Synthesis, FT-IR Spectroscopic Studies and *in vitro* FRAP Assay of some Chalcone Derivatives and their Metal Complexes

[1]Adebayo Tajudeen Bale, [2]Kehinde Dele Daramola, [3]Wahab Adesina Osunniran

[1,2,3] Department of Chemistry and Industrial Chemistry, Faculty of Pure and Applied Sciences, Kwara State University, Malete. P.M.B. 1530, Ilorin, Nigeria.

Correspondence: adebayo.bale@kwasu.edu.ng

Abstract

A variety of substituted chalcones, namely, 3"-aminochalcone, 2",4"-dichlorochalcone, 2"-methoxychalcone, 4"-methoxychalcone and 2"-hydroxychalcone have been synthesized by Claisen-Schmidt condensation and characterised by melting point and FT-IR spectroscopic technique. The tin(II), cadmium(II) and copper(II)) complexes of the 2"-hydroxychalcone (4E2HC) with the formula [M(L)$_2$] were subsequently prepared. The 2"-hydroxychalcone and its metal complexes were evaluated for in vitro radical scavenging activity by carrying out ferric reducing antioxidant power (FRAP) assay. In almost all cases, the metal complexes exhibited higher activities than the ligand (chalcone). Notable activity (11.63 µM) was observed for the cadmium complex of the 4-ethoxy-2"-hydroxychalcone at 10 µg/mL. Limited structure–activity relationship (SAR) was established by considering the effect of different groups attached to the aryl rings and the metal centers on varying antioxidant activity. The enhanced activity may be due to the presence of electron-rich metals and electron-releasing ethoxy (-OC$_2$H$_5$) and hydroxy (–OH) groups. The confirmed bioactive compounds from this study can be used as effective template in medicinal chemistry for drug discovery.

Keywords: Chalcones, Metal complexes, Spectroscopic, Antioxidant, Structure-activity relationship

1. Introduction

Chalcones are considered as precursors of an important group of natural products known as the polyphenolic compounds (Al-Mamary *et al*., 2012). They are α,β-unsaturated ketones consisting of two aromatic rings having diverse array of substituents (Gomes *et al*., 2017). The presence of reactive α,β-unsaturated carbonyl function in chalcones is found to be responsible for their biological activity (Bucha *et al.*, 2014). They possess conjugated double bonds and a completely delocalized π-electron system on both the aromatic rings. These group of compounds have been used as precursors for the synthesis and biosynthesis of flavonoids and many other organic compounds such as pyrazolines, isoxazoles, pyrimidines and an assortment of heterocyclic compounds (Gomes *et al*., 2017).

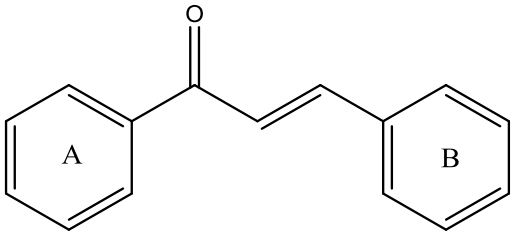
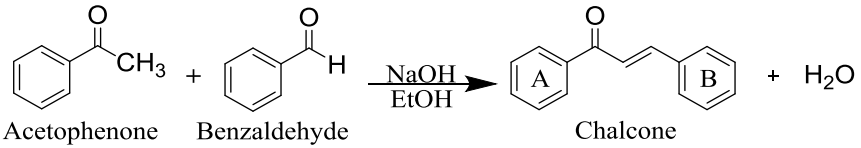


Figure 1: Basic structure of chalcone (A=acetophenone; B=benzaldehyde)

The presence of reactive α,β-unsaturated carbonyl function in chalcones is found to be responsible for their antibacterial and antifungal activities (Bucha *et al.*, 2014). Their flexible structure allows them to possess a large number of biological activities (Lui *et al.*, 2013). Chalcone has been reported to exhibit a broad spectrum of antibacterial (Rachmale, 2012), antifungal (Lahtchev *et al.*, 2008), antitumor (Sharma *et al.*, 2016), anticancer (Ngameni *et al.*, 2021), anti-inflammatory (Zhang *et al*., 2010) and antioxidant activity (Narsinghani *et al*., 2013). Some chalcone-based

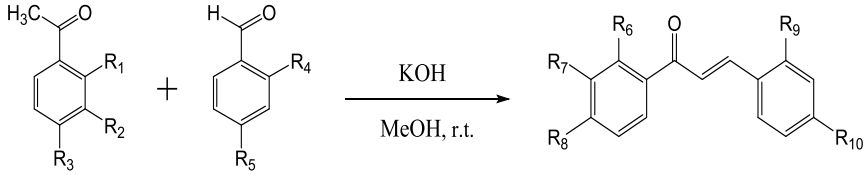
compounds have been approved for clinical use. For example, metochalcone was approved and marketed as a choleretic drug, while sofalcone was used as an antiulcer and mucoprotective drug (Batovska et al., 2010; Sahu et al., 2012). Chalcones can be obtained from a range of reactions including Suzuki coupling reaction, carbonylative Heck reaction, ultrasound irradiation, microwave irradiation, solvent-free reaction, Friedel-crafts acylation and Wittig reaction (Bukhari, Jasamai, Jantan & Ahmad, 2013). However, Claisen-Schmidt condensation (Scheme 1) is the commonly used synthetic approach in which the reaction is carried out in an alkaline alcoholic medium at room temperature (Kaur & Narasimhan, 2018). The aim of this study is to synthesize and evaluate the ferric reducing antioxidant power (FRAP) potentials of chalcones and their metal complexes.



Scheme 1: Chalcone synthesis *via* Claisen-Schmidt condensation

2. Materials and Methods

Chalcone derivatives were synthesized by Claisen-Schmidt condensation reaction. The Solvent used (methanol) was 99.8 % analytical grade and reagents used (substituted acetophenones and substituted benzaldehydes) were purchased from Aldrich chemical company limited. The reaction progress and purity of products were monitored by TLC which was performed with silica-gel coated plate (suspended in n-hexane-ethyl acetate). The plates were viewed under UV lamp. Melting points were determined using Stuart SMP10 melting point apparatus (0 to 300 ^{o}C). FT-IR spectroscopy (400 to 4000 cm^{-1}) was carried out on all the chalcone derivatives and their metal complexes.



4H3AC; R_1= H, R_2= NH_2, R_3= H, R_4= H, R_5= OH, R_6= H, R_7= NH_2 R_8= H, R_9= H, R_{10}= OH
4E3AC; R_1= H, R_2= NH_2, R_3= H, R_4= H, R_5= OC_2H_5, R_6= H, R_7= NH_2 R_8= H, R_9= H, R_{10}= OC_2H_5
4M3AC: R_1= H, R_2= NH_2, R_3= H, R_4= H, R_5= OCH_3, R_6= H, R_7= NH_2 R_8= H, R_9= H, R_{10}= OCH_3
2H24DC; R_1= Cl, R_2= H, R_3= Cl, R_4= OH, R_5= H, R_6= Cl, R_7= H, R_8= Cl, R_9= OH, R_{10}= H
4H24DC; R_1= Cl, R_2= H, R_3= Cl, R_4= H, R_5= OH, R_6= Cl, R_7= H, R_8= Cl, R_9= H, R_{10}= OH
4E2MC; R_1= OCH_3, R_2= H, R_3= H, R_4= H, R_5= OC_2H_5, R_6= OCH_3, R_7= H R_8= H, R_9= H, R_{10}= OC_2H_5
4E4MC; R_1= H, R_2= H, R_3= OCH_3, R_4= H, R_5= OC_2H_5, R_6= H, R_7= H R_8= OCH_3, R_9= H, R_{10}= OC_2H_5
4E2HC; R_1= OH, R_2= H, R_3= H, R_4= H, R_5= OC_2H_5, R_6= OH, R_7= H R_8= H, R_9= H, R_{10}= OC_2H_5

Scheme 2: Synthesis of chalcone derivatives.

2.1. Synthesis of chalcone by mechanochemical method (grinding)

2.1.1. Synthesis of 4-hydroxy-3-aminochalcone (4H3AC)

0.14 g (1 mmol) of 3-aminoacetophenone was grinded with 0.1 g of potassium hydroxide (KOH) for 20 min using mortar and pestle. 0.12 g (1 mmol) of 4-hydroxybenzaldehyde was added and grinded for 3 h.

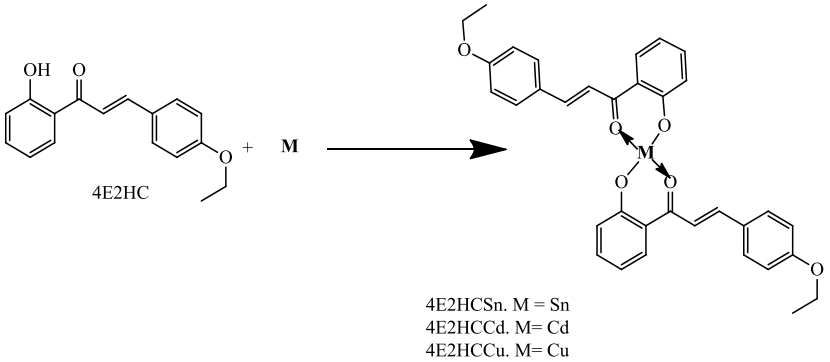
2.2. Synthesis of chalcones by Claisen-Schmidt condensation

2.2.1 Synthesis of 4E3AC, 4M3AC, 2H24DC, 4H24DC, 4E4MC, 4E2MC and 4E2HC

In a round bottom flask, 1 mmol of substituted acetophenone was dissolved in 20 mL of methanol. While stirring, 3 mL of 60 % potassium hydroxide (KOH) was added drop wise and stirred for 30 min. 1 mmol of substituted benzaldehyde was added and stirred for 10 to 15 h. The solid formed was filtered, washed with methanol and then air-dried.

2.3. Synthesis of the metal complexes of 4E2HC (4E2HCSn, 4E2HCCd and 4E2HCCu)

The chalcone (4E2HC) and the metal salts {(Cd(II), Cu(II) and Sn(II)} were grinded together in 2:1 ratio.



Scheme 3: Synthesis of metal complexes

2.4. Ferric Reducing Antioxidant Power (FRAP) Assay of the 4E2HC and its Metal Complexes

FRAP solutions were prepared as described previously (Benzie and Szeto, 1999). The FRAP working solution was prepared by mixing 10-volumes of acetate buffer (300 mM, pH 3.6) with 1-volume of TPTZ (40 mM dissolved with 40 mM HCl) and 1-volume of ferric chloride (20 mM in water). Microplate FRAP assay was performed according to previous reports with minor modifications (Tsao *et al*., 2003). Sample solutions (20 µL) were added directly to the 96-well microplate followed by 280 µL of working FRAP solution. The mixtures were shaken, incubated at 37 ^{o}C in the dark for 30 min and then A593 readings were recorded using a microplate reader.

3. Results and Discussion

Substituted chalcones namely 3”-aminochalcone, 2”,4”-dichlorochalcone, 2”-methoxychalcone, 4”-methoxychalcone and 2”-hydroxychalcone have been synthesized by Claisen-Schmidt

condensation and characterized by melting point and FT-IR spectroscopic technique. The tin(II), cadmium(II) and copper(II)) complexes of the 2”-hydroxychalcone (4E2HC) with the formula $[M(L)_2]$ were prepared. 4E3AC, 4M3AC, 2H24DC and 4E2HC were obtained in high yield ranging in between 80 and 96 %. 4E2HC had the maximum yield, reaching 96 %. 4H3AC, 4E2MC, 4E4MC, 4E2HCCd and 4E2HCCu had yield ranging in between 50 and 75 %, 4H24DC and 4E2HCSn had yield less than 50 %. Metal complexes exhibited higher melting points compared to the ligand (4E2HC). 4E2HCSn had the highest melting point (246 to 248 ^{o}C) followed by 4E2HCCd (190-191 ^{o}C) (Table 2). Syam *et al.*, (2012) synthesized unsubstituted chalcone and reported its melting point to be 56-57 ^{o}C. Increase in the melting points of the substituted chalcones and metal complexes may be due to the presence of aryl substituents and the metal center.

Table 1: Physical parameters of the chalcones and the metal complexes

S/N	Compound code	Chemical formula	Colour observed	% Yield	Melting point (^{o}C)
1	4H3AC	$C_{15}H_{13}NO_2$	Dark-yellow	75	100-102
2	4E3AC	$C_{17}H_{17}NO_2$	Yellow	85	99-100
3	4M3AC	$C_{15}H_{15}NO_2$	Yellow	80	126-128
4	2H24DC	$C_{15}H_{10}Cl_2O_2$	Orange	84	175-177
5	4H24DC	$C_{15}H_{10}Cl_2O_2$	Brown	46	179-181
6	4E2MC	$C_{18}H_{18}O_3$	Creamy white	54	99-101
7	4E4MC	$C_{18}H_{18}O_3$	Creamy white	71	108-110
8	4E2HC	$C_{17}H_{16}O_3$	Yellow	96	200-202
9	4E2HCSn	$C_{34}H_{30}O_6Sn$	Brown	29	246-248
10	4E2HCCd	$C_{34}H_{30}O_6Cd$	Greenish-yellow	60	190 – 191
11	4E2HCCu	$C_{34}H_{30}O_6Cu$	Lemon-green	62	120 – 122

Table 2: Diagnostic infrared absorption bands of the chalcones and the metal complexes

S/N	Compound code	IR bands (cm^{-1})
1	4H3AC	3469(OH), 3370(N-H), 1669(C=O), 1597(C=C)
2	4E3AC	1656(C=O), 1603(C=C)
3	4M3AC	1654(C=O), 1606(C=C)
4	2H24DC	3384(OH), 1616(C=O), 1583(C=C)
5	4H24DC	3336(OH), 1601(C=O), 1581(C=C)
6	4E2MC	1654(C=O), 1599(C=C)
7	4E4MC	1655(C=O), 1598(C=C)
8	4E2HC	3451(OH), 1665(C=O), 1600(C=C)
9	4E2HCSn	3456(OH), 1636(C=O), 1582(C=C), 580(Sn-O)
10	4E2HCCd	3319(OH), 1635(C=O), 1561(C=C), 580(Cd-O)
11	4E2HCCu	3449(OH), 1601(C=O), 1529(C=C), 580(Cu-O)

Absorption bands of C=O and C=C in metal complexes (4E2HCSn, 4E2HCCd and 4E2HCCu) shifted to lower wavelength compared to the ligand (4E2HC) due to the deprotonation of the hydroxy (-OH) group and subsequent coordination to the metal center (Table 2).

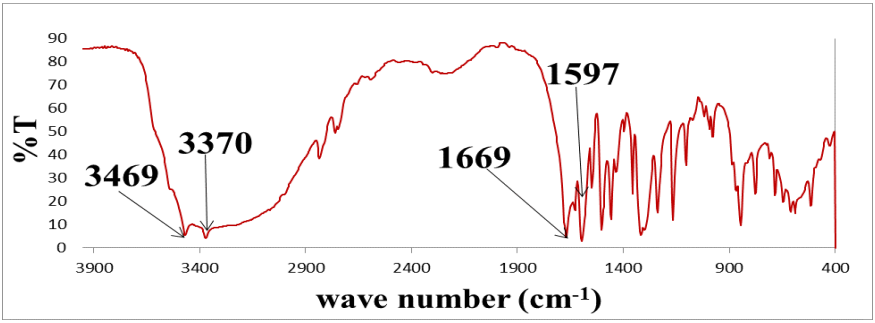


Figure 2: FT-IR spectrum of 4-hydroxy-3”-aminochalcone (4H3AC)

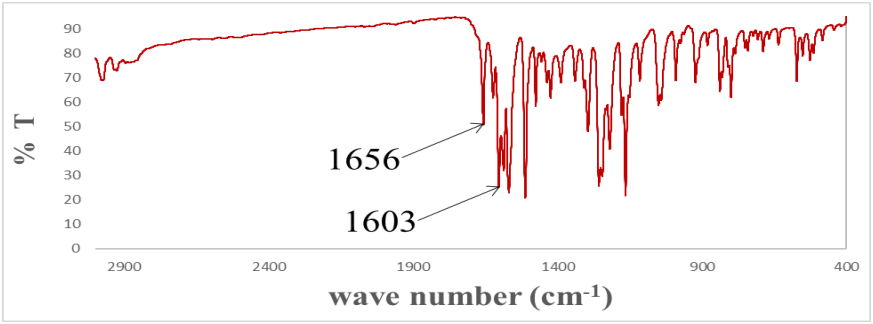


Figure 3: FT-IR spectrum of 4-ethoxy-3”-aminochalcone (4E3AC)

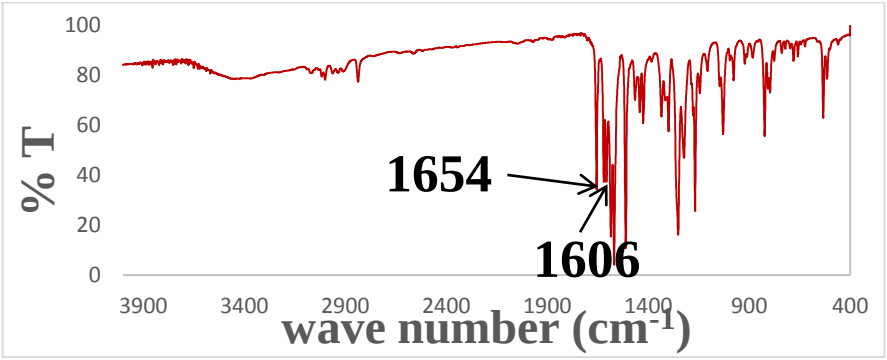


Figure 4: FT-IR spectrum of 4-methoxy-3”-aminochalcone (4M3AC)

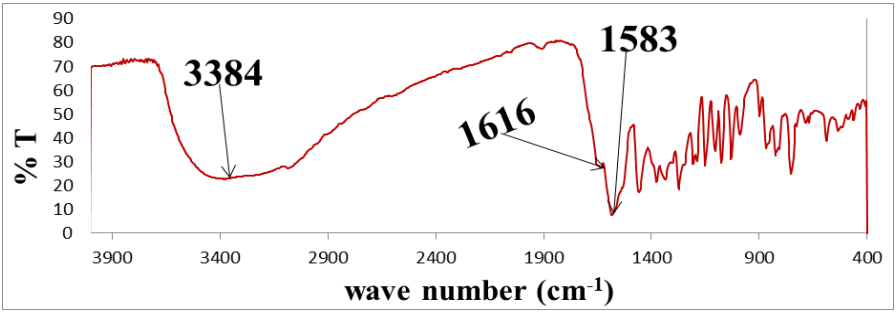


Figure 5: FT-IR spectrum of 2-hydroxy-2”,4”-dichlorochacone (2H24DC)

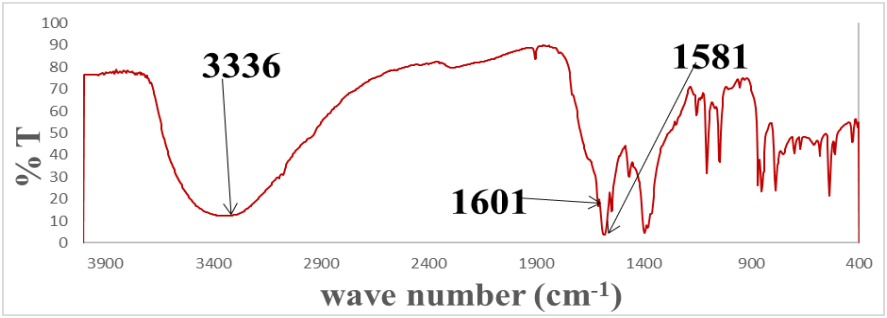


Figure 6: FT-IR spectrum of 4-hydroxy-2",4"-dichlorochacone (4H24DC)

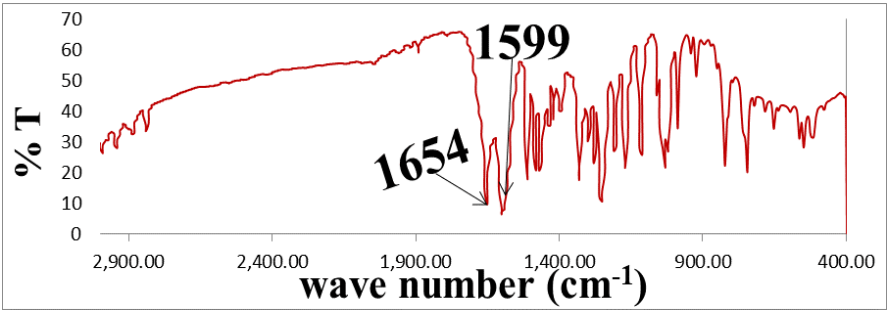


Figure 7: FT-IR spectrum of 4-ethoxy-2"-methoxychalcone (4E2MC)

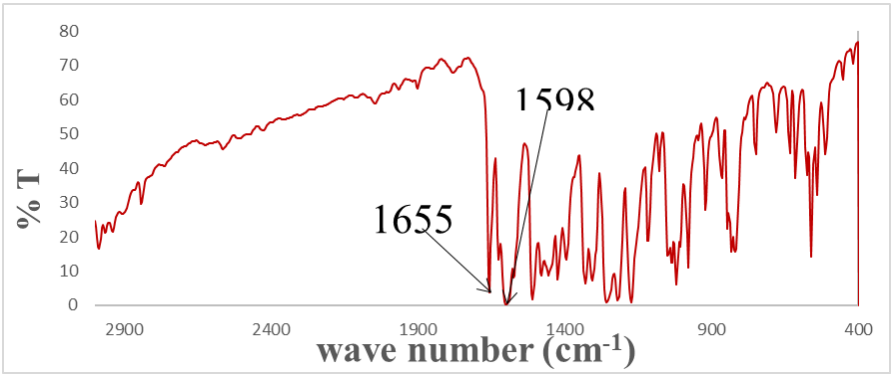


Figure 8: FT-IR spectrum of 4-ethoxy-4"-methoxychalcone (4E4MC)

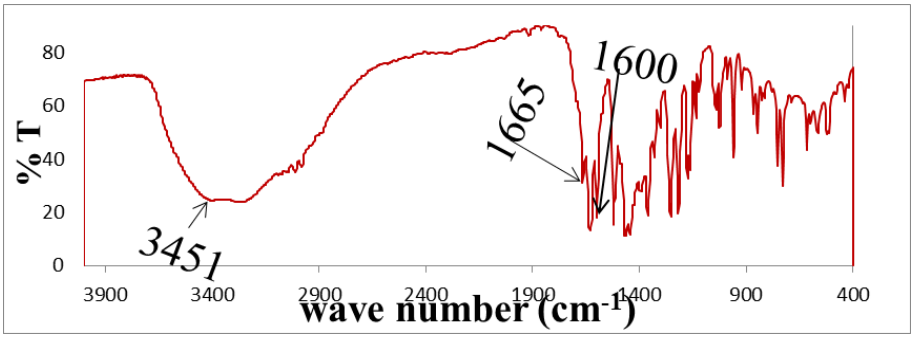


Figure 9: FT-IR spectrum of 4-ethoxy-2”-hydroxychalcone (4E2HC)

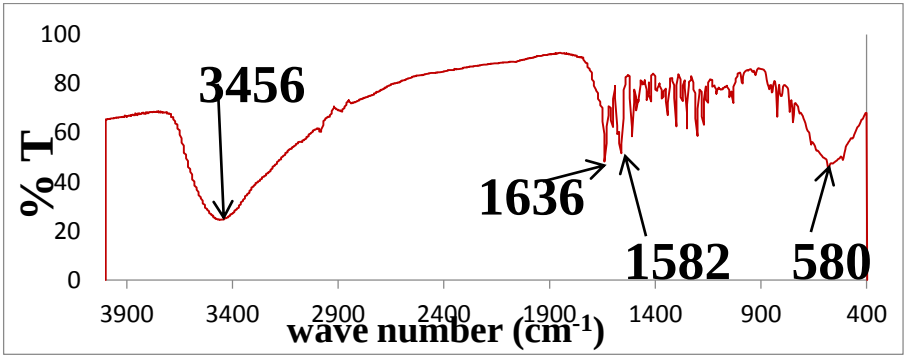


Figure 10: FT-IR spectrum of tin(II) complex of 4-ethoxy-2”-hydroxychalcone (4E2HCSn)

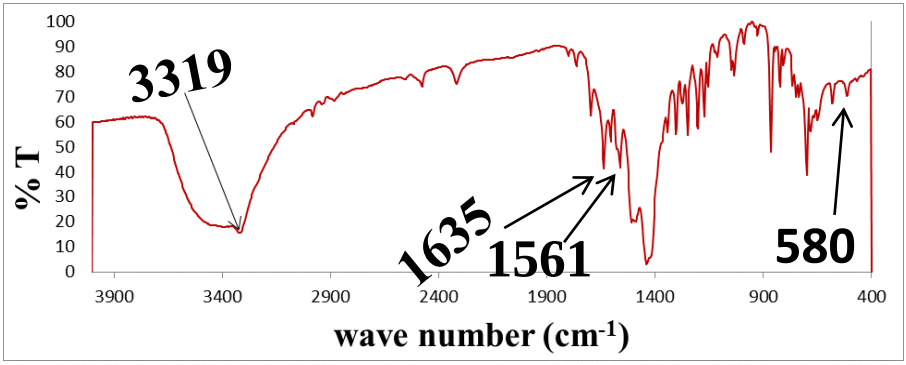


Figure 11: FT-IR spectrum of cadmium(II) complex of 4-ethoxy-2”-hydroxychalcone (4E2HCCd)

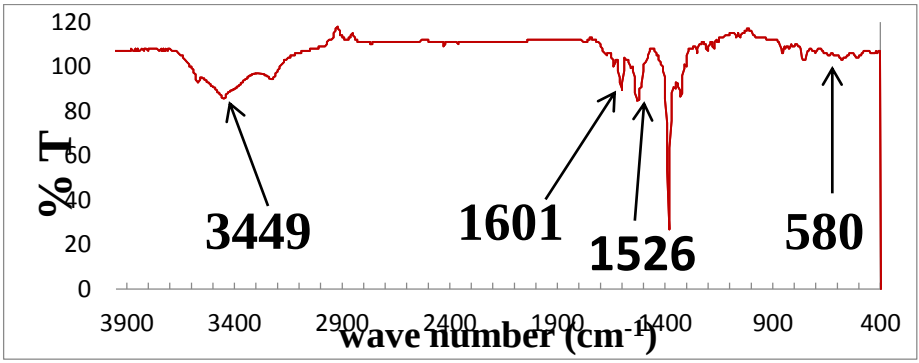


Figure 12: FT-IR spectrum of copper(II) complex of 4-ethoxy-2"-hydroxychalcone (4E2HCCu)

3.1 *In vitro* antioxidant (FRAP) activity studies of the ligand (4E2HC) and its metal complexes

The result in table 3 shows the *in vitro* radical scavenging activities of the ligand (4E2HC) and its metal complexes (4E2HCSn, 4E2HCCd and 4E2HCCu). Ferric reducing antioxidant power (FRAP) assay in the principle of reduction of ferric-tripyridyltriazine (Fe^{3+} TPTZ) to ferrous tripyridyltriazine (Fe^{2+} TPTZ) was carried out. In almost all cases, the metal complexes exhibited higher activities than the ligand (chalcone). Notable activity (11.63 µM) was observed for the cadmium complex of the 4-ethoxy-2"-hydroxychalcone at 10 µg/mL which compares favourably with the ascorbic acid. The enhanced activity may be due to the presence of electron-rich metal and electron-releasing ethoxy (-OC_2H_5) and hydroxy (–OH) groups. This observation was similar to the results obtained by Al-Mamary *et al*., (2014).

Table 3: Results of the *in vitro* radical scavenging (FRAP) activities of the ligand (4E2HC) and its metal complexes

Conc. (µg/mL)	Antioxidant activity (µM)			
	4E2HC	4E2HCSn	4E2HCCd	4E2HCCu
	Ascorbic acid			
10	10.58824	11.41176	11.62745	11.29412
	16.31373			
20	10.7451	12.15686	12.03922	11.64706
	17.17647			
50	11.03922	12.37255	12.27451	12.86275
	63.54902			
100	11.09804	12.80392	12.31373	12.03922
	185.7451			
150	11.7451	13.05882	13.35294	12.68627
	195.8627			

Conclusion

Substituted chalcone derivatives were synthesized by Claisen-Schmidt condensation reaction. The Cu(II), Cd(II) and Sn(II) complexes of the chalcone (4E2HC) were subsequently prepared. The chalcone derivatives and their metal complexes were identified from their physical and spectral data. The ligand (4E2HC) and its metal complexes were screened for antioxidant activity using FRAP method. In almost all cases, the metal complexes exhibited higher activities than the ligand (chalcone). The cadmium complex of 4E2HC showed notable activity (11.63 µM) at 10 µg/mL comparable to the ascorbic acid. These compounds are viable templates in drug design and discovery.

References

Al-Mamary, M., Al-Mikhlafi, S. A. and Jaadan, B. (2014). Synthesis and biological activities of some chalcone

derivatives. *International Journal of Chemistry and Pharmaceutical Sciences*, ***5***(2), 61-68.

Benzie, I. F. and Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, ***47***(2), 633-636.

Bukhari, S.N.A., Jasamai, M., Jantan, I. and Ahmad, W. (2013). Review of Methods and Various Catalysts Used for Chalcone Synthesis. *Mini-Reviews in Organic Chemistry*. ***10***, 73-83.

Gomes, M. N., Muratov, E. N., Pereira, M., Peixoto, J. C., Rosseto, L. P., Cravo, P. V. and Neves, B. J. (2017). Chalcone derivatives: promising starting points for drug design. *Molecules*, ***22***(8), 1210.

Henderson, T., Nigam, P. S. and Owusu-Apenten, R. K. (2015). A universally calibrated microplate ferric reducing antioxidant power (FRAP) assay for foods and applications to Manuka honey. *Food Chemistry*, ***174***, 119-123.

Jimenez-Alvarez, D., Giuffrida, F., Vanrobaeys, F., Golay, P. A., Cotting, C., Lardeau, A. and Keely, B. J. (2008). High-throughput methods to assess lipophilic and hydrophilic antioxidant capacity of food extracts in vitro. *Journal of Agricultural and Food Chemistry*, ***56***(10), 3470-3477.

Kaur, H. and Narasimhan, B. (2018). Synthesis, Characterization, Antimicrobial and Antioxidant Potential of Diazenyl Chalcones. *Current Topics in Medicinal Chemistry*, ***18***(10), 844-856.

Lahtchev, K. L., Batovska, D. I., St P, P., Ubiyvovk, V. M. and Sibirny, A. A. (2008). Antifungal activity of chalcones: A mechanistic study using various yeast strains. *European Journal of Medicinal Chemistry*, ***43***(10), 2220-2228.

Lin, Y., Lu, Q., Chen, C., Wang, B., Guo, L., Chen, C. and Dong, L. (2021). A synthetic chalcone derivative, compound 39, alleviates lipopolysaccharide-induced acute lung injury in mice. *European Journal of Pharmacology*, ***891***, 173730.

Liu, J., Chen, C., Wu, F. and Zhao, L. (2013). Microwave- Assisted Synthesis and Tyrosinase Inhibitory Activity of Chalcone Derivatives. *Chemical Biology & Drug Design*, ***82***(1), 39-47.

Narsinghani, T., Sharma, M. C., & Bhargav, S. (2013). Synthesis, docking studies and antioxidant activity of some chalcone and aurone derivatives. *Medicinal Chemistry Research*, 22(9), 4059-4068.

Ngameni, B., Cedric, K., Mbaveng, A. T., Erdoğan, M., Simo, I., Kuete, V. and Daştan, A. (2021). Design, synthesis, characterization, and anticancer activity of a novel series of O-substituted chalcone derivatives. *Bioorganic & Medicinal Chemistry Letters*, ***35***, 127827.

Patil, R. B., Sawant, S. D. and Thombare, P. A. (2012). Design, synthesis and pharmacological evaluation of chromenones and related analogues. *International Journal of PharmTech Research*, ***4***, 375-381.

Rachmale, P. M. (2012). Synthesis and antimicrobial activity of some chalcone derivatives and their copper complexes. *International Journal of Pharmaceutical Sciences and Research*, ***3***(3), 901.

Sahu, K. N., Balbhadra, S. S., Choudhary, J., and Kohli, V. D. (2012). Exploring Pharmacological Significance of Chalcone Scaffold: A Review. *Current Medicinal Chemistry*, ***19***(2), 209-225.

Sharma, R., Kumar, R., Kodwani, R., Kapoor, S., Khare, A., Bansal, R. and Kumar, S. (2016). A review on mechanisms of anti tumor activity of chalcones. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, ***16***(2), 200-211.

Sharma, V. S. and Patel, R. B. (2017). Design and investigation of calamatic liquid crystals: Schiff base (–CH=N), chalcone (–CO–CH=CH–), and ester (–COO–) linkage group contain rigid rod shape with various terminal parts. *Molecular Crystals and Liquid Crystals*, ***643***(1), 141-158.

Syam, S., Abdelwahab, S. I., Al-Mamary, M. A. and Mohan, S. (2012). Synthesis of chalcones with anticancer activities. *Molecules*, ***17***(6), 6179-6195.

Tsao, R., Yang, R. and Young, J. C. (2003). Antioxidant isoflavones in osage orange, Maclura pomifera (Raf.) Schneid. *Journal of Agricultural and Food Chemistry*, ***51***(22), 6445-6451.

Vishwanadham, Y., Kumaraswamy, T., Suman, A., Patil Prathima, D. V. and Samhitha, T. (2013). A review on chalcones and its importance. *Pharmatutor Magazine*, ***1***(2), 54-59.

Zhang, X. W., Zhao, D. H., Quan, Y. C., Sun, L. P., Yin, X. M. and Guan, L. P. (2010). Synthesis and evaluation of antiinflammatory activity of substituted chalcone derivatives. *Medicinal Chemistry Research*, ***19***(4), 403-412.