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SYNTHESIS, CHARACERIZATION AND ANTIOXIDANT ACTIVITY OF CURCUMINOID COMPLEXES

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Abstract

Since an ancient day, people have used plants and their extracts to cure various diseases. Plants and their extracts are containing several bio-active compounds, which possess different biological activities. Curcumin/their derivatives have a much biological activity such as anti-oxidant, anti-inflammatory, anti-cancer, anti-bacterial, etc. Poor bioavailability is one of the drawback curcumin. To enhance it's this issue we synthesized curcuminoid copper complexes. The synthesized ligand and complexes are well characterized by spectroscopic techniques. The complex and ligand synthesized to study its antioxidant activity of the curcumin. The three-dimensional structure was optimized using the Gaussian software.

Introduction

The most active component of turmeric (Fig 1) is curcumin, which makes up 2 to 5% of the spice curcumin, giving the yellow color to turmeric. The structure of curcumin ($C_{21}H_{20}O_6$) was described in 1910 by Lampe and Milobedeska Extensive research within the last half a century has proven that most of these activities, once associated with turmeric, are due to curcumin. Further work by the same group in 1913 resulted in the synthesis of the compound [1].

Fig 1: Appearance of turmeric powder.

Subsequently, Srinivasan separated and quantified the components of curcumin by chromatography [2]. Even though curcumin is the major component, it contains many powerful antioxidants and anti-inflammatory compounds. A wide range of biological activities of curcumin has been establishing a good relationship with the structure of this natural compound. Presence of the unsaturated diketone, methoxy group and phenolic groups of curcumin was donated proton easily and exhibits reducing phenomenon [4,5]. Curcumin can able to stop lipid peroxidation chain reaction by neutralizing the free radical from ROS [6]. The polar structure and low molecular weight of curcumin increase its penetration ability through the blood-brain barrier (BBB) [7]. It is reported that the curcumin has excellent antioxidant property than well-established endogenous antioxidants such as vitamin C and E [8-10]. Curcumin is one of the superior molecules that possess both diketone and a hydroxyl group in a single molecule which results in greater protection against oxidants. It neutralizes free radicals *via* donating of the proton from the phenolic group and getting converted to a stable dehydrated product due to resonance [11]. In the presence of *O*-methoxy group in curcumin processes effective radical scavenging ability compared to other curcuminoids [12,13]. Studies on biomarkers of oxidative stress have clearly confirmed that the curcumin has strong antioxidant potential *in vivo* and *in vitro* level [14, 15]. Curcumin has been shown to reduce lipid peroxidation, protein oxidation, free radical toxicity and to modulate antioxidant enzymes such as superoxide dismutase (SOD), glutathione s-transferase (GST), glutathione peroxidase (GPx) in different tissues and model

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systems [16,17]. Curcumin is protecting endogenous antioxidant through gene regulation exclusively it can activate Nrf2, a redox-sensitive transcription factor [18]. This transactivation of the Nrf2 by curcumin is increased by increasing its dosage compared to free radicals trapping molecule ferulic acid which does not induce Nrf2 transactivation.

Studies have reported that curcumin regulates Nrf2 mediated -glutamylcysteine synthetase (-GCS), it's a key enzyme in GSH biosynthesis [19,20]. It also modulates anti-oxidant enzymes such as heme oxygenase-1 (HO1) and peroxygenases (PON1). HO1 catalyzes the degradation of heme to carbon monoxide, iron, and biliverdin [21], and PON1 is responsible to protect LDL and HDL oxidation from free radicals. A study has reported that intake of curcumin by turmeric is decreased the HDL and LDL peroxidation in 30 healthy human volunteers ranging in age from 40 to 90 years [22]. A recent days laboratory reported that supplementation of curcumin in rats reduced the susceptibility of LDL oxidation, enhanced PON1 activity. Curcumin has also been reported to prevent depletion of inherited antioxidants of the body thus enriching the immunity of body against various toxicities [23,24].

Anticancer activity

Curcumin provides action against various cancers at different stages including suppression of the cell proliferation and tumerogensis inhibition. Abnormal intake of the curcumin for cancer is a risk to skin, stomach, colon, liver and mammary gland[25-28]. There are many mechanisms was proposed for the action of the curcumin against cancer cells such as cell cycle (G1/S) arrest, apoptotic induction, and mitotic block. Agarwal *et al* report, curcumin effectively suppressed the expression of cyclin D1 to halt the cell cycle in the G1/S phase and inhibited the growth of head and neck squamous cell carcinoma [29]. Curcumin produced 50 % growth arrest in K-562 human chronic myelogenous leukemia cells at the concentration of the 20 µg/mL. Besides this, curcumin stops proliferation and growth of tumor through regulating transcription factors, cell cycle proteins, enzymes, cell surface adhesion protein, and cytokines [30].

Curcumin also reported to deactivate some important enzymes which are help to cells lives such as nuclear factor-kB (NF-kB), early growth response gene-1(Egr-1), activating protein-1 (AP-1) activity, expression of cyclin D1, lipoxygenase (LOX), cyclooxygenase2 (COX2), nitric oxide synthase (NOS), chemokines, tumor necrosis factor (TNF) and some cell surface adhesion molecules [31]. NF-kB plays a crucial role in various stages of carcinogenetic path way, this is getting remarkable attention as a target for various cancer drugs. It regulates various receptor and transcriptional factors of genes that induce tumor and are involved in inflammation, cell proliferation and angiogenesis [32]. Nrf-2 and -catenin pathway regulation by curcumin has also been reported. Antioxidant property of curcumin has also been documented to mediate the anti-cancer activity of curcumin [33,34].

Curcumin-induced apoptosis of malignant cell has been reported to be more promising in eliminating pre-neoplastic cells from colon, liver, breast, and leukemia [35-38]. This apoptosis process is a complicated phenomenon and we can't clearly understand curcumin apoptosis process. Some of the reports are available, they are found curcumin is regulated through receptor and mitochondrial-mediated pathway including various anti-apoptotic protein expression [39,40]. Curcumin has also been reported to act against metastasis through inhibiting the pro-inflammatory chemokines by tumor cells [41,42]. A coordination complex is a complex in which the metal atoms are bonded *via* coordinate bond with organic or inorganic molecules. This organic and inorganic molecule is called ligands. Depending on the number of electron pairs that could be donated, ligands are classified as monodentate ligands, bidentate ligands and poly dentate. Poly dentate ligands are known as chelating agents. In 1893, Werner was the first to propose correct structures for coordination compounds containing complex ions, in which a central transition metal atom is surrounded by neutral or anionic ligands. For instance, For example, it was known that cobalt forms a complex with ammonia to form hexamminecobalt(III) chloride complex, with formula $CoCl_3 \bullet 6NH_3$, but the nature of the bond between cobalt and ammonia is surprising. Werner proposed the structure of this complex is $[Co(NH_3)_6]Cl_3$. Co^{3+} ion is a center metal ions and six NH_3 are coordinate to cobalt at the vertices of an octahedron. The three Cl^- are dissociated as

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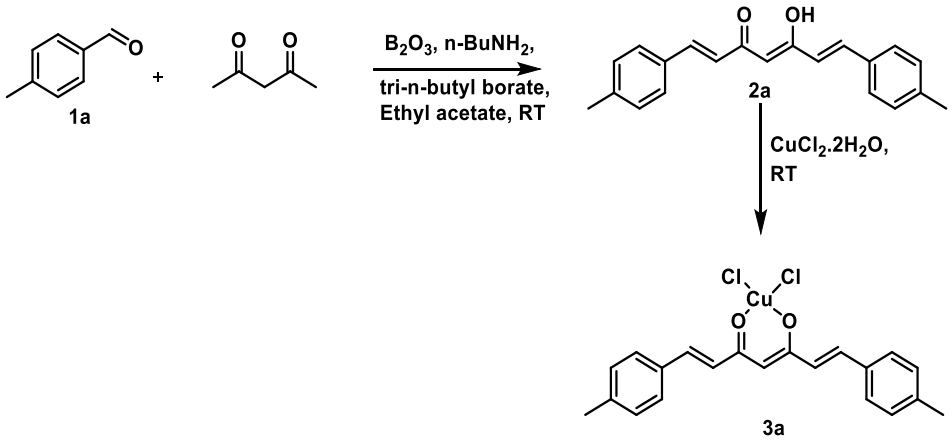
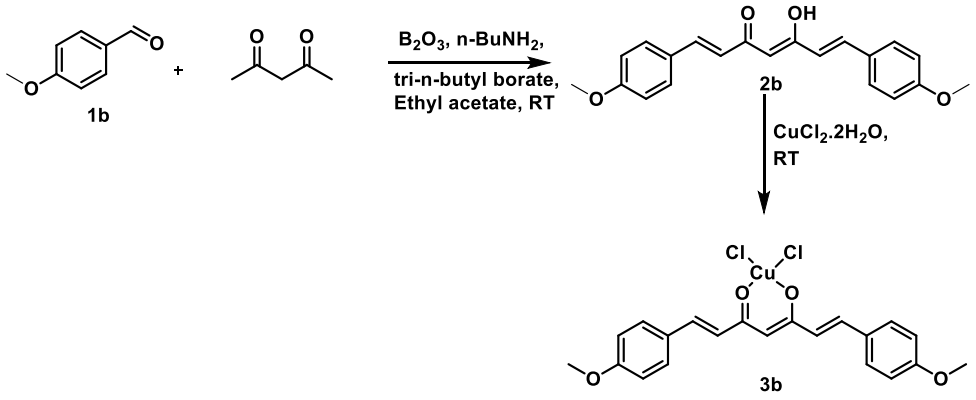
free ions, which was confirmed by measuring the conductivity of the compound in aqueous solution, and also by chloride anion analysis using precipitation with silver nitrate. Later, magnetic susceptibility analysis was also used to confirm Werner's proposal for the chemical nature of $CoCl_3 \bullet 6NH_3$. After that many metal complexes are synthesized and reported based on Werner's theory. These complexes are also used for a medical purpose such as cis-platin is used for cancer therapy. In cis-platin, two molecules of ammonia and two chloride molecules are bind with platinum to form square planner complex. Not only in the medical field, in our biological system has much coordination complex acted as enzyme and proteins. The well-discussed examples are hemoglobin from animals and chlorophyll from plants, without the presence of these two complexes, we can't live on earth. In 1975, Wood made a comment that *"biochemistry is the coordination chemistry of living systems*".

Results and discussion

Synthesis of Curcuminoids (ligand) and Curcumin Copper complexes



Scheme 1: a Synthetic route for complex 1.



Scheme 2: a Synthetic route for complex 2.

We have adopted the previously reported protocol to synthesize curcuminoids complexes (**Scheme 1 a**nd **2**) *via* the aldol condensation of an aldehyde with acetylacetone. To avoid the formation of Knoevenagal product at the C-3 position of acetylacetone, the boron complex was prepared from the acetylacetone and boric anhydride. The reaction of boron complex with corresponding aromatic aldehyde (*p*-tolualdehyde and *p*-methoxy benzaldehyde) in the presence of n-butyl amine and tri-n-butyl borate afforded the ligand (**1a** and **1b**). The final complexes **(2a** and **2b)** were prepared from the corresponding curcumin with copper chloride dehydrate in presence of methanol as the solvent. The synthesized ligands were characterized by ^{1}H NMR and ^{13} C NMR. The metal complexes were characterized by mass spectroscopy.

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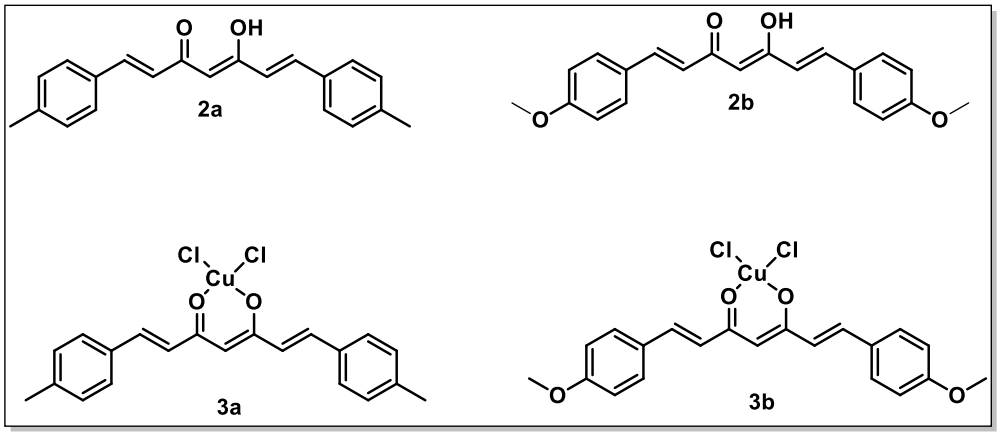

Fig 18: List of synthesized ligand complexes

Biology Studies

DPPH radical scavenging activity

The free radial scavenging activity of the synthesized ligands and its copper complexes were studied by a well-known method, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay described by [1]. Hatano et al. suggested that this is a very simplest method for estimating free radical scavenging activity of bioactive compounds in methanol solution [2]. The DPPH reagent evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants [3]. In general, DPPH (2,2diphenyl-1-picrylhydrazyl) is a purple color as stable radical. On the addition of the synthesized compounds to it will be changed other. Which means the synthesized compound have radical scavenging properties and gives a protonated form of DPPH radial visibly in yellow color. This basic principle was utilized in this DPPH assay [4] (Fig 19).

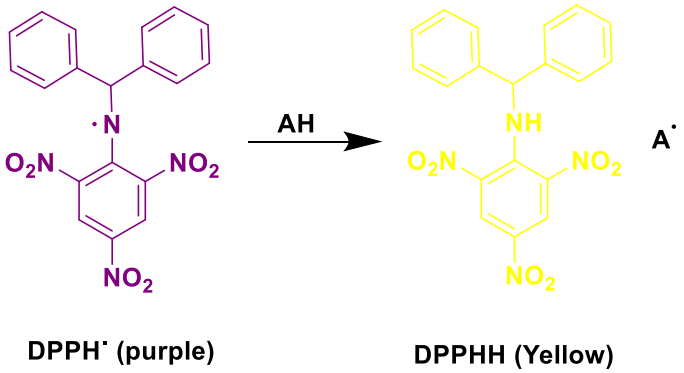

Fig 19: Mechanism of DPPH free radical scavenging activity.

This study shows ligands have less activity compared to the complex and standard. Copper complexes show excellent activity than the standard. In general, curcumin has same activity but this activity was increased after complex formation. In complex Cu^{2+} ions also plays a crucial role in enhancing radical scavenging from DPPH free radical, it may also quench the radical and from the stable complex with curcumin. The Free radical scavenging of ligand, complex and standard ascorbic acid is represented in Fig 20.

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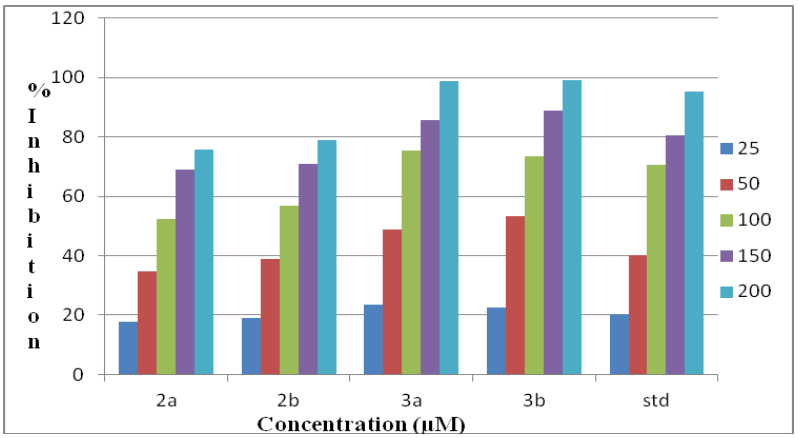

Fig 20: DPPH scavenging activity of ligands and complexes.

H_2O_2 scavenging activity

This is also one of good scavenging method like DPPH radical scavenging method. In this method, hydrogen peroxide was used instead of DPPH [5,6]. This result also shows good H_2O_2 scavenging activity of complexes compared to ligands. The synthesized ligands and its complexes are effectively scavenging the DPPH radical than the hydrogen peroxide. The hydrogen peroxide scavenging activity of ligand and complexes were represented in Fig 21.

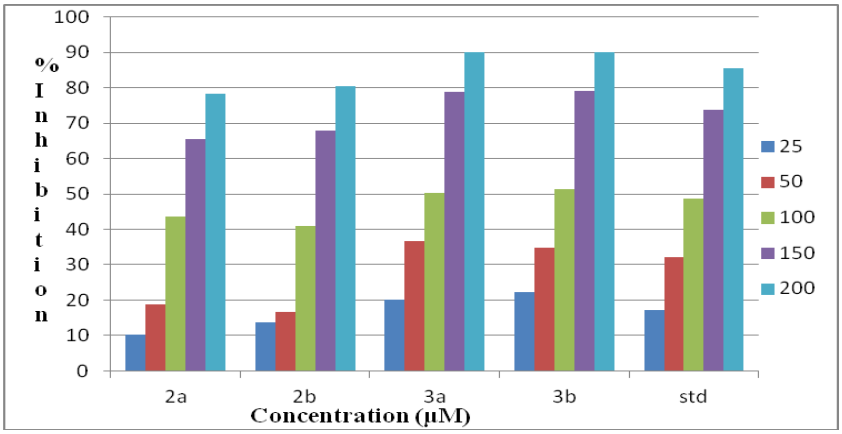

Fig 21: H_2O_2 scavenging activity of ligands and complexes.

Computational Studies

To the further understanding of the formation of the complexes and surface electronic configuration, we performed Density Functional Theory (DFT) calculations. Ligand MeCu shows equal electron distribution of the HOMO and LUMO with energy gab is 3.58 eV. After complex formation, the metal center acted as a HOMO and this electron density was almost transfer into ligands this is due to the strong complex formation. The energy gab is also reduced significantly is 1.7 eV, this is clearly indicated that copper metal ion has more binding affectivity with ligand MeCu.

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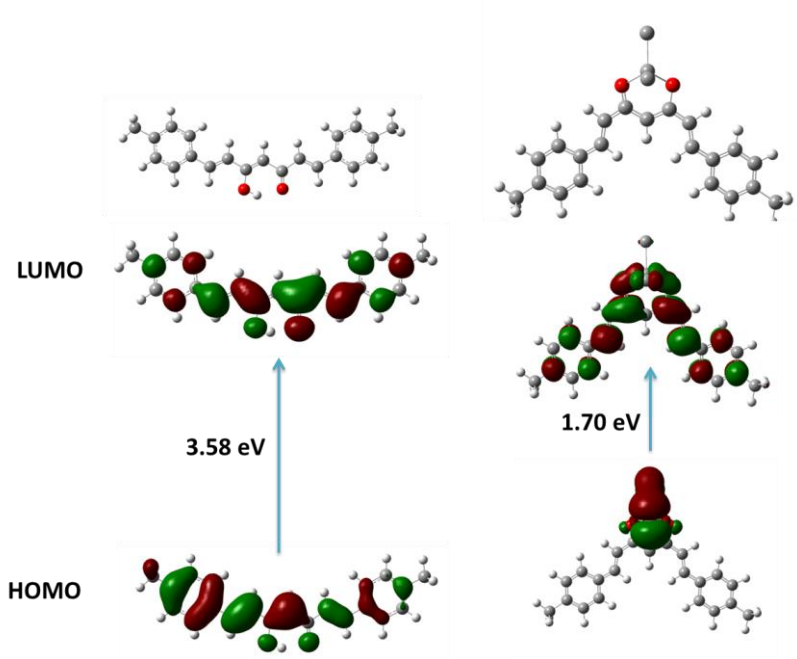

Fig 22: Frontier molecular orbitals of ligand 1 and complex 1

Ligand 2 MeOCu shows high electron density for α,β-unsaturated carbonyl and phenyl ring act as HOMO. In LUMO the electron density was spread over the molecule except for phenyl ring. HOMO has more electron distribution at phenyl ring compared to LUMO. The complex showed a HOMO at whole molecules but at LUMO the electron density is completely transferred into the metal center. This is indicated that strong chelation effect occurs between the MeOcu and copper metal ions. Due to this strong complex formation energy gab also decreased suddenly. The energy gab between the HOMO and LUMO is 3.47 eV for ligand and 0.86 eV for the complex.

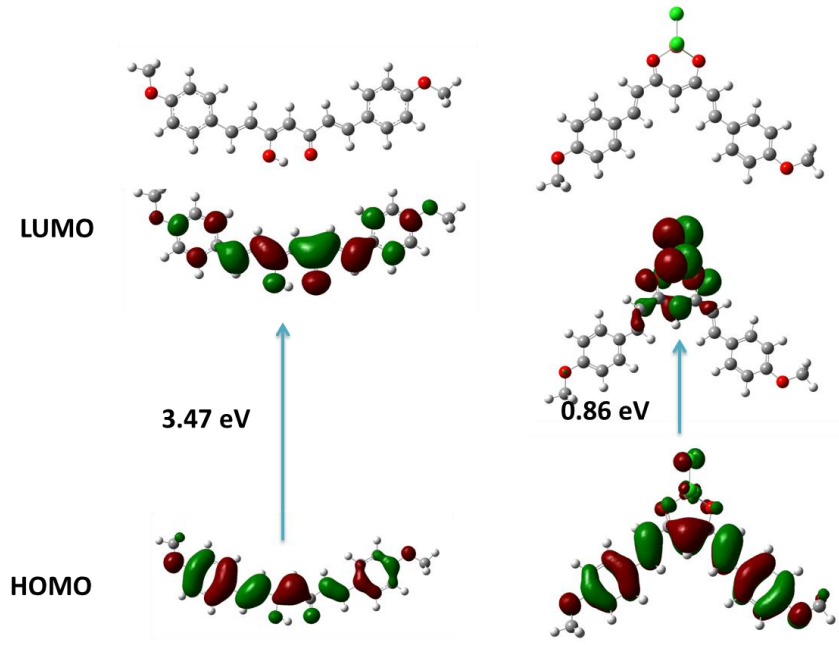

Fig 23: Frontier molecular orbitals of ligand 2 and complex 2.

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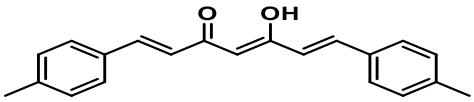
Experimental section

Synthesis of curcumin derivatives(1a and 1b)

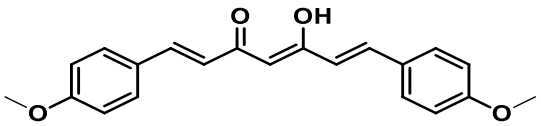
A mixture of Boric anhydride (0.186 g, 2.68 mmol), and 2,4-pentanedione (0.42 ml, 4.0 mmol) was dissolved in ethyl acetate (15 ml) and stirred for 30 min at room temperature under nitrogen atmosphere. Then the corresponding aromatic aldehyde (1 g, 6.7 mmol) and tri *n*-butyl borate (4.00 g, 17.4 mmol) was added to the reaction mixture and stirred for an hour. Then n-Butyl amine was added drop wise over 15 min and again stirred for 3 hours at ambient temperature. After completion of the reaction warmed hydrochloric acid (60 ˚C) (15 mL, 0.4 N) was added to the reaction mixture and stirring was continued for an hour. Then the reaction mixture was diluted with water and extracted with ethyl acetate (50 mL). The ethyl acetate layer was washed with water (50 mL) and brine solution (50 mL). The organic layer was separated and dried over anhydrous sodium sulfate and evaporated under reduced pressures to give the desired product. The obtained product was purified by column chromatography.

(1E, 4E, 6E)-5-hydroxy-1, 7-di-p-tolylhepta-1,4, 6-tries-3-one(1a):



Yield 80%, yellow solid. ^{1}H NMR (300MHz $CDCl_3$): δ ppm: 2.38 (s, 6H), 5.82 (s, 1H), 6.56 (d, 2H, *J*= 15.6Hz), 7.19(d, 4H, *J*= 8.1Hz), 7.44 (d, 4H, *J*= 8.1Hz), 7.61 (d, 2H, *J*= 15.6Hz). ^{13}C NMR (75MHz $CDCl_3$): δ ppm: 21.36, 101.48, 122.92, 127.99, 129.56, 132.06, 140.43, 140.47, 183.25.

(1E, 4Z, 6E)-5-hydroxy-1, 7-bis (4-methoxyphenyl) hepta-1, 4, 6-tries-3-one(1b):



Yield 70%, yellow solid. ^{1}H NMR (300MHz $CDCl_3$): δ ppm: 3.84 (s, 6H), 5.78 (s, 1H), 6.47 (d, 2H, *J*= 15.6Hz), 6.90 (d, 4H, *J*= 8.7Hz), 7.49 (d, 4H, *J*= 8.7Hz), 7.59 (d, 2H, *J* =15.9Hz). ^{13}C NMR (75MHz $CDCl_3$): δ ppm: 55.53, 101.27, 114.51, 121.92, 127.93, 129.90, 140.24, 161.39, 183.47.

Biological studies

DPPH radical scavenging activity

The synthesized ligands and complexes are prepared solutions with various concentrations such as 25, 50, 100, 150 and 200 □M. These solutions are added to the 0.1 mM of DPPH solution in methanol medium. After incubation of the particular hours, the solutions are recorded for absorbance spectroscopy. The same concentration of the standard solution also prepared and incubated like synthesized complexes. In bare DPPH radical solution exhibit the sharp band at 518 nm. After incubation of the ligands and complexes solutions, the DPPH radical band was monitored. Based on the absorbance spectrum we calculate the radical scavenging activity of the complexes and ligands using the formula:

DPPH inhibition effect % = $(A_c\text{-}A_s/A_c)$ x 100

Where,

A_c- Absorbance of the control

A_s-Absorbance of the sample

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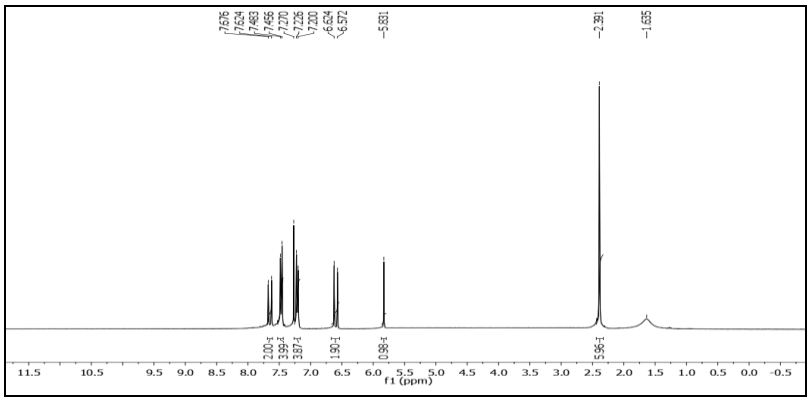
Hydrogen peroxide scavenging activity

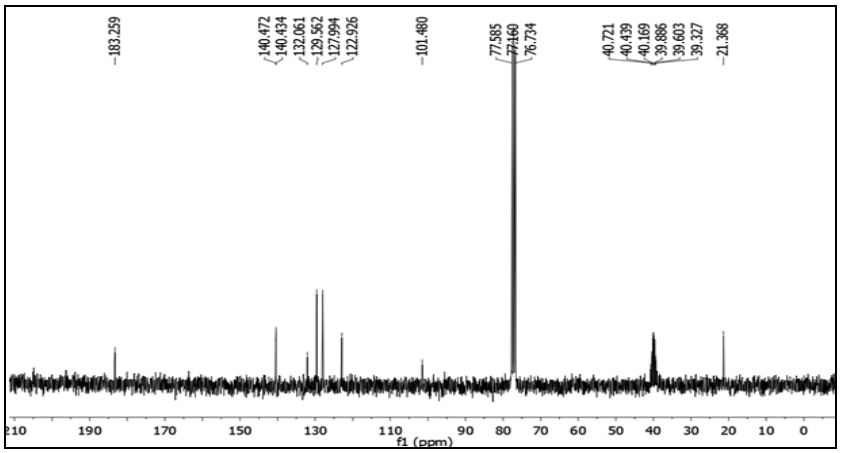
2.0 mM solution of H_2O_2 was prepared in phosphate buffer (0.2 M, PH = 7.4). The different concentrations of synthesized compounds and ascorbic acid were (10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL) added to the hydrogen peroxide solution (0.6 mL) in phosphate buffer. The total solution is made up of 4 mL of phosphate buffer. The same solution without the compound was taken as a negative control. The absorption of hydrogen peroxide recorded at 230 nm and the phosphate buffer was taken as blank. % Inhibition = [(blank-test)/blank] x 100.

Computational studies

All the computational calculations including representation of Highest occupied molecular orbital (HOMO) and Lowest unoccupied molecular orbital in the checkpoint files was performed with the Gaussian 09W program using density functional theory. The chemical structure of the compound was optimized with B3LYP and 6.311 ++ G(d,p)/LANL2DZ basis set. The Gauss view software package was used to visualize the computed structures including HOMO and LUMO representation.



^{1}H NMR (300MHz, $CDCl_3$) Spectrum of compound 2a



^{13}C NMR (75MHz, $CDCl_3$+DMSO-D_6) Spectrum of compound 2a

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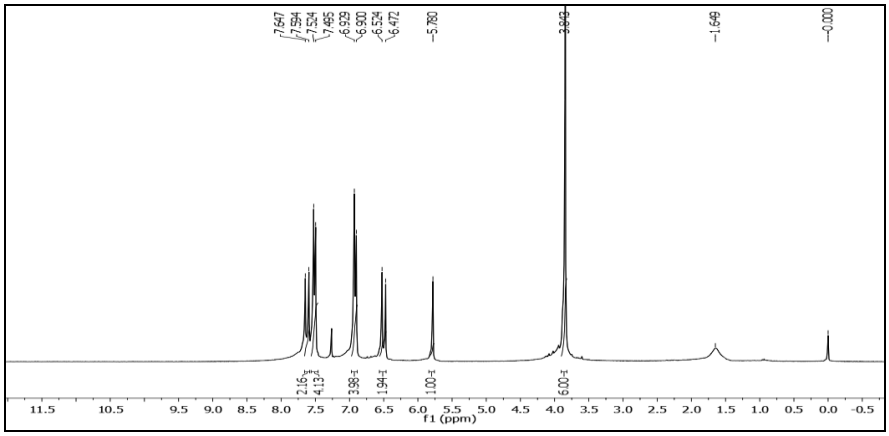
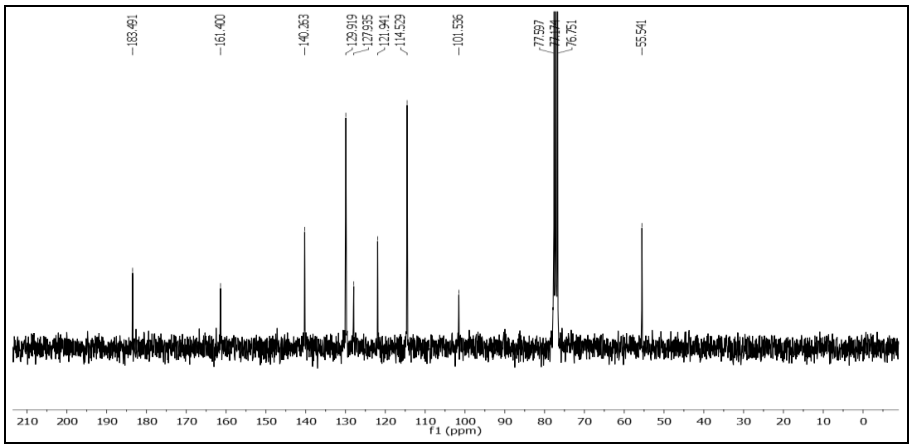


^{1}H NMR (300MHz, $CDCl_3$) Spectrum of compound 2b



^{13}C NMR (75MHz, $CDCl_3$) Spectrum of compound 2b

Conclusion

In this thesis, we have synthesized two ligands based on the small modification of the curcumin and its copper metal complexes. These ligands and metal complexes are characterized and studied its anti-oxidant activity with standard antioxidants. The anti-oxidant activity was carried out in a two different scavenging method. One is DPPH radical scavenging and another one is hydrogen peroxide scavenging method. In both methods the ligands and complexes have efficient scavenging activity, in particular, complexes has more activity than the ligands. The structure of the ligand and complexes were evaluated by theoretical studies using Gaussian 09W programme. This study gives information about structural and electronic motion of the ground and excited state of the ligand and complex. The energy gap shows that the complex formation is very facile because it has very low energy between HOMO and LUMO.

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