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Efficient Synthesis of DHA Transition Metal Chelates as Potent Antioxidants, Enzyme Inhibitor and Antimicrobial Agents †

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Abstract: A large in vitro biological screening and an efficient with easy access to a family of transition metal complexes of dehydroacetic acid (**DHA**) are reported. The obtained complexes (**1-4**) with some transition metal of interest: **Ni (II), Co (II), Zn (II), Mn (II)** respectively, were fully characterized by MP, UV-Vis and FT-IR spectroscopy; several in vitro biological tests were performed on this series of compounds to explore its therapeutically potential in order to continue further investigations and exploring it as new target drugs. In this case, enzymatic activity: as urease inhibitors and antioxidant activities: ABTS radical scavenging activity, β-carotène linoleic acid bleaching activity, Ferrous ions binding effect, Copper and ferrous chelating activity, gave good values of IC50 for all studied complexes **1-4** in range of 8.20 ±0.39-10.62 ±0.01 µg/mL for urease inhibiting test better than DHA and used standard Thiourea (IC50 = 11.57 ± 0,68 µg/mL), interesting results are also obtained for compound **2** in ability of chelating ferrous ions with an IC50 = 14.53 ± 0.92 µg/mL, comparing with tested standard EDTA (CI50 = 8.80 ± 0.47 µg/mL), for all cited applications complex **4** is mostly a hit, while antimicrobial activity gave better results with free ligand DHA, discussion on molecular structure and predicted SAR will be given.

Keywords: DHA; transition metal complexes; biological activities

1. Introduction

Actually, there is a growing interest on DHA derivatives compounds due to its several active sites and a great capacity of chelating [1–4], in addition to an establish antibacterial, antifungal, anti-inflammatory and anticancerous activities; several researches were performed particularly on its metal complexes [5].

In fact, DHA presents 4 active centers [6]: C3 position is highly nucleophilic, it is the site of electrophilic attacks with conservation of pyronic structure such as in enols reactivity.

In the opposite C2, C4 and C6 positions are sites of nucleophilic attacks which lead generally to open pyron cycle and formation of other cyclations of new heterocyclic systems. C5 is inactive centre (Figure 1).

By the other hand, transition metals are characterized by an incomplete under layer (d) which insured electronic transitions and high melting point. It is well establish that inorganic elements play an essential role in vital process, physico-chimical and biological proprieties of organic and metallic moieties increased when gathering into organometallic complexes, in addition to that our research group had already identified and published a number of DHA's chelates as potential class of antimicrobian [7,8]. Thus, this manuscript describes assessment of its in vitro urease inhibition and antioxidant activities, which to the best of our knowledge, has been never reported, in addition to antimicrobial effect, it seems to be interesting to study both DHA and complexes' derivatives structurally and biologically to establish a relationship between molecular structure, electronic proprieties and medicinal effect and how structure modulation by chelating could affect or increase therapeutic response, this could be a great challenge.

The present work investigated DHA and chelates as potent bioactive compounds by several in vitro biological applications and study the effect of coordination on therapeutic response to lead to promising therapeutically agents.

2. Material and methods

All chemicals and reagents used in this work were of analytical grade and purchased from Sigma-Aldrich Company Ltd. (Germany), Melting points were determined with a digital melting point apparatus using capillary technique. Infrared (IR) spectra were recorded with a Shimadzu FTIR-8010M spectrometer between 400 and 4000 cm-1 (KBr disks), UV spectra were recorded with a Thermo Evolution 100 Nicolet spectrophotometer. Enzymatic and antioxidant tests were performed using a microplate reader (*Multi-mode* EnSpire ᴿ PerkinElmer).

2.1. Chemistry

All studied complexes (1–4) were synthesized according to a published procedure [7]. Briefly, to 2 equiv of DHA dissolved in acetone, 1 equiv of the corresponding metal salt was added. The solution was stirred 1h30min at room temperature for Co, Mn and Ni complexes, and 6h for Zn complex. The resulting solid was recovered by filtration and washed with cold water, dried in air, then recristlyzed by slow evaporation at room temperature from dimethylsulfoxyde or dimethylformamide.

2.2. Biological Methods

All tested in vitro *biological applications on* DHA and chelates were realized in the range of 3.125–200 μg for urease inhibiting activity, 12.5–800 μg for antioxidant and antimicrobial activities.

2.2.1. Urease inhibiting activity essay

Urease inhibiting essay was realized according to published protocol [9] without modification.

2.2.2. ATBS scavenging activity

The ATBS scavenging activity test was performed by published method [10]. using BHA and BHT as reference standards.

2.2.3.β-carotène linoleic acid bleaching activity

β-carotène linoleic acid bleaching activity was tested with published protocol [11] using BHT and BHA as positive control.

2.2.4. Fe binding test

The Fe-binding effects was evaluated by published protocol [12].

2.2.5. Ferrous ions chelating activity

Ferrous ions chelating quantitative activity was realized by a published method [13].

2.2.6. Copper chelating activity CCA

Copper chelating activity CCA was performed according to published protocol [14] using EDTA as positive control.

2.2.7. Antimicrobial activity

The antimicrobial susceptibility and resistance tests of tested compounds were carried out according to the Agar disk-diffusion testing developed in 1940 [15] against four referential strains Gram-negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603 and Gram-positive *Staphylococcus aureus* ATCC 25923 and one fungi *Trichoderma harzianum Rifai*; for comparison Gentamicine (10μg /disc), and Nalidixique (30 μg/disc) were used as standards.

Discs (Whatman No. 1, 6 mm diameter) are impregnated with each extract and then applied to the surface of the specifique agar plates which have been seeded by spreading the microbial suspension. The seeding is carried out in such a way to ensure a homogeneous distribution of the bacteria. The petri dishes are incubated during 24 h at the appropriate temperature 37 °C in the laboratory oven, and the resulting inhibition zone diameter was measured in millimeters.

Antimicrobial activity is determined in terms of the diameter of the inhibition zone produced around the discs.

2.3. Statistical analysis of data

Statistical analyses were performed using Excel 2018 software, measurements were realized in triplicate. $p < 0.05$ is regarded as statistically significant.

3. Results and discussion

3.1. Synthesis

Studied chelates (1–4) were efficiently synthesized according to green chemistry pathways: room temperature, air atmosphere, base catalyst and water purification; leading to target compounds in one pot reactions with good yields.

3.1.1. Bis(3-acetyl-6-methyl-2-oxo-2H-pyran-4-olato)bis(dimethyl sulfoxide) cobalt(II)

[Co(DHA)2.2DMSO] (1). Yield 81%, mp 260 °C, pink solid. IR spectrum, ν, cm-1:1670 (C=O,lactones), 1627 (C=O, acetyl), 3377 (C-O, hydroxyl), 595 (O-M), 1050 (C-O-C). UV λ, nm: 251, 267, 296.

3.1.2. Bis(3-acetyl-6-methyl-2-oxo-2H-pyran-4-olato)bis(dimethyl formamide) nickel(II)

[Ni(DHA)2.2DMF] **(2)**. Yield 72%, mp = 243 °C, green solid, IR spectrum, ν, cm–1:1667 (C=O,lactones), 1583 (C=O, acetyl), 3377 (C-O, hydroxyl), 600 (O-M), 1010 (C-O-C). UV λ, nm: 253, 264, 296.

3.1.3. Bis(3-acetyl-6-methyl-2-oxo-2H-pyran-4-olato)bis(dimethyl formamide) zinc(II)

[Zn(DHA)2.2DMF] **(3).** Yield: 91%, mp = 178 °C, white solid, IR spectrum, ν, cm–1:1670 (C=O,lactones), 1575 (C=O, acetyl), 3377 (C-O, hydroxyl), 620 (O-M), 1000 (C-O-C). UV λ, nm: 251, 267, 285.

3.1.4. Bis(3-acetyl-6-methyl-2-oxo-2H-pyran-4-olato)bis(dimethyl formamide) manganese (II)

[Mn(DHA)2.2DMF] **(3).** Yield: 43%, mp > 260°C, white solid, IR spectrum, ν, cm–1:1693 (C=O,lactones), 1597 (C=O, acetyl), 3360 (C-O, hydroxyl), 575 (O-M), 990 (C-O-C). UV λ, nm: 252, 263, 313.

3.2. In vitro urease inhibitory activity

Urease is a metal containing enzyme that catalyzes the hydrolysis of urea into ammonia and carbamate [16,17]. Ureases are widespread in nature among plants, bacteria, fungi, algae and invertebrates [9]. Depending of its origin, Urease is associated to a lot of human diseases: infections of gastrointestinal and urinary tract [18], such as stomach cancer and the peptic ulcer [19]. Hepatic encephalopathy, urolithiasis, urinary catheter encrustation, pyelonephritis and hepatic coma [20].

Uresase inhibiting activity by DHA and chelates (1–4) started at low concentration (3.125–6.25 μg) in a dose depending-response relationship fashion and irreversible way, furthermore IC_{50} values exhibit a very high and close inhibitory capacity of all analogs in the range of 8.2 ± 0.39–10.62 ± 0,01 μg/mL in comparison with DHA moiety (IC_{50} >200μg/mL) and used standard thiourea (IC_{50} = 11.57 ± 0.68 μg/mL), best activity is reported for compound 4 with an IC_{50} = 8.2 ± 0.39μg/mL.

3.3. In vitro antioxidant activity

Antioxidant activity can be evaluated by different approaches: direct way by scavenging different radicals: ABTS, B-carotene which are responsible of many diseases.

And indirect way for example it has been well documented that ROS (especially _O2□ radical anions and _OH radicals) can be generated via Fe^{2+} catalysis according to Fenton reaction.

Fe^{2+}-binding (or chelating) can therefore efficiently reduce the generation of ROS and has been developed as a therapeutic approach for many diseases related to ROS [21].

3.3.1. ABTS scavenging activity

This method is based on producing a two electron oxidation of ABTS to the radicalization, addition of antioxidants to the pre-formed radical cation reduces it into ABTS.

A dose depending-response behavior is observed with all tested compounds, in term of efficiency, DHA's chelate 4 is the most active of the series with an IC50 = 258. 36 ± 4. 62 μg/mL, which still weak when compared with tested standards BHT and BHA (1.29 ± 0.30 μg/mL 1.81 ± 0.10 μg/mL respectively), DHA and 1–2 complexes with an IC50 around 800 μg/mL exhibit no significant results for this activity.

3 is found to be inactive in the studied game of concentrations. From the obtained results, we can deduce that mechanism of action for this application is conducted mainly by charge transfer characterizing compound 4 from remain compounds.

3.3.2. β-. carotène linoleic acid bleaching activity

Obtained results for this application indicate also a dosedepending-response behavior and exhibit chelate 4 as a hit, starting it activity at low concentration 12,5 μg with an IC50= 22.69 ± 0.86 μg/mL but still however weaker than tested standards BHA and BHT(1.05 ± 0.03 μg/mL 0.91 ± 0.01 μg/mL respectively).

DHA moiety with an IC50= 77.07 ± 2.14 μg/mL is more active than its chelates 1,3 (220.87 ± 6.07 μg/mL 185.72 ± 3.19 μg/mL respectively), 2 is found to be inactive in the studied game of concentrations.

3.3.3. Ferrous ions binding effect

This test allows to appreciate ability of studied compounds to bound ferrous ions, it is a preliminary qualitative test, so increasing doses is not significant.

From obtained results, it appears that Fe^{+2} ions absorb strongly approximately at 420 nm.

All tested compounds chelating to Fe^{+2} ions had UV absorptions around 450 nm with varying degrees, in fact DHA and chelates 1,3,4 had higher Fe^{2+}-binding ability than 2.

DHA and chelates 1–4 free Fe-binding have no absorption in this area.

This indicate that DHA and transition metal derivatives 1–4 are potentially able to bind Fe ions and could undergo Fe^{+2}- binding to inhibit the generation of O_2.

3.3.4. Ferrous ions chelating activity

Quantitative evaluation of tested compounds for Ferrous ions chelating activity gave following results: only chelate 1 chaw interesting activity for this test, beginning to chelate Ferrous ions at 12,5µg with an IC50 =14.53 ± 9.02 µg/mL close to tested standard EDTA (IC50 =8.80 ± 0.47 µg/mL), remaining compounds DHA and 3,4 complexes had no significant activity with an IC50 > 800 µg/mL.

Chelate 2 as predicted by the previous test was inactive for this application.

3.3.5. Copper (CCA) chelating activity

Copper chelating activity was also tested quantitatively, results indicated a dosedepending-response behavior for all tested compounds and exhibit chelate 4 as a hit again giving relatively the best activity with an IC50 = 106.36 ± 3.88 µg/mL when used standard EDTA had an IC50= 59.04 ± 0.56 µg/mL, the rest of compounds had mostly a close activity with an IC50 in the range of 129.07 ± 5.93–179.83 ± 6.74 µg/mL.

Tested compounds gave better results for chelating Copper ions than ferrous ions.

3.3.6. Antimicrobial activity

Investigations on antibacterial potential of DHA and chelates against the four referential strains, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus*, gave good to moderate activity against all tested bacterial strains.

However, we can note that DHA exhibits a high activity against all tested strains with bactericidal effect ≤ 0.195 mg/mL better than corresponding chelates, and all chelates (1)-(4) have nearly the same bactericidal effect (≤ 0.195 mg/mL) in case of *E.coli* bacteria. Besides, complex (1) shows a very good activity against S. *aureus,* complex (2) gave a moderate activity against all tested bacteria, complex (3) has a very good activity against *K.pneumonia* and *P.aeruginosa* and a moderate one against the other tested bacteria.

Antifungal activity against *Trichoderma harzianum Rifai* revealed a weak inhibiting effect about 10mm even at high dose for all tested compounds.

3.4. Activity and structure relationship

Activity relationship has been studied at molecular level on the basis of binding interactions and electronic structure of the active analogs [22].

- Uréase was the first enzyme being crystallized, but its mechanism of action still rather unknown, by the other hand; all prior studies on enzym-inhibitor complexes report that urease active center is a pseudo paramagnetic octahedral containing 2 Ni atoms [23], and according to literature only functional groups containing O, N and S could give bidendate, tridentate ligands or chelates able to form octahedral complexes with Ni atoms active center [24].

Zerner model [25,26] supports our theory about the role of carbonyl group (Figure 1) in binding and deactivating the 2Ni atoms inhibiting urease enzyme by the same way in an irreversible mechanism via covalent bonds. Furthermore, the presence of transition metals improved inhibiting ability and capacity of DHA moiety by charge transfer acting as co-factor or catalysts.

- Concerning SAR of studied compounds and antioxidant activities; chelating improved mainly therapeutic response, and total effects of these compounds can be attributed to the fact that their scavenging or chelating activities could involve several antioxidant pathways, including Fe2+-binding [27], H-donating, ET [28], proton transfer [29], and even radical adduct formation (RAF) [30]. To understand transition metals' chelates antioxidant SAR, central cation environment must be well known, because it is all molecule that react not only metal.

Therefore, we can suppose that complex 4 gave almost higher antioxidant activity because of Mn(II) incomplete under layer d with 3 valence electron which could play at the same time charge transfer as well as facilitating H+ hydroxyl group lability or both.

- Antimicrobial activity

Investigations on antibacterial potential of DHA and chelates, exhibits DHA free ligands as better antimicrobial agent than its corresponding chelates, which indicates that it doesn't follow the same

mechanism of action in all biological applications, we can suppose that in case of antimicrobial activity the role of hydroxyl function in DHA moiety is determinant, and according to a previous publication [8] hydrogne binding with bacteria membrane insure and increase its reactivity.

4. Figures, Tables and Schemes

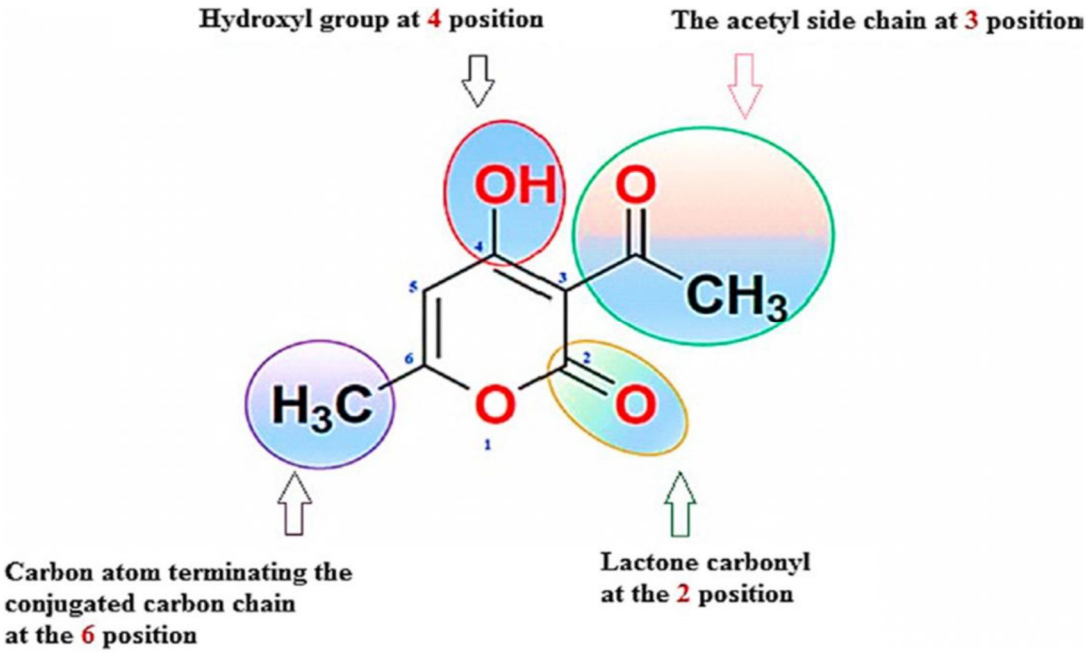

Figure 1. DHA's active centers.

5. Conclusion

Dehydroacetic acid and its transition metal complexes **(1–4)** were efficiently synthesized, characterized and fully screened for over than 20 in vitro biological activities, which exhibit a high urease inhibiting capacity for all chelates, Mn chelate as a hit for antioxidant activity and DHA free ligand as better antimicrobial agent. Discussion on molecular structures and comparison with observed effect helped to explain the structure activity relationship that may or not improve observed therapeutically effect of Dehydroacetic acid by chelating in comparison with DHA free ligand, and suppose that tested compounds adopt different mechanism of action depending on biological application.

In regards of these promising results, kinetic studies, pharmacomodulation of tested oraganometallic complexes to increase medicinal effect and in vivo preclinical tests, are recommended as future investigations.

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Dhanraj. C.J, Nair. M.S.;. Application of Schiff bases and their metal complexes-A Review. Journal of Coord. Chemestry, 2009. 62 : 4018.
2. Hadjipalu. L, Dimitra.J, Athina.A &Geronikaki. Chem Abstr., 1998. 129 :144.
3. Lei Shi, W-J Mao, Y.Yang, H-L Zhu. Synthesis, characterization, and biological activity of a Schiff-base Zn (II) complex . J. of Coord. Chem., 2009. 62: 3471.
4. Tarafder, M.T.H.; Ali, M.A.; Azahari, D.J.; Wee, K.; Silong, S.; Crouse, K.A.; Transition Metal Chemistry. 2000. 25. 456.
5. Casabó.J, Marquet.J, Moreno-Mañas.M, Prior.M, Teixidor.F, Florencio.F, Martínez-Carrera.S, García-Blanco.S .(1987). Transition-metal complexes with dehydroacetic acid: Crystal structure of bis (3 - acetyl - 4 - hydroxy - 6 - methyl - 2 - pyrone) cobalt(II) bis(dimethylformamide).Polyhedron.Vol 6, 1235-1238.
6. Stephen, J.F.; Marcus, E. *The Journal of Organic Chemistry*. 1969. *34* (9), 2527-2534 DOI: 10.1021/jo01261a011
7. Marir, A., Mouas, T. N., Anak, B., Jeanneau, E., Djedouani, A., Aribi-Zouioueche, L., & Rabilloud, F. (2020). *Cobalt(II), Nickel(II) and Zinc(II) complexes based on DHA: Synthesis, X-ray crystal structure, antibacterial activity and DFT computational studies. Journal of Molecular Structure, 128353*. doi:10.1016/j.molstruc.2020.128353
8. Chiter, C., Bouchama, A., Mouas, T. N., Allal, H., Yahiaoui, M., Warad, I., … Djedouani, A. (2020). *Synthesis, crystal structure, spectroscopic and hirshfeld surface analysis, NCI-RDG, DFT computations and antibacterial activity of new asymmetrical azines. Journal of Molecular Structure, 128376*. doi:10.1016/j.molstruc.2020.128376
09. Taha, M. ; Ullah, H. ; Muhammad Ramadhan Al Muqarrabun, L. ; Naseem Khan, M. ; Rahim, F. ; Ahmat, N. ; Javid, M.T. ; Ali, M. ; Mohammed Khan, K. ; . Bisindolylmethanethiosemicarbazides as potential inhibitors of urease: Synthesis and molecular modeling studies. Bioorganic & Medicinal Chemistry . 2018. 26. 152–160. https://doi.org/10.1016/j.bmc.2017.11.028Get ri
10. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.;, Rice-Evans, C.;. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 1999. 26, 1231–1237.
11. Tepe, B.; Sokmen, M.; Akpulat, H. A.; Daferera, D.; Polissiou, M.; Sokmen, A.; Antioxidative activity of the essential oils of Thymus sipyleus subsp. sipyleus var. sipyleus and Thymus sipyleus subsp. sipyleus var. rosulans, Journal of Food Engineering,Volume 66, Issue 4, 2005, Pages 447-454.
12. Li, X.; Jiang, Q.; Wang, T.; Liu, J.; Chen, D. Comparison of the Antioxidant Effects of Quercitrin and Isoquercitrin: Understanding the Role of the 6″-OH Group. *Molecules* 2016, *21*, 1246. https://doi.org/10.3390/molecules21091246
13. Dinis, P.; Pineda, M.; Aguilar, M.;. Spectrophotometric quantity of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. Anal. Biochem. 1999. 269, 337–341
14. Sánchez- Vioque, R.; Polissiou, M.; Astraka, K.; de los Mozos-Pascual, M.; Tarantilis, P.; Herraiz-Penalver, D., Santana-Méridas,O.;. Polyphenol composition and antioxidant and metal chelating activities of the solid residues from the essential oil industry Industrial. Industrial Crops and Products. 2013. 49:150–159.
15. Heatley, N. G. (1944) 'A method for the assay of penicillin', *Biochemical Journal*. Portland Press Ltd., 38(1), pp. 61–65. doi: 10.1042/bj0380061.
16. Mobley H, Hausinger R. Microbiol Rev. 1989;53:85–108.
17. Karplus PA, Pearson MA, Hausinger RP. Acc Chem Res. 1997;30:330–337.
18. Collins CM, D'Orazio SE. Mol Microbiol. 1993;9:907–913.
19. Montecucco C, Rappuoli R. Nat Rev Mol Cell Biol. 2001;2:457–466.
20 Hanif M, Saleem M, Hussain MT, et al. J Braz Chem Soc. 2012;23:854–860.
21. a) Fang, Y.Z.; Zheng, R.L. Reactive oxygen species in theory and application of free radical biology, 1st ed.; Science Press: Beijing, China, 2002; p. 124.
b) Devos, D.; Moreau, C.; Devedjian, J.C.; Kluza, J.; Petrault, M.; Laloux, C.; Jonneaux, A.; Ryckewaert, G.; Garçon, G.; Rouaix, N.; et al. Targeting chelatable Iron as a therapeutic modality in Parkinson's disease. Antioxid. Redox Signal. 2014, 21, 195–210. [CrossRef] [PubMed]
22. Boukerzaza, G.. Synthèse, Analyse Structurale et Criblage Biologique de Nouveaux Complexes Organométalliques. Mémoire de Master2, Biochimie Appliquée UFMC1, Route Ain El Bey Constantine 25000 Algeria, 26/06/2019.
23. Clark. PA, Wilcox. DE. (1989). Magnetic properties of the nickel enzymes urease, nickelsubstituted carboxypeptidase A and nickel-substituted carbonic anhydrase, *Inorg. Chem*., *28*, 1326-1333.
24. Amtul.Z, Atta-ur-Rahman, R.A. Siddiqui, M.I. Choudhary. (2002). Chemistry and Mechanism of Urease Inhibition. Current Medicinal Chemistry.Vol 9, 1323-1348.
25. Blakeley. RL, Hinds. JA, Kunze. HA, Webb. EC. (1969). Jack bean urease (EC 3.5. 1.5).Demonstration of a carbamoyl-transfer reaction and inhibition by hydroxamic acids. *Biochemistry*. *8*, 1991-2000.
26. Dixon.N-E, Gazzola.C, Blakely.R-L, Zerner.B.(1975). Jack-bean urease (E.C. 3.5.1.5.3).A metalloenzyme. A simple biological role for nickel. Journal of the American Chemical Society.97 :4131-4133.

27. Fisher, A.E.; Hague, T.A.; Clarke, C.L.; Naughton, D.P. Catalytic superoxide scavenging by metal complexes of the calcium chelator EGTA and contrast agent EHPG. Biochem. Biophys. Res. Commun. 2004, 323, 163–167. [CrossRef] [PubMed]
28. Holtomo, O.; Nsangou, M.; Fifen, J.J.; Motapon, O. DFT study of the effect of solvent on the H- atom transfer involved in the scavenging of the free radicals _HO2 and _O2□ by caffeic acid phenethyl ester and some of its derivatives. J. Mol. Model. 2014, 20, 2509. [CrossRef] [PubMed]
29. Bielski, B.H.J.; Cabelli, D.E.; Arudi, R.L.; Ross, A.B. Reactivity of HO2/O2 □ radicals in aqueous solution. J. Phys. Chem. Ref. Data 1985, 14, 1041–1100. [CrossRef]
30. Andrew, B.D.; Thomas, N. Rapid reaction of superoxide within sulin-tyrosyl radicals to generate a hydroperoxide with subsequent glutathione addition. Free Radic. Biol. Med. 2014, 70, 86–95.