Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Identification and Antioxidant Activity of Di and Tri-Organotin Complexes Derived from Cinnamic Acid

Inas J. Mahdi [a], Nahlah Salman Saddam [a], Angham G. Hadi [a], Sadiq J. Baqir [b], Yahya F. Al-Khafaji [a*], and Ahmed S. Abbas [a]

[a] *Department of Chemistry, College of Science, University of Babylon, Babylon, Iraq.*

[b] *Medical Laboratories Techniques Department, Al-Mustaqbal University College, Babylon Iraq*

Abstract

In this study, di and triorganotin compounds derived from Cinnamic acid were prepared by condensation reaction to obtain the corresponding complexes (**1**-**4**) with high yields. These complexes were also diagnosed with several techniques, including infrared spectroscopy, $Sn^{119,}$and ^{1}H NMR, in addition to elemental analysis of the elements. These complexes were applied to find out the anti-oxidative activity of cinnamic acid and the prepared complexes by using DDPH and CUPRAC techniques. The results of the antioxidant activity in both ways showed that the prepared complexes are more effective than the ligand from which they are derived. Also, complex **1** showed more antioxidant activity than other complexes.

Keywords: Cinnamic acid, antioxidant activity, CUPRAC method, DPPH method, ligand

1. Introduction

The chemistry of free radicals has received a lot of attention recently. There is much evidence that free radicals in the cell nucleus and molecular membranes cause oxidative damage to biomolecules such as proteins, lipids, and nucleic acids. Maintaining a balanced mix of free radicals and antioxidants is essential for long-term health.Then, oxidative stress processes can be controlled for the prevention and treatment of a wide range of diseases, including diabetes, atherosclerosis, coronary artery disease, cancer, infections, liver disease, Cardiovascular disease, cataracts, nephrotoxicity, and other neurodegenerative diseases associated with aging. Antiviral activity has been demonstrated in a variety of natural antioxidants. Flavonoids, such as (+)-catechin, luteolin, apigenin, quercetin, and quercetin 7-rhamnoside, have been shown to be effective in MERS infection (PEDV) and infectious gastroenteritis virus (TGEV) [1-3].Antioxidants may show to be an effective option to treat diseases caused by coronaviruses in the absence of suitable medicines for SARS and MERS-CoV [4]. Plants, such as edible vegetables, fruits, spices, and herbs rich in vitamins, phenolic compounds, carotenoids, and microelements, are the principal sources of natural antioxidants [5-7]. However, it is important to note that antioxidant activity varies depending on the types and morphological components of natural resources. Synthetic antioxidants are chemically manufactured substances that are added to food as preservatives to assist prevent lipid oxidation because they do not exist naturally [8].The oxygen radical absorption (ORAC) method [9], determination of total phenol content (TPC) [10], DPPH (1,1′-diphenyl-2-picrylhydrazyl) [11], Antioxidant Equivalent Capacity in Trolux [12], Iron Reducing Antioxidant Capacity [13], CUPRAC[14], and Determination of Total Reducing Power (TRP)[15] are all chemical assays used to evaluate the antioxidant activity.Organic tin (IV) compounds have been discovered to have a wide range of biological functions [16-21].Because these compounds have better biological activity, those with organic tin(IV) carboxyl radical Complexes have garnered considerable study. In comparison to other organic tin (IV) complexes [22-32], which have different bonds. Using the DPPH and CUPRAC methods, we investigated the antioxidant activity of various organotin(IV) Cinnamate complexes in this work.

2.Experimental Section

2.1 Preparation of Tri-organotin(IV) Complexes(1-2)

4mmol of the ligand (Cinnamic acid) with NaOH (4mmol) wasdissolved in methanol (40 mL)and stirred for 1hr at room temperature. (4mmol) of (1.54g or 1.302g) from tri-phenyl tin chloride(Ph_3SnCl) or tri-butyl tin chloride(Bu_3SnCl) was added as solid to the first mixture, and the mixture was refluxed for 4 hours with continuously stirred [33-35]. The white precipitated NaCl was filtered off and the solvent was evaporated under a

*Corresponding author e-mail: sci.yahya.alkhafaji@uobabylon.edu.iq, Yahya F. Al-Khafaji

Receive Date: 11 June 2022, **Revise Date:** 23 July 2022m **Accept Date:** 08 August 2022, **First Publish Date:** 08 August 2022
DOI: 10.21608/EJCHEM.2022.144119.6285

vacuum. The resultantprecipitate was gathered and recrystallized to provide **1** and **2**complexes.

2.2 ***Preparation of di-organotin(IV) Complexes (3-4)***

4mmol of the Cinnamic acid and NaOH (4mmol) were dissolved in methanol 30 ml and stirred for 1hr at room temperature. (2mmol of 0.44g or 0.61g) of dimethyl tindichloride(Me_2SnCl_2 or dibutyltin dichloride(Bu_2SnCl_2)was added as solid to the first mixture, and the mixture was left to reflux for 4 hours with continuously stirred [36-38]. The solid precipitated NaCl was filtered off and the solvent was evaporated under a vacuum. The resultant precipitate was gathered and recrystallized to provide **3** and **4**complexes.

2.3 Antioxidant Activity Tests

a) DPPH technique

Antioxidant activity was measured using the DPPH technique, as described by others [1-3]. The compounds were dissolved in methanol at different concentrations of 2; 4; 8; 16, and 32 M, respectively. DPPH (0.1 mM in methanol) was added to each test solution and carefully mixed. After 30 minutes, the solution was discarded. A UV-vis spectrophotometer was used to test the mixture's absorbance at a wavelength of 517 nm. The proportion of inhibition against DPPH was used to calculate antioxidant activity. The percentage inhibition was calculated using equation (1);

$$\textbf{Inhibition Percentage} = \left[\frac{\textbf{Cotrol Absorbance} - \textbf{Sample Absorbance}}{\textbf{Control Absorbance}}\right] \times \mathbf{100} \qquad (\mathbf{1})$$

b) CUPRAC Method

Antioxidant activity test by CUPRAC method was performed according to the method used by others [22].

$$Total\ antioidants\ levals = Conce.of\ STD\ \left(\frac{mmole}{L}\right) \qquad (2)$$

3.Results and Discussion

3.1 Synthesis of Organotin(IV) Complexes 1–4

The tri and di-organotin(IV) complexes 1-4 were obtained by refluxing reaction ofmethanolic solutions of tri and di- organotin chloride with Cinnamic acid as a ligand (L) (Figures1 and 2) under reflux for four hours in yield percentage of 79,82,77 and 83 respectively.

All the synthesized complexes were well illustrated with spectroscopy techniques of FTIR [39], NMR (^{1}H, and Sn^{119}) [40-42] in addition to elemental analysis. The comes about of each investigation are arranged inTables 1-3.

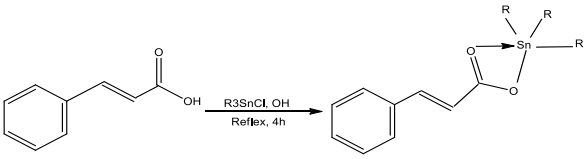

Figure 1. Complex 1 and 2 Preparation

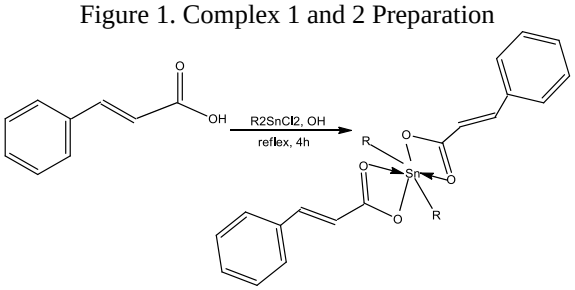

Figure 2. Preparation of complex 3 and 4

Table 1. Physical Analysis Data of Complexes 1–4.

Compound	R	color	Yield %	M.p./ °C	Elemental analysis % Calculated (Found)	
					C	H
L	-	white	-	174-176	72.96(72.15)	5.44(5.12)
1	Ph_3	white	80	195-193	40.66(41.18)	2.65(2.16)
2	Bu_3	off-white	73	184-186	57.69(56.45)	7.84(8.85)
3	Me_2	white	75	186-188	54.22(52.91)	4.55(5.89)
4	Bu_2	off-white	79	197-199	59.23(58.40)	6.12(5.82)

Table 2. FTIR Spectral Data of Complexes 1–4.

Sn (IV)Complex	C-O	C=C	Sn-C	Sn-O
1	1610	1543	460	525
2	1621	1545	468	528
3	1612	1543	472	525
4	1612	1547	469	525

Table 3. NMR Spectral data (^{1}H and ^{119}Sn) of ligand and 1–4 Complexes.

Sn(IV) Complex	^{1}H-NMR	^{119}Sn-NMR
L	12.50(s, 1H, COOH), 6.84-7.78(1H, HC=C), 6.39-6.55 (m, 5H, Ar).	--
1	7.95(m, 5H, Ph), 6.96-7.62(1H, HC=C), 6.82-6.89 (m, 5H, Ar).	-181
2	6.95-7.64(1H, HC=C), 6.92-6.97 (m, 5H, Ar), 6.34-6.48(s, 1H, CH-CO_2H), 0.14-2.69(Bu).	-165
3	6.99-7.63(1H, HC=C), 6.96-6.95 (m, 5H, Ar), 3.18 (d, CH_2), 1.78 (s, Me).	-2٢2
4	7.21-7.66(1H, HC=C), 6.96-6.98(m, 5H, Ar), 0.80-2.09(Bu).	-270

Singlet signals may be seen in the ^{119}Sn-NMR spectra of samples 1-4, but they are substantially weaker than those for the corresponding organotin(IV) salts (Table 3). The chemical shift, however, depends on the complex's geometry [40,41], and these shifts are in line with the theory that there is an increase in the amount of tin atoms coordinated within the complexes (i.e., tin nuclear shielding) [42].

3.2Antioxidant Activity

The four manufactured complexes were examined in various quantities in the antioxidant activity analysis using the two procedures outlined. After obtaining the absorbance in each measurement, the percent inhibition can be computed; the results are also, displayed in Figures3 and 4.

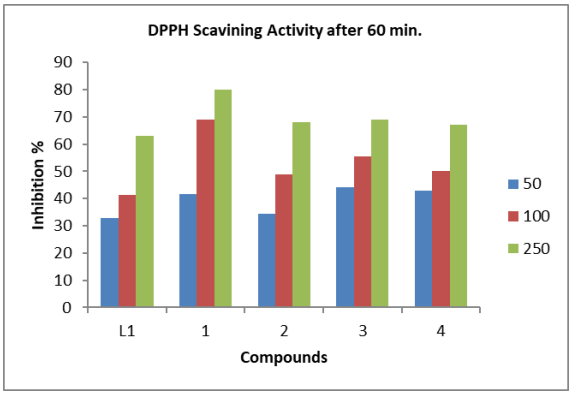

Figure 3. DPPH scavenging activity of Cinnamic acid and its complexes at 250 µg/mL DMSO solutions at T = 60 min

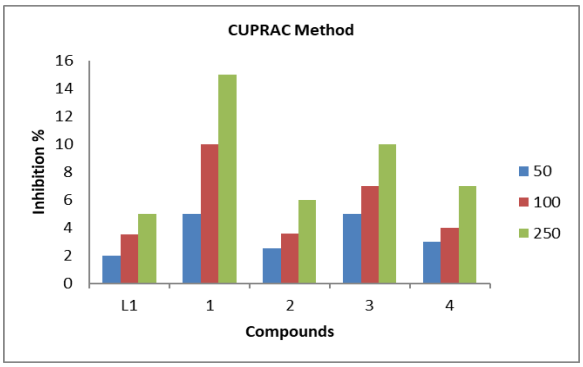

Figure 4. CUPRAC Method activity of Cinnamic acid and its complexes at T = 60 min

The results showed a high antioxidant activity of the complexes prepared from Cinnamic acid and organic tin salts compared to ligand alone, and this is due to the presence of the tin element, which caused an increase in the antioxidant activity [43-46]. Also, complex **1** (triphenyl tincarboxylate) showed higher activity than the rest of the prepared complexes, and this may be due to the presence of three phenolic groups and an increase in the aromatic content of the complex compared to the rest of the complexes.

4. Conclusion

The reaction of Cinnamic acid as a ligand with tri and di-organotin salts yielded four organotin (IV) complexes. The antioxidant activity of the Cinnamic acid and its organotin(IV)complexes was determined using the DPPH and CUPRAC techniques. The antioxidant activity of the organotin(IV) complexes was found to be higher than that of the ligand using two approaches.

5. Acknowledgements

The authors would like to thank Babylon University and Al-Mustaqbal University College for the kind support.

6. Conflict Of Interest

The authors have no conflicts of interest regarding this investigation.

7. Author Contributions

A. G. H, and I.J.M conducted the experiment, N.S.S and S.J. B conducted the DFT calculations, and A.G.H and Y.F wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

8. REFERENCES

[1] Choi, H.J., Kim, J.H., Lee, C.H.,Ahn, Y.J.,Song, J.H., Baek, S.H., Kwon, D.H. 2009, Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. *Antiviral Res.* 81, 77–81. [CrossRef] [PubMed]. https://doi.org/10.1016/j.antiviral.2008.10.002

[2] Song, J.H., Shim, J.K., Choi, H.J., 2011, Quercetin 7-rhamnoside reduces porcine epidemic diarrhea virus replication via the

independent pathway of viral-induced reactive oxygen species. *Virol. J.* 8, 460. [CrossRef] [PubMed]. http://www.virologyj.com/content/8/1/460

[3] Liang, W., He, L., Ning, P., Lin, J., Li, H., Lin, Z., Kang, K., Zhang, Y. 2015, (+)-Catechin inhibition of transmissible gastroenteritis coronavirus in swine testicular cells is involved its antioxidation. *Res. Vet. Sci.* 103, 28–33. [CrossRef]. https://doi.org/10.1016/j.rvsc.2015.09.009

[4] Diniz, L.R.L., Bezerra Filho, C.D.S.M., Fielding, B.C., de Sousa, D.P., 2020, Natural Antioxidants: A Review of Studies on Human and Animal Coronavirus. *Oxid. Med. Cell. Longev.* 1–14. [CrossRef] [PubMed]. https://doi.org/10.1155/2020/3173281

[5] Bansal, S., Choudhary, S., Sharma, M., Kumar, S.S., Lohan, S., Bhardwaj, V., Syan, N., Jyoti, S. 2013, Tea: A native source of antimicrobial agents. *Food Res. Int.* 53, 568–584. [CrossRef]. https://doi.org/10.1016/j.foodres.2013.01.032

[6] Jiang, J., Xiong, Y.L., 2016, Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: *A review. Meat Sci.*, 120, 107–117. [CrossRef] [PubMed]. https://doi.org/10.1016/j.meatsci.2016.04.005

[7] Lü, J.-M., Lin, P.H., Yao, Q., Chen, C., 2009, Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems., *J. Cell. Mol. Med.* 14, 840–860. [CrossRef].

[8] Simec, M. G., Porter, K. M., 1980, Autoxidation in Foods and Biological Systems, *Plenum Press*, New York, https://doi.org/10.1007/978-1-4757-9351-2

[9] Čiž M., Čížová H., Denev P., Kratchanova M., Slavov A., Lojek A., 2010, Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control*, 21:518–523. https://doi.org/10.1016/j.foodcont.2009.07.017

[10] Prior R. L., Wu X., Schaich K., 2005, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem*, 53(10):4290–4302. https://doi.org/10.1021/jf0502698

[11] Brand-Williams W., Cuvelier M. E., Berset C., 1995, Use of free radical method to evaluate antioxidant activity. *LebensmWiss Technol*, 28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

[12] Arnao, M. B., Cano A., Acosta M., 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem*, 73:239–244. https://doi.org/10.1016/S0308-8146(00)00324-1

[13] Benzie I. F. F., Strain J. J., 1996, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*, 239:70–76. https://doi.org/10.1006/abio.1996.0292

[14] Apak, R., Güçlü, K., Ozyürek, M., Karademir, S.E., Altun, M., 2005, Total antioxidant capacity assay of human serum using copper(II)-neocuproine as chromogenic oxidant: The CUPRAC method. *Free Radic. Res.* 39, 949–961. [CrossRef] [PubMed]. https://doi.org/10.1080/10715760500210145

[15] Negi, P. S., Chauhan, A. S., Sadia, G. A., Rohinishree, Y.S., Ramteke, R.S., 2005, Antioxidant and antibacterial activities of various Seabuckthorn (Hippophaerhamnoides L.) seed extracts. *Food Chem*, 92:119–124. https://doi.org/10.1016/j.foodchem.2004.07.009

[16] Affan, M. A., Foo, S.W., Jusoh, I., Hanapi, S. and Tiekink, E. R. T., 2009, *Inorg. Chim. Acta*, 362 5031. https://doi.org/10.1016/j.ica.2009.08.010

[17] Alama, A., Tasso, B., Novelli, F. and Sparatore, F., 2009, *Drug Discov. Today*, 14 500. (2009), https://doi.org/10.1016/j.drudis.2009.02.002

[18] Kang, W., Wu, X. and Huang, J., 2009, *J. Organometal. Chem.*, 694 2402. https://doi.org/10.1016/j.jorganchem.2009.03.025

[19] Suhartati, T., Herasari, D., Pandiangan, K. D., &Hadi, S., 2021, Synthesis, characterization, and antioxidant activity of some organotin (IV) 2-nitrobenzoate using the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method. In: *Journal of Physics: Conference Series*. IOP Publishing, p. 012098.https://doi:10.1088/1742-6596/1751/1/012098

[20] HADI, Angham, G., Jawad, K., Ahmed, D. S., & Yousif., 2019, Synthesis and biological activities of organotin (IV) carboxylates: a review. *Systematic Reviews in Pharmacy*, 10.1: 26-31.http://doi:10.5530/srp.2019.1.5

[21] HADI, Angham, G.,JAWAD, Khudheir,YOUSIF, Emad, 2019, Medicinal Applications of Organotin (IV) Complexes and Its Important Place as Effective Antitumor Agents. *Indian Journal of Public Health Research & Development*, 10.6.https://doi.org/10.5958/0976-5506.2019.01449.9

[22] Pellerito, L. and Nagy, L., 2002, *Coor. Chem. Rev.*, 224 111.

https://doi.org/10.1016/S0010-8545(01)00399-X

[23] Gleeson, B., Claffey, J., Ertler, D., Hogan, M., Müller-Bunz, H., Paradisi, F., Wallis, D. and Tacke, M. (2008),*Polyhedron*, 27 3619. https://doi.org/10.1016/j.poly.2008.09.009

[24] HADI, Sutopo, Fenska, M. D., Noviany, N., Satria, H., Simanjuntak, W., & Naseer, M. M., 2021, Synthesis and antimalarial activity of some triphenyltin (IV) aminobenzoate compounds against Plasmodium falciparum. *Main Group Metal Chemistry*, 44.1: 256-260.https://doi.org/10.1515/mgmc-2021-0028

[25] SARI, Widia, QUDUS, HardokoInsan, HADI, Sutopo, 2020, The Chemical Reactivity Study of Organotin (IV) 4-aminobenzoates Using Cyclic Voltammetry and Antioxidant Activity Test by the DPPH Method. *Revista de Chimie*, 71.10: 28-37.

[26] HADI, Sutopo, NOVIANY, Noviany, RILYANTI, Mita, 2018, In vitro antimalarial activity of some organotin (IV) 2-nitrobenzoate compounds against Plasmodium falciparum. *Macedonian Journal of Chemistry and Chemical Engineering*, 37.2.

[27] SZORCSIK, 1, A., Nagy, L., Gajda-Schrantz, K., Pellerito, L., Nagy, E., &Edelmann, F., 2002, Structural studies on organotin (IV) complexes formed with ligands containing {S, N, O} donor atoms. *Journal of radioanalytical and nuclear chemistry*, 252.3: 523-530. https://doi.org/10.1023/a:1015802820423

[28] HADI, Sutopo, Simanjuntak, W., Qudus, H. I., Yandri, Y., &Herasari, D., 2021, In Vitro Antimicrobial Activity Study of Some Organotin (IV) Chlorobenzoates against Staphylococcus aureus and Escherichia coli. *Journal of Advanced Pharmacy Education & Research [JAPER]*, 11.2: 17-22.

[29] HADI, Sutopo, Fenska, M. D., Wijaya, R. A., Noviany, N., &Suhartati, T., 2020, Antimalarial Activity of Some Organotin (IV) Chlorobenzoate Compounds against Plasmodium falciparum. *Mediterranean Journal of Chemistry*, 10.3: 213-219.https://doi.org/10.13171/mjc0200312162sh

[30] SUTOPO, Hadi, Noviany, N., Hardoko, I. Q., &Pakorn, W. A.,2019, The Potency Study of Organotin (IV) 3-Nitrobenzoate Compounds as Antimalarial Agents. In: *Journal of Physics: Conf. Series*. IOP Publishing, p. 1-7.http://doi:10.1088/1742-6596/1338/1/012012

[31] HANSCH, Corwin, VERMA, Rajeshwar, P., 2009, Larvicidal activities of some organotin compounds on mosquito larvae: A QSAR study. *European journal of medicinal chemistry*, 44.1: 260-273. https://doi.org/10.1016/j.ejmech.2008.02.040

[32] Hadi, A. G., Hassen, T. F., & Mahdi, I. J., 2021, Synthesis, characterization, and antioxidant material activities of organotin (IV) carboxylates with tin-para methoxy benzoic acid. *Materials Today: Proceedings*, https://doi.org/10.1016/j.matpr.2021.09.469

[33] HADI, Angham, G., Jawad, K., Yousif, E., El-Hiti, G. A., Alotaibi, M. H., & Ahmed, D. S., 2019, Synthesis of telmisartan organotin (IV) complexes and their use as carbon dioxide capture media. *Molecules*, 24.8: 1631. http://doi:10.3390/molecules24081631

[34] HADI, Angham, G., Yousif, E., El-Hiti, G. A., Ahmed, D. S., Jawad, K., Alotaibi, M. H., & Hashim, H., 2019, Long-term effect of ultraviolet irradiation on poly (vinyl chloride) films containing naproxen diorganotin (IV) complexes. *Molecules*, 24.13: 2396.http://doi:10.3390/molecules24132396

[35] HADI, Angham, G., Jawad, K., El-Hiti, G. A., Alotaibi, M. H., Ahmed, A. A., Ahmed, D. S., & Yousif, E., 2019, Photostabilization of poly (vinyl chloride) by organotin (IV) compounds against photodegradation. *Molecules*, 24.19: 3557.http://doi:10.3390/molecules24193557

[36] HADI, Angham, G., Baqir, S. J., Ahmed, D. S., El-Hiti, G. A., Hashim, H., Ahmed, A., ... & Yousif, 2021, Substituted Organotin Complexes of 4-Methoxybenzoic Acid for Reduction of Poly (vinyl Chloride) Photodegradation. *Polymers*, 13.22: 3946.https://doi.org/10.3390/polym13223946

[37] AHMED, Ahmed, El-Hiti, G. A., Hadi, A. G., Ahmed, D. S., Baashen, M. A., Hashim, H., & Yousif, E., 2021, Photostabilization of poly (vinyl chloride) films blended with organotin complexes of mefenamic acid for outdoor applications. *Applied Sciences*, 11.6: 2853.https://doi.org/10.3390/app11062853

[38] JASEM, Hadeer, Hadi, A. G., El-Hiti, G. A., Baashen, M. A., Hashim, H., Ahmed, A. A., ... & Yousif, E., 2021, Tin-Naphthalene Sulfonic Acid Complexes as Photostabilizers for Poly (vinyl chloride). *Molecules*, 26.12: 3629. DOI: 10.3390/MOLECULES26123629

[39] Akram, M.A.; Nazir, T.; Taha, N.; Adil, A.; Sarfraz, M.; Nazir, S.R., 2015, Designing, development and formulation of mouth disintegrating telmisartan tablet with extended release profile using response surface methodology. *J. Bioequiv*., 7, 262–266. http://dx.doi.org/10.4172/jbb.1000250 [CrossRef]

[40] Pejchal, V.; Holeˇcek, J.; Nádvorník, M.; Lyˇcka, 1995, A. 13C and 119Sn-NMR Spectra of some mono-n-butyltin(IV) compounds. *Collect. Czech. Chem. Commun.*, 60, 1492–1501. https://doi.org/10.1135/cccc19951492 [CrossRef]

[41] Shahid, K.; Ali, S.; Shahzadi, S.; Badshah, A.; Khan, K.M.; Maharvi, G.M., 2003, Organotin(IV) complexes of aniline derivatives. I. Synthesis, spectral and antibacterial studies of di- and triorganotin(IV) derivatives of 4-bromomaleanilic acid. Synth. React. *Inorg. Met. Org. Chem.*, 33, 1221–1235. https://doi.org/10.1081/SIM-120023490 [CrossRef]

[42] Rehman, W.; Baloch, M.K.; Badshah, A.; Ali, S., 2005, Synthesis and characterization of biologically potent di-organotin(IV) complexes of mono-methyl glutarate. *J. Chin. Chem. Soc*., 52, 231–236. https://doi.org/10.1002/jccs.200500034 [CrossRef]

[43] Hashim, D. J., Falah, T. F., &Hadi, A. G., 2021, Enhanced Antioxidant Activity of Di- and Triorganotin Complexes Derived from Mefenamic Acid. *Egyptian Journal of Chemistry*.https://doi.org/10.21608/EJCHEM.2021.82292.4056

[44] Saddam, N. S., Hadi, A. G., & Mohammed, A. A., 2021, Enhanced antioxidant activity of chitosan extracted from fish shells materials. *Materials Today: Proceedings*. https://doi.org/10.1016/j.matpr.2021.09.460

[45] Hadi, A. G., Zaooli, R. H., Ahmed, D. S., & Yousif, E., 2021, Anti-oxidant Activity of Naproxen and Its Diorganotin Complexes. *International Journal of Drug Delivery*, *11*(2), 383-385.

[46] HadeerJasem, A. G. H., 2021, Enhanced the Antioxidant Activity of 1-Amino-2-naphthol-4-Sulfonic acid by Complexation with Organotin (IV) Compounds. *Annals of the Romanian Society for Cell Biology*, *25*(6), 6182-6193.