons highlighted significant increase of 6-glycosylation at the expense of C3 which stabilized at intermediate relative glycosylation level (**Figure 6b1**). However, C25 was not favored in bidesmosylation system (**Figures 6b2, 6b3**). However, weights' ellipses in C25 vs C6 showed positive inclination indicating that 6-glycosylation could be favorable factor for 25-glycosylation. Such hypothesis found checking in tridesmosylation:

Tridesmosylation implied further decrease of relative 3-glycosylation in favor of C25-glycosylation (**Figure 6c2**). However, C6-glycosylation slightly decreased by maintaining relatively high level indicating its open (alternative) role for glycosylation for tridesmoside formation (**Figure 6c3**). Systematic intra-molecular positive trend between C6- and C25-glycosylations was highlighted by positively inclined weights' ellipses contrary to negative states in C6 and C25 vs C3 plots (**Figures 6c1-6c3**).

Highlighted sequential glycosylation mechanisms revealed in agreement with [4-8]:

- The promiscuity characterizing glycosyl-transferases of saponins.
- The initial hydroxylation occurring at C3 during aglycone formation from 2,3-oxidosqualene.

Conclusions: Simplex simulation approach provides useful chemometrics tool for extraction and visualization of inter-atomic factors governing molecular diversity in a large metabolic dataset (population). It can be applied on other structural criteria than desmosylation leading to multiple analysis ways of molecular diversification factors. In consequence, such a computational tool has wide perspective applications in metabolomics.

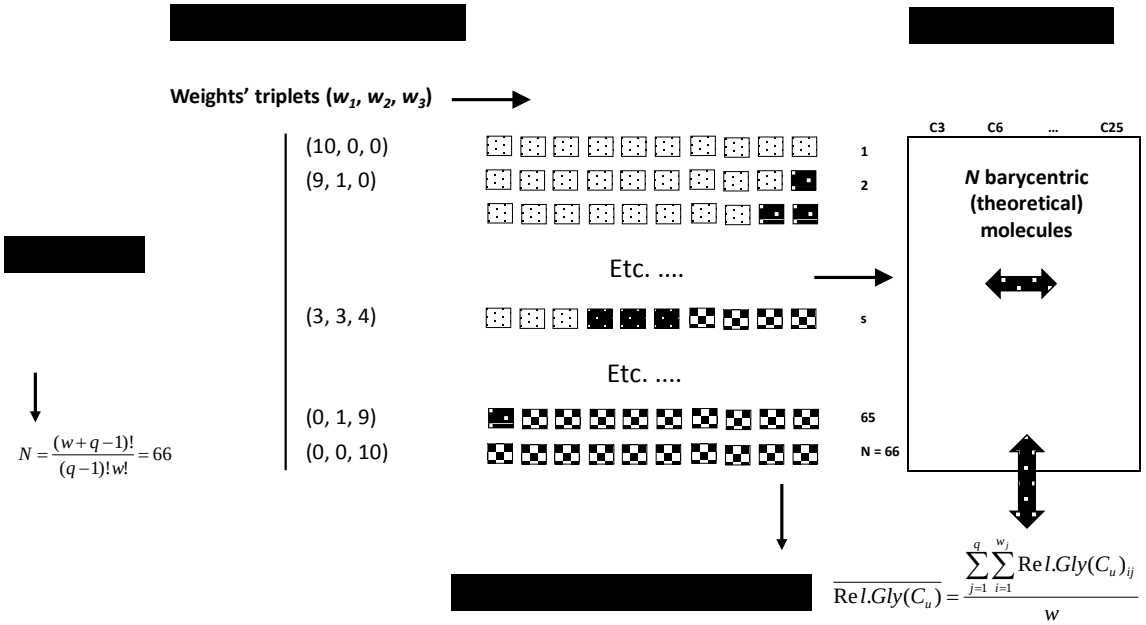

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Figure 4. Principle of the simplex approach based on Scheffé's matrix (**a**) and combining structural information of three molecular clusters (**c**) to simulate a response matrix of barycentric molecules (**e**) by averaging the contributive molecules to combinations (**d**). Required number of combinations is calculated from the number *q* of clusters and the whole weight *w* of mixture (**b**).

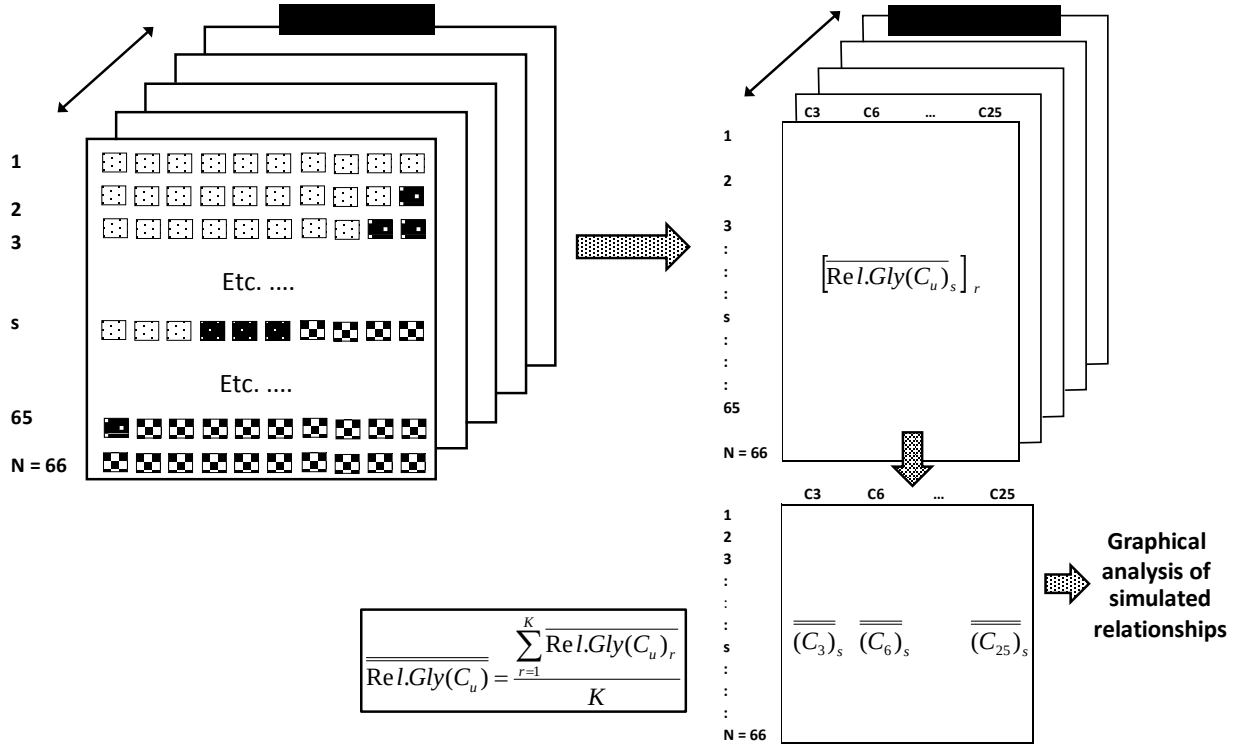

Figure 5. Iteration of Scheffé's mixture design (**a**) and its response matrix of barycentric molecules (**b**) to calculate a final smoothed matrix (**c**) used for graphical analysis of relationships between carbons leading to highlight molecular diversification mechanisms (**d**).

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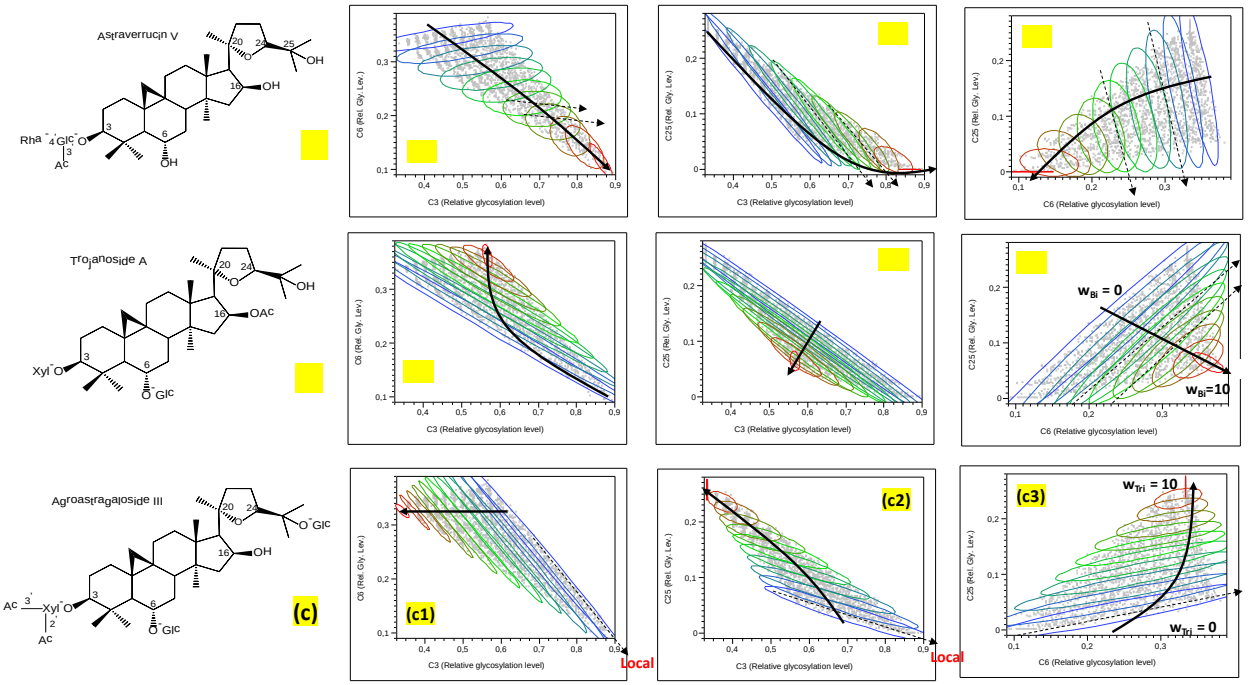

Figure 6. Smoothed relationships between three glycosylated carbons (C3, C6, C25) of *Astragalus* saponins showing global trajectories and local variations associated to metabolic diversification factors at inter- and intra-molecular scales, respectively.

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Immune protection against *Trypanosoma cruzi* induced by TcVac1 vaccine in a murine model using an intradermal/electroporation protocol

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Abstract

The development of vaccines against Chagas disease during the past years have provided a partial control of *Trypanosoma cruzi* infection. GPI-anchored *T. cruzi* genes are conserved in all *T. cruzi* life cycle stages and were tested as vaccine candidates in previous studies, they elicited humoral and cellular mediated immune responses and controlled parasitemia in mice. Herein we tested multi-component DNA-prime/DNA-boost vaccine

(TcVac1) which comprises two plasmids encoding GPI-anchored genes (TcG2 and TcG4) from *Trypanosoma cruzi*; two plasmids encoding adjuvant cytokines (IL12 and GM-CSF). To identify the best route of vaccine application in BALB/c mouse model, two vaccination protocols were compared; a) intradermal injection/electroporation (IDE), b) intramuscular injection (IM). Humoral immune response was evaluated through assessing titers of anti-TcG2 and TcG4 IgG and IgG subtypes (IgG1, IgG2a and IgG2b) antibodies through ELISA assay, using recombinant TcG2 and TcG4 as sensitizing antigens. Evaluation of immune cellular response was assessed through a lymphocyte proliferation assay, after exposure of vaccinated mice splenocytes to the studied antigens. Finally, histopathological and common clinical signs were carried on for vaccinated and infected mice groups. Results demonstrated higher antibody titers for IDE mice groups with a switch from a Th1 (IgG2b/IgG1>1) to Th2 (IgG2b/IgG1<1) immune profile from pre- to post-infection experimental periods, as well as a higher lymphocyte proliferation favoring IDE> IM mice groups. Histopathological evaluation of experimental mice hearts showed areas of myocardial necrosis and degenerative changes associated with severe inflammatory cell infiltrates for control infected mice groups to slight or moderate infiltrates for vaccinated-infected groups. In conclusion electroporation technique enhances the TcVac1 vaccine uptake leading to high specific immune response in both pre- and post-infection periods compared to the intramuscular technique.

Key words

Trypanosoma cruzi; TcVac1; Electroporation; Chagas Disease; Mice model.

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Descriptors Based on Continuous Indicator Fields for 3D-QSAR Studies

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Abstract: CIF descriptors are based on the concept of Continuous Indicator Fields (CIF) [1], a particular case of Continuous Molecular Fields [2,3]. Each CIF descriptor is defined by an isotropic Gaussian function centered at a specific point in the physical space. The positions of these points can be chosen by applying hierarchical cluster analysis to Cartesian coordinates of all atoms in all molecules in the aligned training set. The value of a CIF descriptor for a molecule is equal to the overlap integral between this function and the sum of analogous Gaussian functions centered on all atoms in the molecule. The resulting matrix of CIF descriptors can be used to build 3D-QSAR models.

There are several advantages of using CIF descriptors over the original methodology of building CIF 3D QSAR models [1]. Firstly, CIF descriptors can efficiently be computed for big data sets. Secondly, any machine learning method, regression or classification; linear or non-linear, can be applied to build 3D-QSAR models. Thirdly, CIF descriptors can be aggregated to form 3D analogs of fragment descriptors, which can be used to interpret 3D-QSAR models from structural viewpoint (see Graphical Abstract).

CIF descriptors are implemented in R scripts and available as a part of the Continuous Molecular Fields project [4]. They were used in conjunction with Support Vector Machines and several other machine learning methods to build 3D-QSAR models for several benchmarking data sets.

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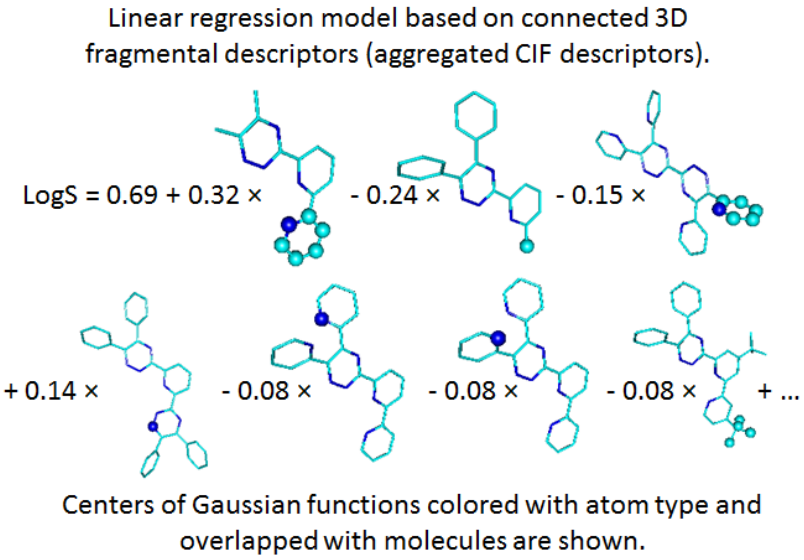
Keywords: 3D-QSAR, continuous indicator fields

Graphical Abstract:



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Acknowledgements: This work was supported by Russian Foundation for Basic Research (Grant 13-07-00511).

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The Use of Energy-Based Neural Networks for Similarity-Based Virtual Screening

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Abstract: Energy-based learning [1] is a common framework for building models in which dependencies between variables are captured by means of a scalar function conventionally called “energy”. The energy-based learning is implemented mostly in recurrent neural networks with symmetric connections between neurons. In this study for the first time, the energy-based neural networks were applied to build structure-activity models. The Hopfield Networks (HN) [2] and the Restricted Boltzmann Machines (RBM) [3,4] were used to build one-class classification models for conducting similarity-based virtual screening [5,6]. The AUC (Area Under Curve) score for ROC curves and 1%-enrichment rates were compared for 20 targets taken from DUD repository. Five different scores were used to assess similarity between each the tested compounds and the training sets of active compounds: the mean and the maximum values of Tanimoto coefficients (Tc-mean and Tc-max, respectively), the energy for Hopfield Networks (HN), the free energy and the reconstruction error for Restricted Boltzmann Machines (RBM-fe and RBM-rec, respectively). The latter score was shown to provide the superior mean predictive performance. Additional advantages of using energy-based neural networks for similarity-based virtual screening over the state-of-the-art similarity searching based on Tanimoto coefficients are: computational efficacy and scalability of prediction procedures, the ability to implicitly reweight structural features and consider their interactions, their “creativity” and compatibility with modern deep learning and artificial intelligence techniques (see reviews in the use of neural networks and deep learning in drug discovery [7-10]).
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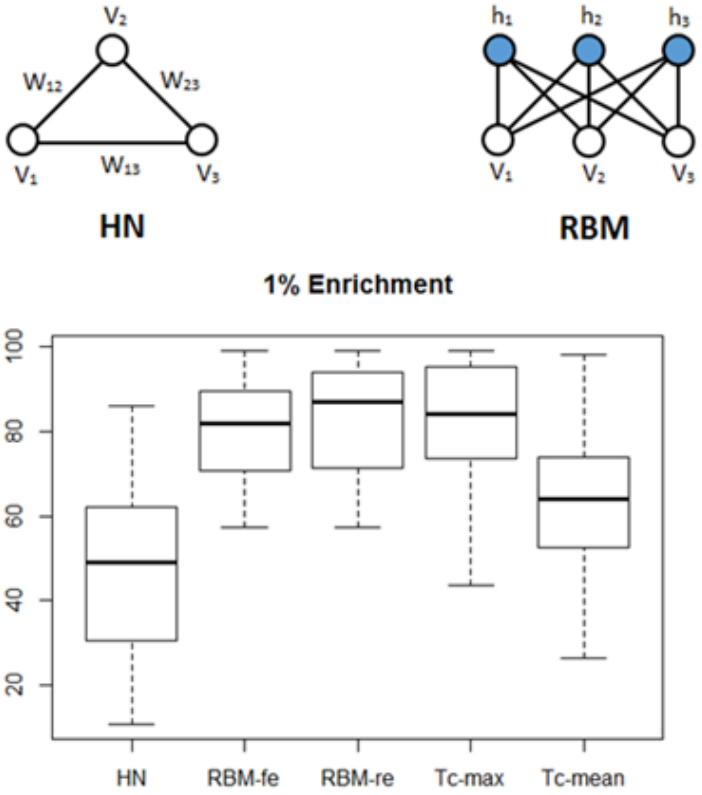
Keywords: neural networks, Hopfield nets, Restricted Boltzmann Machines, similarity searching, virtual screening, one-class classification

Graphical Abstract:



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Study on Optimal Control Strategy of Automatic Transmission Based on Policy Search

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Abstract:

Automatic transmission can shift according to the engine power output and environmental conditions automatically. It is the challenge to reduce the shift jerk and improve the shift quality. A policy search algorithm of reinforcement learning for automatic transmission shift process is proposed. First, algorithm learns from fixed environment set for preliminary strategy. Second, agent interacts with environment and starts online learning for optimal control strategy. Finally, to verify the performance of the algorithm, the simulation study of the shift process under different conditions is carried out. The simulated result demonstrated that the shift jerk can be significantly reduced by applying the optimal control strategy.

Key words: policy search; automatic transmission; optimal control; reinforcement learning

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Conclusions.
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References

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Acknowledgements
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Video Description with Spatio-temporal Feature and Knowledge Transferring

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Abstract:

Describing open-domain video with natural language sequence is a major challenge for computer vision. In this paper, we investigate how to use temporal information and learn linguistic knowledge for video description. Traditional convolutional neural networks (CNN) can only learn powerful spatial features in the videos, but they ignored underlying temporal features. To solve this problem, we extract SIFT flow features to get temporal information. Sequence generator of recent work are solely trained on text from video description datasets, so the sequence generated tend to show linguistic irregularities associated with a restricted language model and small vocabulary. For this, we transfer knowledge from large text corpora and employ word2vec to be the word representation. The experimental results have demonstrated that our model outperforms related work.

Conclusions

In this paper, we propose a model which contains a visual extractor and a sequence generator. First, SIFT flow features are extracted to get temporal information. Through shallow fusion with CNN feature, we can get video visual feature representation. Second, we consider two stacked LSTMs to generate natural language sequence with variable length. In order to integrate linguistic information into the sequence generator, transferring knowledge from large text corpora can generate natural language grammatically. Besides, the experiments show that word2vec is a comparatively better word representation than “one-hot” vector. We evaluate our model on Youtube2Text dataset for METEOR metric. The experiments show that our model can achieve higher METEOR scores than other methods proposed recently. In the future, we would like to exploit a more efficient visual extractor, which contains visual attention mechanism.

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Efficient Actor-critic Algorithm with Dual Piecewise Model Learning

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Abstract: As classic methods for handling continuous action space problem for continuous action space problem in RL, the actor-critic (AC) algorithm and its variants still fail to be sample efficiency. Therefore, we propose a method based on learning two linear models for planning. The two linear models refers to state-based piecewise model and action-based piecewise model, which are determined by the divisions for the state and action space, respectively. Through division, the models are learned more accurately. To accelerate the convergence, the sample near the goal is saved and used to learn the model, the value and the policy to balance the distribution of the samples. On two classic RL benchmarks with continuous MDPs, the proposed method shows the ability of learning an optimal policy by combing both models, and it also outperforms the representative methods in terms of convergence rate and sample efficiency.

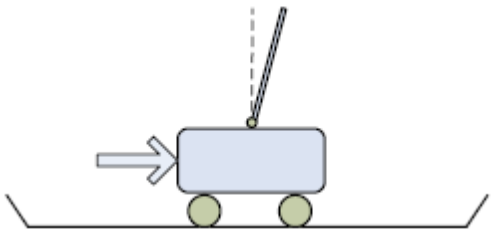


Figure 1. The Pole balancing problem

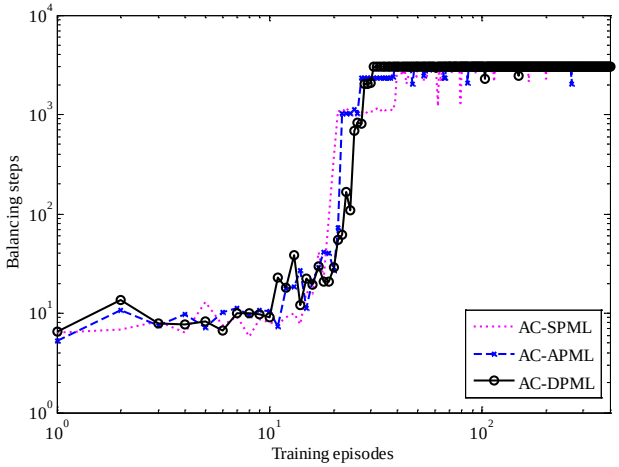
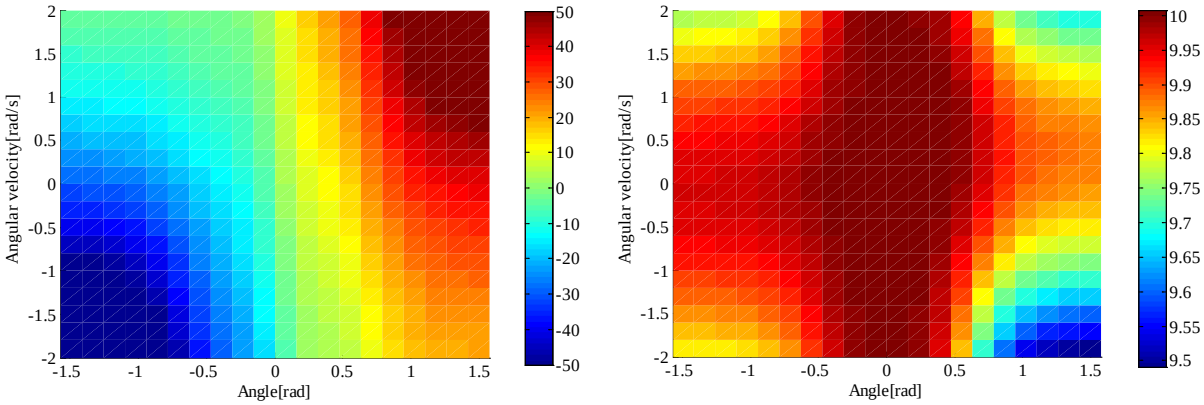


Figure 2. Comparisons of different piecewise models



(a) Final policy obtained after the training is over

(b) Final value function obtained after the training is over

Figure 3. Comparisons of the learned policy and optimal value function

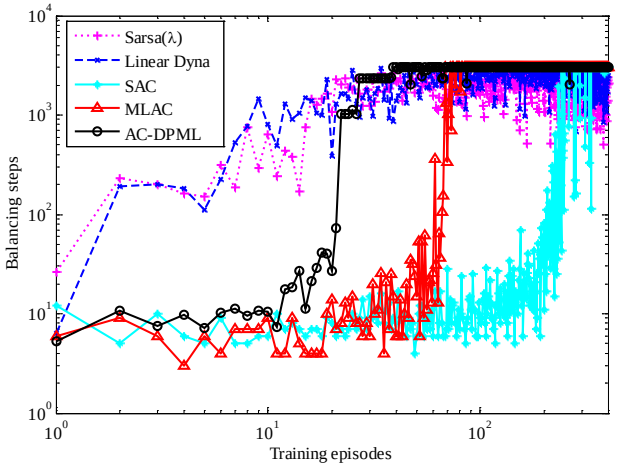


Figure 4. Comparisons of the balancing steps

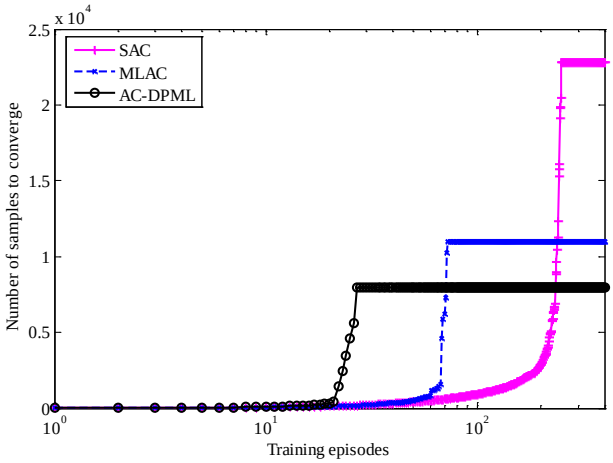


Figure 5. Comparisons of the sample efficiency

Conclusions.

This paper proposes an improved AC algorithm based on two piecewise models, the state-based piecewise model and the action-based piecewise model, to improve the sample efficiency and convergence rate for the problems with continuous state and action spaces. The empirical results show that the two models can cooperate well, additionally, the performance becomes more stable after introducing two piecewise models. In comparison to the discrete action algorithms Sarsa (λ) and linear Dyna as well as the continuous action algorithms SAC and MLAC, AC-DPML behaves well not only in convergence rate but also in sample efficiency. The performances of the discrete action algorithms Sarsa(λ) and linear Dyna do not look as well as those of the compared continuous algorithms. The comparison results between the method with model learning and the one without model learning, e.g., the discrete methods linear Dyna versus Sarsa(λ) or the continuous methods MLAC versus SAC, seem to demonstrate that model learning can improve the performance to a certain extent.

Since the introduction of the piecewise models can really improve the model accuracy, the sample efficiency and the convergence from the experimental results, it would be interesting to apply the two kinds of models to more complex domains, e.g., the inputs are figures or videos, so as to improve the performances for these domains.

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Trajectory-pooled Spatial-temporal Structure of Deep Convolutional Neural Networks for Video Event Recognition

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Abstract:

Video event recognition according to content feature faces great challenges due to complex scenes and blurred actions for surveillance videos. To alleviate these challenges, we propose a spatial-temporal structure of deep Convolutional Neural Networks for video event recognition. By taking advantage of spatial-temporal information, we fine-tune a two-stream Network, then fuse spatial and temporal feature at a convolution layer using a conv fusion method to enforce the consistence of spatial-temporal structure. Based on the two-stream Network and spatial-temporal layer, we obtain a triple-channel structure. We pool the trajectory to the fused convolution layer, as the spatial-temporal channel. At the same time, trajectory-pooling is conducted on one spatial convolution layer and one temporal convolution layer, to form another two channels: spatial channel and temporal channel. To combine the merits of deep feature and hand-crafted feature, we implement trajectory-constrained pooling to HOG and HOF features. Trajectory-pooled HOG and HOF features are concatenated to spatial channel and temporal channel respectively. A fusion method on triple-channel is designed to obtain the final recognition result. The experiments on two surveillance video datasets including VIRAT 1.0 and VIRAT 2.0, which involves a suit of challenging events, such as person loading an object to a vehicle, person opening a vehicle trunk, manifest that the proposed method can achieve superior performance compared with other methods on these event benchmarks.

Our contribution including:

1. We utilize two-stream Network to extract spatial feature and temporal feature, and fuse spatial and temporal feature at a convolution layer by using a conv fusion method, which can enforce the consistence of spatial-temporal structure.

2. To combine the merits of deep feature and hand-crafted feature, we implement trajectory-constrained pooling to HOG and HOF features, which can more accurately represent local feature of the happening actions.

3. We design a trajectory-pooled triple-channel structure. Triple-stream structure can model the spatial-temporal information better.

4. We conduct an extensive set of experiments, which demonstrates that our method can obtain excellent performance.

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Fusing Augmented Spatio-temporal Features for Action Recognition

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Abstract:

Visual features are vitally important for action recognition in videos. However, traditional features fail to effectively recognize actions for two reasons: on one hand, spatial features are not powerful enough to capture appearance information of complex video actions; on the other hand, important temporal details are always ignored when pooling and encoding. In this paper, we present a new architecture that fuses multiple augmented spatio-temporal features. In order to strengthen spatial features, we conduct crop and horizontal flip on original frame images. Then we feed these processed images into deep Two-Stream network to produce robust spatial representations. To get powerful temporal features, we employ fourier temporal pyramid (FTP) to capture three different levels of video context, including short-term level, medium-range level, and global-range level. At last, we fuse these augmented spatio-temporal features using canonical correlation analysis (CCA) method, which is capable to capture the correlation between these features. Experimental results on UCF101 dataset show that our method can achieve excellent performance for action recognition.

Conclusions

In this paper, we propose to fuse multiple augmented spatio-temporal features for better action recognition. The enhanced spatial features are extracted by feeding multiple crop images into VGG networks. Then through a three-level FTP, the features are capable to capture different level temporal context information about action. Finally, the method is capable to improve the performance effectively by CCA fusion. Our experimental results show that the model can achieve comparable accuracy to the state of the art methods.

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Person Re-identification by Null Space Marginal Fisher Analysis

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Abstract:

For better describing pedestrian's appearance, the feature representations used in person re-identification are usually of high dimension - typically amounting to thousands or even higher. However, this incurs the typical Small Sample Size (SSS) problem, i.e., the number of training samples in most re-identification datasets is much smaller than the feature dimension. Although some dimension reduction techniques or metric regularization could be applied to alleviate this problem, they may result in the loss of discriminative power.

In this work, we propose to overcome SSS problem by embedding training samples into a discriminative null space based on Marginal Fisher Analysis (MFA). In such a null space, the within-class distribution of the images of the same pedestrian will shrink to a single point, resulting the extreme fisher analysis criterion. We theoretically analyze the subspace where the discriminant vectors lie on and derive a closed-form solution. Furthermore, we also extend the proposed method to nonlinear domain via the kernel trick. Experiments on VIPeR, PRID450S and 3DPes benchmark datasets show that our method achieves 56.30%, 76.80% and 66.88% rank-1 matching rates respectively, outperforming the state-of-the-art results by 2.74%, 15.38% and 9.59%.

Our contributions including:

1. We develop a novel null space learning method called Null Space Marginal Fisher Analysis (NSMFA) to overcome the Small Sample Size (SSS) problem in person re-identification.
2. To deal with the highly nonlinear patterns of pedestrian appearance, the proposed method is further extended to nonlinear case via the kernel trick, Kernel Null Space Marginal Fisher Analysis (KNSMFA).
3. Experiments on three challenging datasets including VIPeR, PRID450S, and 3DPes, demonstrate that our method improves the state-of-the-art results significantly.

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QSAR Models and Virtual Screening for Discovery of New Analgesic Leads

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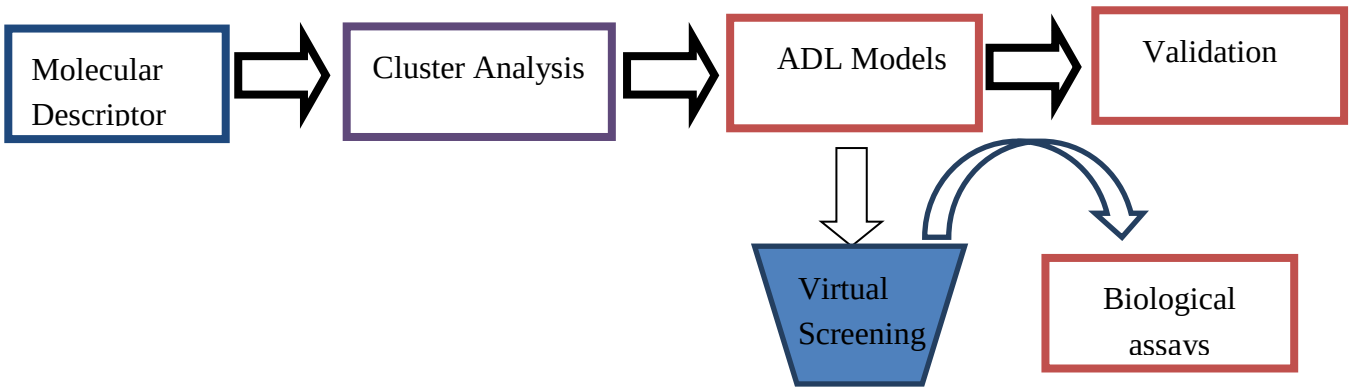
Abstract: The search for new selective pharmacological agents with no significant side-effects is an increasing requirement for the development of new drugs to be used in the treatment of acute and chronic pain. In the present study, a new series of compounds (VAM 1, 6, 10, 11, 12, 2–4) has been screening in QSAR–LDA mathematic models and pharmacologically evaluated. The antinociceptive properties of the new analgesic candidates obtained of virtual screening have been investigated *in vitro* tests. The pre-treatment with the compounds VAM 1, 2-4, 6, 10, 11, 12, showed a potent inhibition of IL-6 on RAW cells. The blocking efficacy of nineteen compounds on several isoforms of voltage-dependent sodium channels, expressed in *Xenopus laevis* oocytes, was tested (Nav1.3, Nav1.5, Nav1.6, Nav1.7, and Nav1.8). An exception was Nav1.6, where VAM 2–4 compound to result in substantial block indicating that acts specifically at this peculiar isoform. Compounds VAM 10 and VAM 2-4 are the most potent antinociceptive agents. These results indicate the potential of the compound VAM 2-4 to treat pain conditions.

Keywords: *In silico* Study, *TOMOCOMD-CARDD* Software, Non-Stochastic and Stochastic Linear Indices, Classification Model, Learning Machine-based QSAR, Analgesic Activity

Graphical Abstract:



Introduction:

The most frequent way to treat pain is by using analgesic drugs. Among the analgesics in use nowadays, those of reference continue to be acetylsalicylic acid and morphine, both isolated in the 19th century. In fact, the classical therapies for pain relief consist mainly in the use of Non-Steroidal Anti-inflamatory Drugs (NSAIDs) and opioids (OP). The first class, whose effects are mediated by the peripheral inhibition of cyclooxygenase (COX) is generally used in the treatment of mild to moderate pain [1]. OP drugs, are used in moderate to severe pain [2]. Both families show quite serious secondary effects such as renal toxicity and gastrointestinal lesions in the case of the NSAIDs or respiratory depression, tolerance and dependence in the case of opioids [3-4].

Current research in pain therapy looks at the discovery of new potent drugs devoid of the limiting side effects of the above-mentioned classes. In light of this virtual (computational) screening of chemical libraries has emerged as a complementary approach to techniques using the classical -*trial and error*- screenings [5-6]. By this means, computational techniques are used to select a reduced number of potentially active compounds from large available chemical or combinatorial libraries [7-9]. This *in silico* procedure will be used here in order to find predictive models that permit us the -rational-selection/identification as well as the design of new analgesics with the required properties.

Taking into consideration the arguments mentioned above, the aims of the present paper are: (1) to use a novel molecular descriptor family, atom-based non-stochastic and stochastic linear indices, in the generation of discriminant functions by linear discriminant analysis (LDA) that permits the classification of chemicals (analgesic and non-analgesic drug-like compounds) in a data set drawn from the literature, (2) to assess the 'biosilico' models by the use of different validation tests, (3) to develop a virtual screening of some *in house* libraries in order to identify potential novel chemical entities (NCEs) with analgesic activity and, (4) to examine the expression of the Nav1.3, Nav1.6, Nav1.7, Nav1.8, and Nav1.9 sodium channels and the electrophysiological properties of the drugs by studying their effects on the inward sodium current (I_{Na}) in *vitro*. (5) to determinate the effects of series of compounds over IL-6 on RAW cells.

Materials and Methods:

The database collected for our study consists of 1190 compounds in total. The active compounds in this set were 572 and 618 organic chemicals, having different clinical uses, were chosen as inactive compounds. In both cases (we consider the structural molecular variability as important goal to assure the quality (from *application domain* point of view) of our QSAR study.

The data stratification was done by using Cluster Analysis. From these chemicals, 902 were chosen to form the training set, being 433 of them active and 469 inactive ones. The great structural variability of the selected training data

makes possible the discovery of lead compounds, not only with determinated mechanisms of analgesic activity, but also with novel modes of action. The remaining subseries, consisting of 139 analgesics and 149 non-analgesics, were prepared as test sets for the external validation of the models (see Figure 1 for more details).

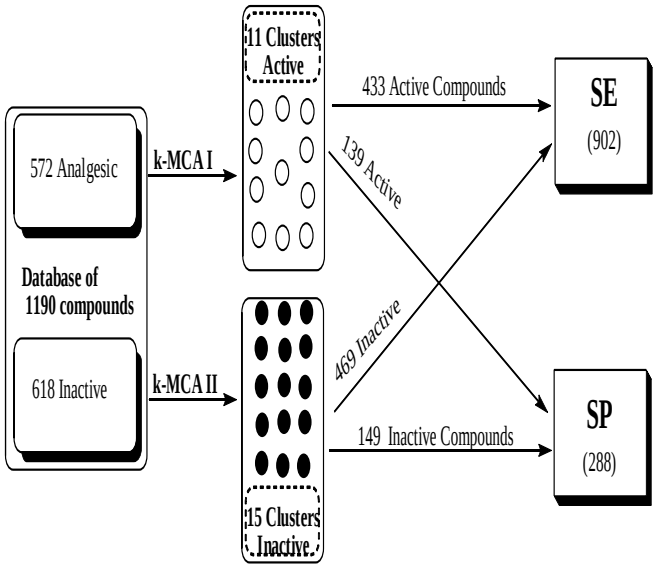


Figure 1. Partition scheme in Training and Test Set

In the present report, we used the *TOMOCOMD-CARDD* software [10]. We performed a hierarchical cluster analysis of the active and inactive series using statistical software package STATISTICA [11]. This procedure permits to select compounds for the training and test sets, in a representative way. This 'rational' design of the training and predicting series allowed us to design both sets, representative of the whole 'experimental universe. LDA was developed to classify compounds as analgesic-like (positive) or no analgesic-like (negative) through LDA, by using non-stochastic and stochastic linear indices as independent variables.

A chemical library, with 145 compounds was evaluated using all the obtained models. From these, nineteen compounds were chosen for biological assays (considered its structural diversity). An electrophysiological experiments *in vitro* was performed to study the effect of different compounds on sodium channels, the whole cell patch-clamp technique was used. The compounds were evaluated at 1, 10 and 100 μM concentrations. Also we carried out the determination of IL-6. The compounds evaluated ion this assay were VAM1, 2, 6, 10, 11, and 12: also at 1, 10 and 100 μM concentrations

Results and Discussion

The main classification-based QSAR equation derived by using forward stepwise LDA and all set of total and local atom-based linear indices computed is shown below:

$$\begin{aligned} AA = & -1.334 + 0.001\ {}^{P}f_{6}^{H}(\bar{x}) - 0.013\ {}^{G}f_{3L}(\bar{x}_{E}) \\ & - 0.278\ {}^{P}f_{3L}^{H}(\bar{x}_{E\text{-}H}) + 0.025\ {}^{K}f_{2L}(\bar{x}_{E}) \\ & - 0.066\ {}^{G}f_{0}^{H}(\bar{x}) - 3.133 \times 10^{-7}\ {}^{M}f_{10}^{H}(\bar{x}) \\ & + 0.021\ {}^{P}f_{5L}^{H}(\bar{x}_{E\text{-}H}) - 0.164\ {}^{P}f_{2}(\bar{x}) \\ & + 0.015\ {}^{V}f_{1}(\bar{x}) + 0.031\ {}^{P}f_{3}(\bar{x}) \end{aligned} \quad \textbf{(1)}$$

$\lambda = 0.40$ $F = 5.95$ $D^2 = 147.88$ $p<0.001$

$C = 0.84$ $Q_{total} = 91.82$ $Spec = 89.61$

$Sen = 93.05$ $fpr = 6.14$

where, ***AA*** refers to Analgesic Activity. $\mathbf{Q_{total}}$ to Accuracy, **Spec** to Specificity, **Sen** to Sensitivity and ***fpr*** refers to false positive rate.

The best of the 13 models obtained is showed in Equation 1. However, their real power and final aim reside at the ability to predict the biological properties of new compounds. Therefore, the use of a test set is essential to assess such a predictive power. The results of this model for the test set were Q_{total} = 88.77, Spec = 87.05, Sen = 89.63 and *fpr* = 9.59.

New analgesic leads were selected using the obtained models for the virtual screening of several databases, only the results of the virtual screening of quinoxalin chemical library will be showed.

Using heterologous expression in *X. laevis* oocytes, we investigated the potency of different compounds for sodium channels. The compounds VAM1, 2-4 and 10 evidence activity over the sodium channels. In the IL-6 determination assay, the compounds that evidence the best dose-answer relationship were VAM 10, 11 and 12. The rest showed good inhibition with 100 uM but the effect was smaller with the other ones. The inhibitory effects is dose dependent in all the cases. The more potent compounds were VAM 10 and VAM11.

Conclusions: Since the models obtained had a wide range application domain they could be useful in the selection of analgesic candidates. In some cases these models are better than those reported by the literature. Most powerful derivates could be obtained from the VAM 2-4 compound by using structure-activity relationship and molecular similitude studies. The compounds VAM-1, VAM-6, VAM-10, VAM-11 and VAM-12 showing *in vitro* analgesic activity, must be considered for further research in order to clarify their mechanism of action possibly related to IL-6.

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An approach toward the identification of new antileishmaniasic compounds.

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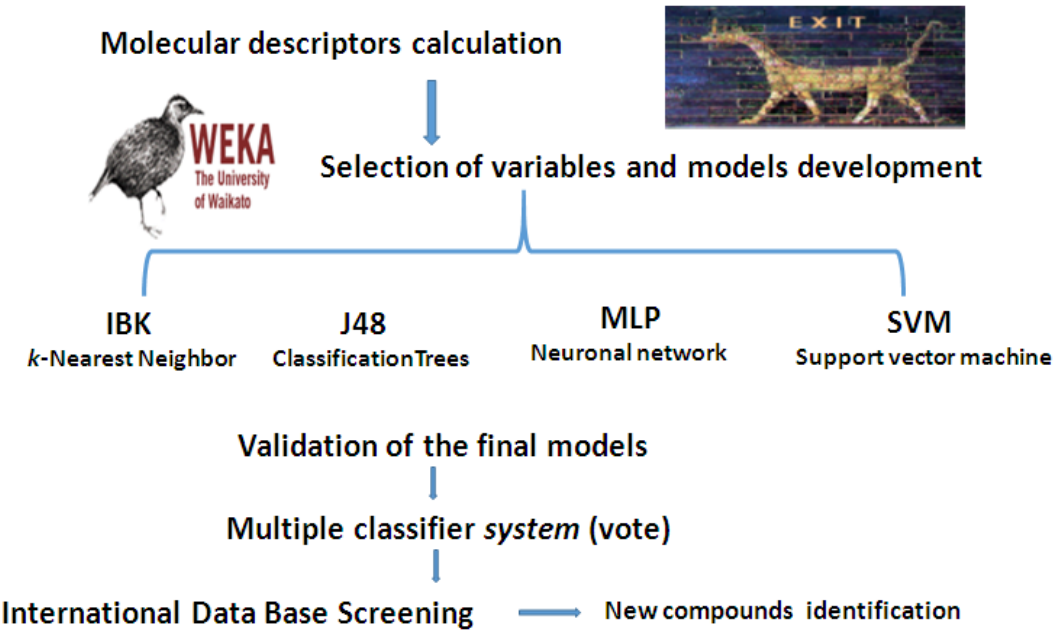
Abstract: Herein we present results of a quantitative structure–activity relationship (QSAR) study to identify new antileishmaniasic compounds (*Leishmania amazonensis*) by using a set of more than 2000 0D-2D Dragon´s molecular descriptors and machine learning techniques. A data set of organic chemicals, with antileishmaniasic activity against promastigote forms of the parasite, is used to develop four QSAR models based on *k*-nearest neighbors, Support Vector Machine, Multi-Layer Perceptron and classification tree techniques. External validation procedures were developed to demonstrate the predictive power of the models. Promastigote´s models correctly classify more than 89% chemicals in both training and external prediction groups, respectively. In addition to the individual techniques an assembled system of majority vote was personalized with the aim of improving the results of the obtained models. To identify new compounds with potential activity against this parasite, a virtual screening was performed using DrugBank international database. There were identified more than five hundred new potential antileishmaniasic compounds. The current results constitute a step forward in the search for efficient ways to discover new antileishmaniasic lead compounds.

Keywords: antileishmaniasic, *Leishmania amazonensis*, machine learning, virtual screening.

Graphical Abstract:



Introduction:

Leishmaniasis, is a disease caused by obligate intracellular protozoa of the genus *Leishmania*, is an old but largely unknown disease that afflicts the World's poorest populations [1]. It presents a broad spectrum of clinical forms and is transmitted to humans and animals through the bite of insects of the *Psychodidae* family [2]. There have been reported by WHO (World Health Organization) more than 20 species of *Leishmania* and between them *Leishmania amazonensis* is of vital importance for the American continent because it is the cause of a wide variety of clinical manifestations, some of them potentially fatal [3-4].

"In silico" methods are useful tools for screening chemicals, especially in early stages of the drug discovery process [5-8]. In the last two decades these studies have played a fundamental role in the development of a number of drugs that are currently on the market [9].

Materials and Methods:

All the compounds included in the research were gathered from published in PubChem bioassays. We select specifically studies carried out against promastigotes of *Leishmania amazonensis*. Different researchers have reported them and publish it in the last years, in several journals with high impact on the Web of Sciences. To verify the structural diversity of the compounds of the database a Cluster Analysis (CA) implemented in the software STATISTICA 8.0 was performed [10].

Models by using k-nearest neighbors (IBK), classification trees (J48), artificial neural network (MLP for its acronym MultiLayer Perceptron) and support vector machine (SMO for Sequential Minimal Optimization) techniques were obtained for promastigote form of the parasite, with the employee of WEKA software [11]. External validation procedures were developed to demonstrate the predictive power of the four resultants models. Virtual screening was performed using DrugBank international database.

Results and Discussion:

A new database of antileishmaniasic compounds with a high degree of structural variability was performed. The parameterization of the structures was carried out using 2489 molecular descriptors 0D-2D implemented in the DRAGON software. WEKA's selection procedures were used to obtain a subset of variables for models development.

Active and inactive compounds were divided into different subsets using k-MCA so we could obtain Training and Test sets following the procedure shown in the Figure 1.

Promastigote´s models correctly classify more than 82% and 80% of chemicals in both training and external prediction groups, respectively. Figure 2 shows the Accuracy percentages obtained for the models SVM results the higher accuracy model followed by IBK and MLP respectively.

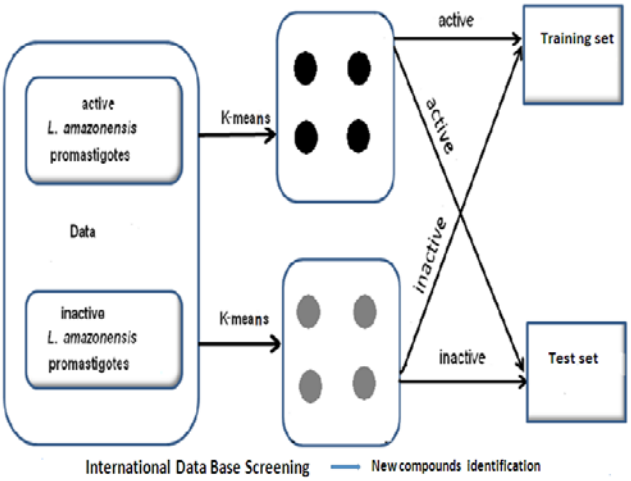


Figure 1 Training and Test Sets obtention procedure.

The external validation of the four models for promastigotes using a new set of 22 compounds previously evaluated in PubChem bioassays showing positive results. In addition to the individual techniques an assembled system of majority voting was personalized with the aim of improving the results of the obtained models [12].

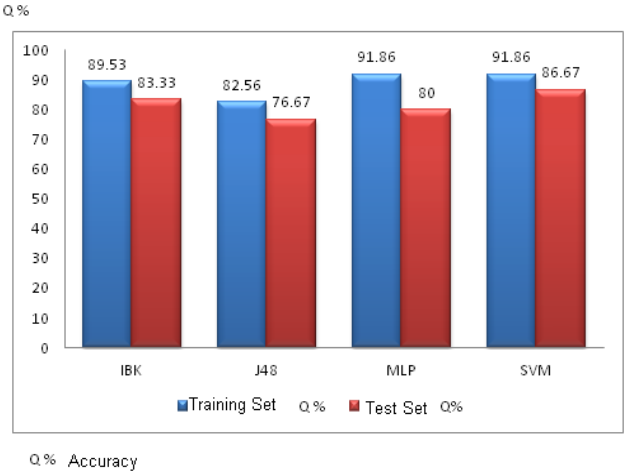


Figure 2. Accuracy percentages obtained for training and test series on final models

To identify new compounds with potential activity against promastigotes forms of *L. amazonensis* a virtual screening using DrugBank international database was performed. There were identified more than five hundred new potential antileishmaniasic compounds. The use of the assembly by the majority vote enabled us to reduce the screening compounds identified as potentially active. These compounds can be experimentally evaluated to corroborate their activity against *L. amazonensis* with favorable repercussion in a time saving and use of chemical reagents, so the current results constitute a step forward in the search for efficient ways to discover new antileishmaniasic lead compounds.

Conclusions

With the use of artificial intelligence techniques we develop four validated models with good statistical parameters. The obtained models were able to identify new compounds with potential activity against promastigote forms of *L. amazonensis* through virtual screening of databases. This work constitutes a useful tool in the search of new leading compounds against this parasite.

Conflicts of Interest: *"The authors declare no conflict of interest"*

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Multiple Linear Regression Model of Thermolysin Inhibitors as Antihypertensive Pattern.

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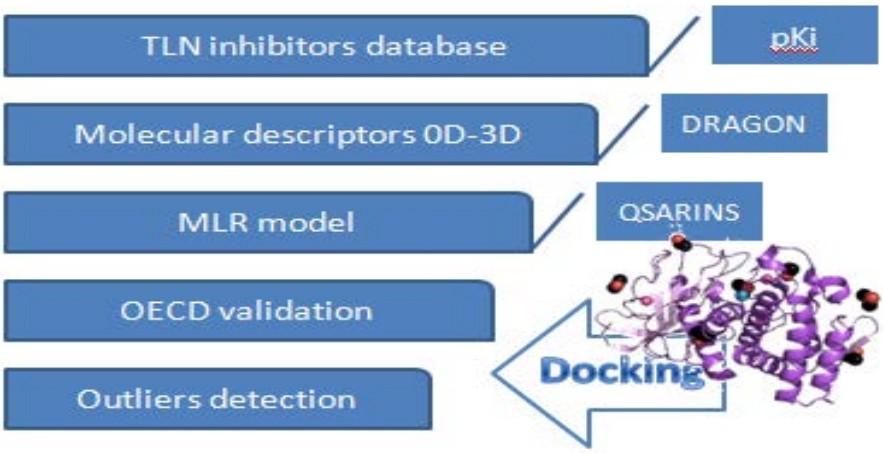
Abstract: Thermolysin is a bacterial proteolytic enzyme, considered by many authors as a pharmacological and biological model of other mammalian enzymes, with similar structural characteristics, such as Angiotensin Converting Enzyme and Neutral Endopeptidase. Inhibitors of these enzymes are considered therapeutic targets for common diseases, such as hypertension and heart failure. In this report, a mathematical model of Multiple Linear Regression, for ordinary least squares, and genetic algorithm, for selection of variables, are developed and implemented in QSARINS software, with appropriate parameters for its fitting. The model is extensively validated according to OECD standards, so that its robustness, stability, low correlation of descriptors and good predictive power are proven. In addition, it is found that the model fit is not the product of a random correlation. Two possible outliers are identified in the model application domain but, in a molecular docking study, they show good activity, so we decide to keep both in our database. The obtained model can be used for the virtual screening of compounds, in order to identify new active molecules.

Keywords: *Antihypertensive; Docking; Multiple Linear Regression; QSARINS; Thermolysin.*

Graphical Abstract:



Introduction:

The zinc-metalloproteinases secreted by the gram-positive thermophilic bacterium *Bacillus thermoproteolyticus* is the prototype of the TLN family and has served as a model system to study the inhibition mechanism of other metalloproteinases. Crystallographic data for TLN and various TLN-inhibitor complexes have been used in efforts to model the active site of other TLN-like enzyme [1,2]. These enzymes play a key role in the biosynthesis and metabolism of different bioactive peptides, so that its inhibitors have considerable potential as therapeutic agents [3]. In addition, Thermolysin presents close structural and functional similarities with several mammalian enzymes that are involved in the control of different physiological functions, like neprilysin (NEP) and angiotensin-converting enzyme (ACE), both involved in the control of blood pressure

The dual inhibition of neprilysin and the angiotensin receptor may represent an attractive therapeutic approach for a wide range of cardiovascular diseases, including hypertension, diabetes and heart or kidney failure, in which vasoconstriction, volume overload and neurohormonal activation play a role in the pathophysiology [4]. The structural and functional similarities between TLN, NEP and ACE indicate that Thermolysin inhibitors may also inhibit ACE and NEP and be putative antihypertensives [5]. Dual NEP/ACE inhibitors repress simultaneously two key enzymes that participate in cardiovascular function regulation [6]. This type of inhibitors exerted typical actions of ACE inhibitors and/or NEP inhibitors, such as dose-dependent inhibition of angiotensin I–induced hypertension, protection of atrial natriuretic factor, and enhancement of diuresis, natriuresis, and cGMP urinary excretion [7].

The QSAR studies are useful tools for screening chemicals, especially in early stages of the drug discovery process [8,9]. If these are properly developed and rigorously validated [10,11], they become outstanding tools to evaluate only those that are most promising [12,13].

Materials and Methods:

The database was collected from reports of the international literature (can be seen in the full paper [14]). The database of 176 experimental compounds was split into training (133 compounds) and prediction (43 compounds). For the calculation of molecular descriptors (all the families of descriptors 0-3D), we used DRAGON Software [15]. In order to obtain the MLR model, we used the QSARINS (QSAR-Insubria) software [10].The model was validated in accordance with the principles established by the OECD [11].

Results and Discussion:

The best MLR model obtained with its statistical parameters is shown below:

$$\textit{p}\mathbf{Ki} = -23.62 + 0.52\text{x}\mathbf{Mor07u} - 20.77\text{x}\mathbf{Mor12v} - 3.12\text{x}\mathbf{R5v}^{+} + 2.87\text{x}\mathbf{R5p}^{+} + 23.58\text{x}\ \mathbf{B01[N–O]}$$

N= 133	R^2= 0.714	R^2_{adj}= 0.702
s= 1.230	F= 63.248	R^2-R^2_{adj}= 0.011
LOF = 1.688	K_{xx}= 0.231	ΔK= 0.100
	$RMSE_{tr}$= 1.202	MAE_{tr}= 0.975
	RSS_{tr}= 192.024	CCC_{tr}= 0.833

The model presented an R^2 of 0.714, so it manifests a proper fit for modeling Thermolysin inhibition. In addition, an R^2_{adj} of 0.702, which is indicative of the convenience to add a new descriptor to the model and, together with the low value of the LOF parameter of 1.688, we can say that no existing overfitting is in the model, as it presents a good fit with minimum number of descriptors. The correlation among the model´s descriptors is low because the value of K_{xx} is small (0.231); this allows us to assume that the model has very little redundant information in the selected descriptors.

In order to validate our model, we followed the OECD regulatory principles to ensure their validity, checking the model performance, i.e., the fitting, stability in the cross-validation and the ability to predict new compounds [11]. The fitting and stability of the model were evaluated using internal validation procedures; first, we take into account the parameters of the cross-validation Leave-One-Out (LOO).

According to the obtained results, it is possible to affirm that the internal predictions are good since the variance explained in the prediction by LOO (Q^2_{LOO}= 0.6868) has a comparable value with R^2= 0.7135 (see Fig. 1) with a small error in the predictions ($RMSE_{cv}$= 1.2563 and MAE_{cv}= 1.0190).

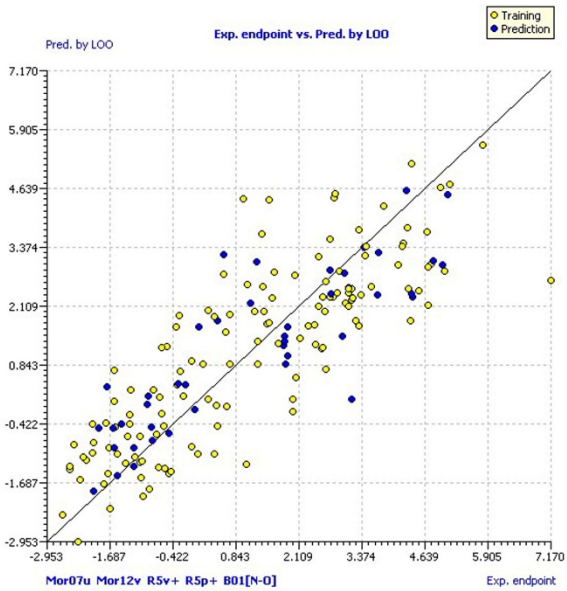


Figure 1: Scatter plot of experimental pKi versus predicted by LOO.

A stronger technique included in the QSARINS is Leaving-Many-Out (LMO), which was developed leaving out the 30% of the dataset to study the behavior of our model. According to this, the model is considered stable because the R^2= 0.7135 and Q^2_{LMO}= 0.6810 values are comparable, and calculation in each iteration of LMO and their averages are comparable to the values of R^2 and Q^2_{LOO} of the model.

We demonstrates that the model was not the result of a casual correlation, using the Y-scrambling procedure, placing the answers at random, so that there was no correlation with descriptors and, as a consequence, the model performance decayed dramatically.

The predictive ability of the model was tested using a series of external predictions (external validation). Using this procedure, we checked the ability of the model to predict new compounds and their statistical parameters showed values equivalent to the model R^2_{ext} = 0.723, $RMSE_{ext}$ = 1.182, MAE_{ext} = 0.91, $PRESS_{ext}$ = 60.091, CCC_{ext} = 0.817.

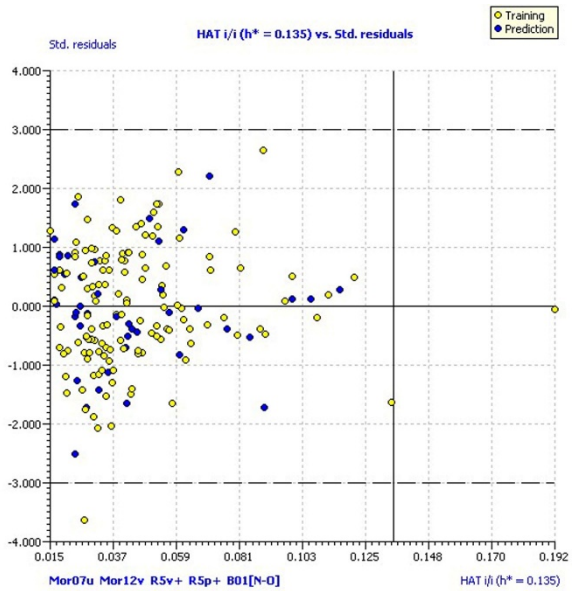


Figure 2: Williams plot. Hat diagonal values versus standardized residuals.

In this study, we used the approach of leverage (*h*) and standardized residuals described in the technical literature [16]. Fig. 2 shows the William graph of the model, where yellow circles represent the compounds of the training and blue circles represent the prediction set. As shown in this figure, most of the compounds are within the applicability domain of the model. There is only one compound (compound 34) with leverage value greater than the critical leverage (*h**= 0.192), but showing residual within the

limits, and one compound (compound 43), having residual off- limits (3.62) though this is within critical leverage. Both cases must be taken into account as potential outlier compounds.
In this sense, we made experiments of molecular docking of these 'outlier' compounds and a group of compounds with similar structural characteristics, since the model provided three-dimensional information of molecules, and it would be interesting to observe their behavior in the active site of Thermolysin. Based on these results, we decided to keep both compounds in the database.

Conclusions: In the present study, a QSAR-RLM model was developed using molecular descriptors of Dragon software, which adequately predicted the inhibitory activity of the enzyme Thermolysin. This model fulfilled all regulatory principles established by the OECD; the robustness of the model was tested through exercised internal validation (LOO, LMO and Y-scrambling), and its predictability was determined with an external prediction set (external validation). Two possible outliers were identified in the model application domain but, in a molecular docking study, they showed good activity, so we decided to keep both in our database.

Acknowledgements:
The authors thank to Prof. Paola Gramatica of the University of Insubria for kindly provide us a free version of QSARINS software to develop this work.

Conflicts of Interest: The authors declare no conflict of interest

Notes:
For the full content of the results presented here, see:
Cañizares-Carmenate, Y., Mena-Ulecia, K., Perera-Sardiña, Y., Torrens, F., Castillo-Garit, J. A. **2016**. **An approach to identify new antihypertensive agents using Thermolysin as model: In silico study based on QSARINS and docking.** *Arab. J. Chem.* (which can be downloaded free of cost using the following link: http://dx.doi.org/10.1016/j.arabjc.2016.10.003)

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CRISPR-Cas Gene Editing: Regulatory Issues and Applications

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Abstract: CRISPR-Cas gene editing methods is an emerging powerful tools in biosciences. Applications of CRISPR to synthetics biology, biotechnology, personalized medicine, drug discovery etc, generates a series of legal and bioethics questions. This generates in turn the necessity of new regulatory issues. In this work we discuss more recent applications of CRIPSR focused on drug discovery with incidence of human genome information. This includes, but is not limited to: resistance-selection studies of antimalarial drugs; new animal models for drug assay, *etc*. We also, discuss the legal regulation and ethical aspects of this area of human genome editing related to drug discovery.

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Keywords: CRISPR-Cas; Drug discovery; Human genome; Gene editing legal issues.

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