



Received on 26 September 2019; received in revised form, 17 March 2020; accepted, 20 March 2020; published 01 September 2020

## SYNTHESIS, SPECTRAL ELUCIDATION, ANTIBACTERIAL, ANTIOXIDANT AND DNA STUDIES OF ONNO TETRADENTATE SCHIFF BASE METAL(II) COMPLEXES DERIVED FROM 2-AMINOPHENOL DERIVATIVES

Kumarasamy Savitha and Subramaniam Vedanayaki \*

Department of Chemistry, Kandaswami Kandar's College, Paramathi Velur, Namakkal - 638182, Tamil Nadu, India.

### Keywords:

Schiff base tetra dentate ligand,  
Square planar, Antimicrobial,  
Antioxidant, DNA studies,  
Antitumour activity

### Correspondence to Author: Subramaniam Vedanayaki

Head and Associate Professor,  
Department of Chemistry,  
Kandaswami Kandar's College,  
Paramathi Velur, Namakkal - 638182,  
Tamil Nadu, India.

**E-mail:** varshuvishal@gmail.com

**ABSTRACT:** The new asymmetrical tetradentate (ONNO) Schiff base (L) 4-chloro- 2- ((E)- (4- ((E)-((2-hydroxy-5-nitrophenyl)imino)methyl)benzylidene) amino)phenol was prepared by the condensation of terephthalaldehyde with 2-amino 4-chlorophenol and 2-amino 4-nitrophenol in 1:1:1 molar ratio in methanol. The homo binuclear metal(II) complexes were synthesized in ligand to metal ratio 2:2 from the template method. The composition, geometry and binding sites of ligand and its complexes were determined by various studies like Elemental, molar conductance, electronic, magnetic moment, FT-IR, <sup>1</sup>H and <sup>13</sup>C-NMR, ESI-Mass, EPR, Thermal, and PXRD. The above spectral studies show that the Schiff is a dibasic tetradentate (ONNO), and its metal complexes possess a square planar structure. The biological applications of all compounds were tested for antibacterial, antioxidant, and DNA cleavage, and the final reports reveal high inhibition activity for metal(II) complexes compared to the ligand. The DNA binding activities of Cu(II) complex was observed by electronic absorption method. Antitumor activity of Cu(II) complex was screened for their cancer-inhibiting ability against MCF-7 human breast cancer cells by using the MTT experiment.

**INTRODUCTION:** The design and preparation of asymmetrical Schiff base ligand derived from the 1:2 ratio condensations of carbonyl group (dialdehyde) with alkyl or aryl primary amines and its derivatives. Tetra dentate Schiff base and its metal complexes played an important role in the progress of coordination chemistry following in a substantial number of publications. The infection treatment was challenged by multidrug resistance in pathogenic organisms and oxidative stress, so the development of newer Schiff base compounds for the management of infections and oxidative stress is warranted <sup>1,2</sup>.

Tetra and polydentate ligands are having nitrogen and oxygen donor atoms in their structure act as chelating agents for the non-transition and transition metal ions, which form stable complexes with transition metal(II) ions <sup>3-5</sup>.

In recent years, the preparation and spectral evaluation of macrocyclic binuclear metal complexes have been more efforts and useful to study the relation between geometry and magnetic exchange interactions. Ligands are used to substrates in the synthesis of many numbers of biologically active and industrial compounds *via* ring closer, cyclo-addition, and replacement reactions <sup>6</sup>. Unsymmetrical Schiff base ligand and its complexes were recommended and used as biological models in understanding irregular binding of peptides and catalyst in chemical process <sup>7</sup>. The macrocyclic chemistry field of metal is developing very fast because it shows the many

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.11(9).4513-23</p> <p>This article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a></p> <p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.11(9).4513-23">http://dx.doi.org/10.13040/IJPSR.0975-8232.11(9).4513-23</a></p>
---	---

pharmacological applications and importance in the area of bioinorganic and medicinal chemistry<sup>8</sup>. Macrocyclic Schiff base complexes give potential applications of many fields in fundamental and applied sciences, stabilization of unusual oxidation states, sensors technology, magnetic resonance imaging, models for biological structures and functions, contract enhancing agents, and metal selective extraction<sup>9</sup>. Schiff base metal complexes allow systematic studies depends on the modified ancillary ligand, which plays the main role in the catalysis; enzymatic reaction, magnetism, and molecular modeling studies are related to the development of bioinorganic chemistry<sup>10</sup>. Metal complexes are reported to have excellent biological applications such as antimicrobial, antioxidant, anti-insecticidal, antifertility, antitubercular, anti-tumor and anti-inflammatory<sup>11-18</sup>. Schiff base ligand and its binuclear metal complexes can cleave the DNA through three kinds of mechanisms like hydrolytic, oxidation, and photolytic cleavages<sup>19</sup>. Cytotoxicity effect of Cu(II) complex has considerable ability due to their bind and cleave DNA, which leads to cell cycle prevention and apoptosis<sup>20, 21</sup>.

Based on the above fact, herein we have reported the synthesis of new asymmetric Schiff base ligand (4-chloro-2-((E)-4-((E)-((2-hydroxy-5-nitrophenyl)imino)methyl)benzylidene)amino)phenol) containing the azomethine (CH=N) and hydroxyl groups (OH) as potent chelating sites and its Co(II), Ni(II) and Cu(II) binuclear metal complexes evaluated by diverse physicochemical technique and their applications were analyzed for antibacterial, antioxidant, DNA studies (cleavage and binding) and antitumor activity (*in-vitro* cytotoxicity).

## EXPERIMENTAL SECTION:

### Analytical and Physical Measurements:

Terephthalaldehyde, 2-amino 4-chlorophenol, 2-amino 4-nitrophenol, metal salts were purchased from Sigma Aldrich. Ethanol, Methanol, DMSO, DMF, and Acetone were purchased from Loba and Merck chemicals. The purity of all compounds was tested by TLC.

C, H and N elements were carried out on a Thermo Finningan Flash EA 1112 series elemental analyzer. The molar conductance of compounds

was measured at room temperature using by ELICO CM 180 Conductivity Bridge. The magnetic moment values of compounds were calculated using Gouy balance calibrated with Hg[Co(SCN)<sub>4</sub>] method at room temperature. UV spectral studies were measured using the Perkin-Elmer Lambda-25 spectrometer in the range of 200-800 nm. FT-IR spectra of compounds were recorded on a Shimadzu FT-IR-8300 spectrometer using KBr pellet experiment in range 4000-400 cm<sup>-1</sup>. <sup>1</sup>H and <sup>13</sup>C-NMR spectra were recorded on a BRUKER ADVANCED III 4000 MHz spectrometer using TMS as an internal reference. Electro Spray Ionization of mass spectra was obtained on a Perkin-Elmer R MU-6E instrument in the mode of positive. The ESR spectra were recorded on the JES-FA200 ESR spectrometer with X-band frequency at room temperature. Thermal analyses were measured at 0°-1000 °C using SDT-Q600 V20.9 Build 20 thermal analyzer in an oxygen atmosphere. The Powder-XRD spectra were carried out on a Perkin-Elmer TA/SDT-2960 and Philips 3701 instrument.

### Synthesis of Asymmetrical Tetra Dentate Schiff Base Ligand:

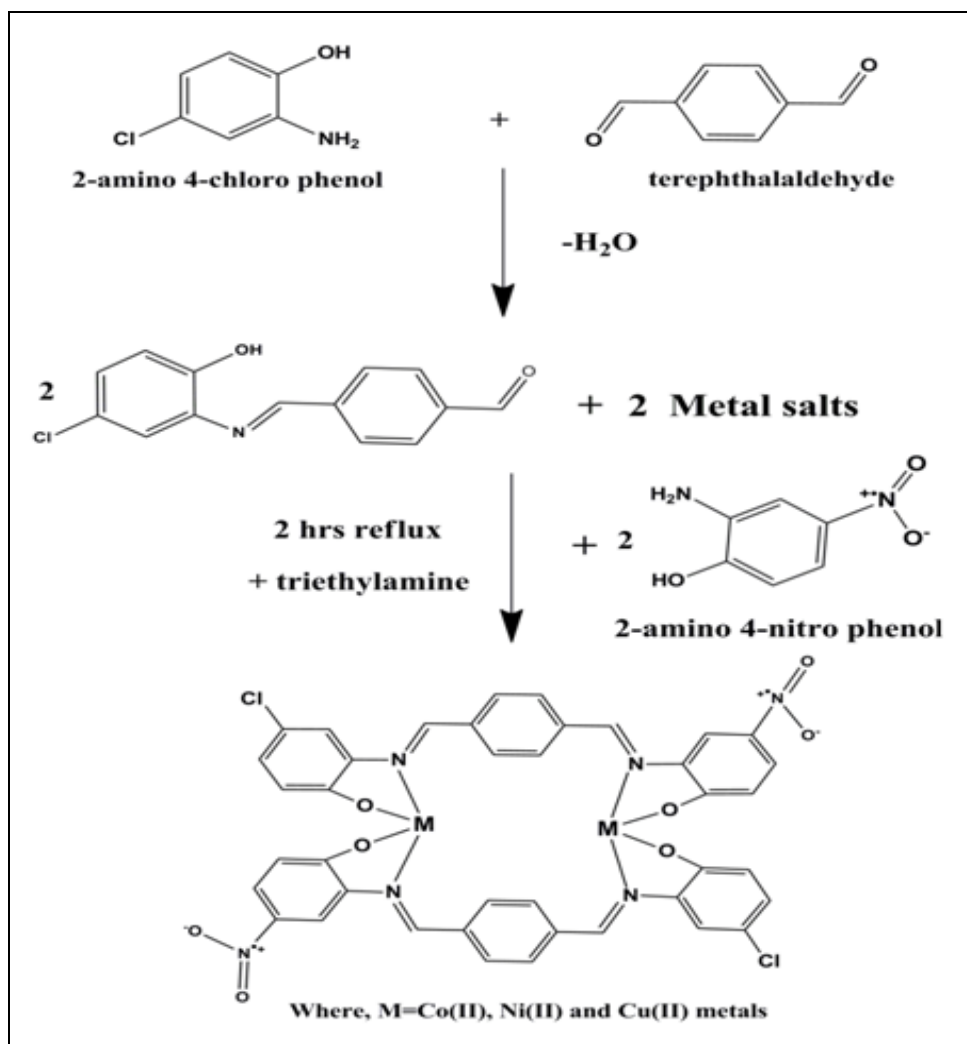
The mixture of 1mmole of terephthalaldehyde with 1mmole of 2-amino 4-chlorophenol and 1mmole of 2-amino 4-nitrophenol were dissolved in methanol. The solution was kept under stirring for 2 h, the formed light yellow precipitate was separated by filtration, washed and purified by methanol solvent. The Schiff base solid was recrystallized from ethanol. Yellow solid, Molecular weight- 395.80, Melting point-240 °C, Yield- 80%, IR (KBr cm<sup>-1</sup>): 3339 (OH), 1623 (C=N), 1289 (C-O); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>, δ, ppm): 8.85 (s, 1H, C=N), 10.1 (s, 1H, OH), 6.5-8.2 (m, Ar-H); <sup>13</sup>C-NMR (100MHz, CDCl<sub>3</sub>): 113, 115, 116, 117, 118, 119, 123, 127, 129, 130, 138, 139, 140, 141, 143, 150, 161; Elemental analysis: C<sub>20</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>4</sub> Calculated values: C- 69.14, H- 4.70, N- 7.68; Found values: C- 68.86, H- 4.50, N- 7.98; ESI-Mass: m/z: (M+1)<sup>+</sup> 396.

### Synthesis of Binuclear Metal(II) Complexes:

The homo binuclear metal(II) complexes were synthesized by using template method<sup>22</sup>. The mixture of terephthalaldehyde (2mmole) with 2-amino 4-chlorophenol (2mmole) and 2-amino 4-nitrophenol (2mmole) was dissolved in methanol, which was added to the methanolic solution of

metal salts (2mmole) like Co, Ni, and Cu. The mixture was stirred, and a few drops of triethylamine were added to the mixture. It was stirred for 1 h and under reflux for 3 h. The product was partly evaporated, cooled at room temperature,

the obtained metal complexes were separated by filtration, washed with methanol and diethyl ether, stored at room temperature. The structure of metal complexes was shown in **Scheme 1**.



**SCHEME 1: METAL COMPLEXES PREPARATION FROM TEMPLATE METHOD**

**Schiff Base Binuclear Co(II) Complex (Co<sub>2</sub>L<sub>2</sub>):**

Brownish black solid, Molecular weight- 994.11, Melting point->300 °C, Yield- 78%, IR (KBr cm<sup>-1</sup>): 1602 (C=N), 1340 (C-O), 577 (M-O), 425 (M-N); Elemental analysis: C<sub>46</sub>H<sub>42</sub>Cl<sub>2</sub>Co<sub>2</sub>N<sub>6</sub>O<sub>8</sub> Calculated values: C- 55.49, H- 4.25, N- 8.44, M- 11.84; Found values: C- 55.80, H- 4.51, N-4.18, M-11.34; Molar conductance (Ω<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>) 10.5.

**Schiff Base Binuclear Ni(II) Complex (Ni<sub>2</sub>L<sub>2</sub>):**

Reddish yellow solid, Molecular weight- 992.11, Melting point->300 °C, Yield- 76%, IR (KBr cm<sup>-1</sup>): 1598 (C=N), 1338 (C-O), 548 (M-O), 438 (M-N); Elemental analysis: C<sub>46</sub>H<sub>42</sub>Cl<sub>2</sub>Ni<sub>2</sub>O<sub>8</sub> Calculated values: C- 55.52, H- 4.25, N- 8.44, M- 11.80:

Found values: C- 55.12, H- 4.13, N- 8.21, M-11.41; Molar conductance (Ω<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>) 11.4.

**Schiff Base Binuclear Cu(II) Complex (Cu<sub>2</sub>L<sub>2</sub>):**

Black solid, Molecular weight- 1004.86, Melting point->300 °C, Yield- 79%, IR (KBr cm<sup>-1</sup>): 1606 (C=N), 1344 (C-O), 529 (M-O), 473 (M-N); Elemental analysis: C<sub>46</sub>H<sub>42</sub>Cl<sub>2</sub>Cu<sub>2</sub>N<sub>6</sub>O<sub>8</sub> Calculated values: C- 54.98, H- 4.21, N- 8.36, M- 12.65; Found values: C- 54.35, H- 4.10, N- 8.04, M- 12.22; Molar conductance (Ω<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>) 13.6.

**Pharmacological Studies:**

**Antibacterial Activity:** *In-vitro* antibacterial activity was performed by the Disc-agar well

diffusion experiment. The asymmetric tetradentate Schiff base ligand and its homo binuclear complexes were screened against bacteria of Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative (*K. pneumoniae*, *E. coli*). Tetracycline was used as a standard drug for antibacterial activity, and the tested compounds were dissolved in DMSO. The antibacterial activities were maintained in nutrient agar well plates at 4 °C. The 100 µL concentration of culture supernatant was placed on agar well plate, and then it was incubated for 24 h at 37 °C. The antibacterial activity was determined by measuring the diameter of the zone, indicating complete inhibition<sup>23</sup>.

**Antioxidant Activity:** 4-chloro-2-((E)-(4-((E)-((2-hydroxy-5-nitrophenyl)imino)methyl)benzylidene)amino)phenol and its metal (Co, Ni, Cu) complexes were investigated by using its free radical scavenging activity on the stable DPPH free radicals described in the literature<sup>24</sup>. The scavenging activity investigates the antiradical power of an antioxidant by calculating the decrease in the absorbance wavelength of DPPH at 510 nm. The ligand and its metal complexes exhibit DPPH free radical scavenging activity at different concentrations (like 10, 20, 30, 40, and 50 µL). The percentage of DPPH free radical scavenging ability was measured by using the following formula

$$\% = \frac{A_c - A_s}{A_c} \times 100$$

Where  $A_c$  is the absorbance of the control and  $A_s$  is the absorbance of the tested sample.  $IC_{50}$  values were calculated for ligand and its complexes, which showed significant ability, and it is defined as concentration sufficient to generate 50% of maximum scavenging activity. Ascorbic acid was used as a standard.

### DNA Binding Studies:

**Electronic Absorption Spectroscopy:** Electron absorption spectroscopy is one of the most useful techniques to determine the interaction between the metal complexes with DNA from the stock solution of calf thymus (CT)<sup>25</sup>. DNA was prepared by 5mM Tris-HCl/20mM NaCl buffer (pH=7.2) at room temperature. The stock solution was stored at 4 °C and used up to 4 days. The buffer solution of CT-DNA gave a ratio of ~1.8-1.9 and the UV absorbance at 260 to 280 nm. The concentration of CT-DNA was determined using the known molar

extinction coefficient value of  $6600 \text{ M}^{-1} \text{ cm}^{-1}$  at absorption intensity is 260 nm. The stock solution concentrations of the complex were prepared by dissolving the Cu complex in DMSO solvent. All the experiments were maintained at proper dilution with the corresponding buffer to the required concentration. The binding constant ( $K_b$ ) was calculated by using the equation:

$$[\text{DNA}] / (\epsilon_a - \epsilon_f) = [\text{DNA}] / (\epsilon_a - \epsilon_f) + 1 / K_b (\epsilon_a - \epsilon_f)$$

Where [DNA] is the molar concentration of DNA in base pairs,  $\epsilon_a$ ,  $\epsilon_f$  are apparent extinction coefficient, the  $K_b$  values were obtained from the ratio between the equation of  $\text{DNA} / (\epsilon_a - \epsilon_f)$  versus [DNA] in each case.

**Agarose Gel Electrophoresis Assay:** The cleavage of supercoiled pBR322 DNA was investigated by gel electrophoresis method<sup>26</sup>. The agarose gel electrophoresis studies were carried out incubation of the mixtures containing 20 µL pBR322 DNA, 50 mM of NaCl, 50 mM of metal complexes, and 50 mM  $\text{H}_2\text{O}_2$  in Tris-HCl buffer (pH=7.4) at 37 °C for 1 h. After incubation, the sample compounds were electrophoresed at 60 °C for 2 h on 1% of agarose gel using TAE (Tris-Acetic acid-EDTA) buffer (pH=8.0). After 0.5 µg/ml of ethidium bromide was used, the gel was stained. Thus all the experiments were performed at room temperature and photographed under UV light at 360 nm.

**Antitumor activity:** *In-vitro* cytotoxicity was carried out on an MCF-7 cell line in which cell viability was tested using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) activity<sup>27</sup>. *In-vitro* effect of growth inhibition of Cu(II) complex was measured by spectrophotometric experiment. This experiment was used to determine the MTT conversion into "formazan" by living cells, and it was maintained in a 96-well microplate having DMEM/F12 plain media supplemented with 10% heat-inactivated FBS (Fetal Bovine Serum) having 5% of a mixture of 100 µg/mL of streptomycin and 100 units/ml of penicillin were incubated at 37 °C for 24 h in the presence of 5%  $\text{CO}_2$ . In this study, various concentrations of 10, 20, 40, 80, 160 and 320 µL of the stock solution were prepared in DMSO, which were added to relevant wells having 100 µL of the DMSO medium. After incubation, 100 µL of MTT

stock solution (5mg/10ml of MTT in PBS) was added to each well and further incubated for 4 hrs. After the supernatant was removed, the plates were gently shaken to form solubilize formazan crystals by adding 100  $\mu$ L of DMSO. The absorbance was measured at wavelength 590 nm by a microplate reader. The activity was performed in triplicate and used to calculate the mean.

**RESULTS AND DISCUSSION:** The synthesized ligand and their metal complexes were investigated by diverse physicochemical methods. The resultant compounds are soluble in DMF and DMSO, partially soluble in ethanol and methanol.

#### Elemental and Molar Conductivity Analysis:

The composition of C, H, and N are analyzed by CHNS elemental analyzer. The molar conductance of ligand and their complexes were measured at 25  $^{\circ}$ C in DMF solution ( $10^{-3}$ M) indicates low

conductance values (10.5 to 13.6), which shows that the complexes are non-electrolytic nature<sup>28,29</sup>.

**Electronic Absorption Spectral Data:** The UV-Vis spectral data of the ligand and its complexes were measured within a 200-800 nm wavelength at room temperature in the DMSO solution. The electronic spectra of the mixed ligand exhibit absorption bands at 296 and 384 nm, which is referred to the aromatic benzene rings ( $\pi \rightarrow \pi^*$ ) and imine group ( $n \rightarrow \pi^*$ ) respectively<sup>30</sup>.

The diffuse reflectance spectrum of the macrocyclic metal complexes of Co(II), Ni(II) and Cu(II) shows bands at 444, 452 and 414 nm, which may be attributed to the ligand to metal co-ordinations (L $\rightarrow$ M transitions) in square planar geometry of the compounds (given in **Table 1**). The magnetic moment and absorption spectra is used to determine the geometry of the metal complexes<sup>31</sup>.

**TABLE 1: UV SPECTRUM OF LIGAND AND ITS METAL(II) COMPLEXES**

Compounds	$\pi - \pi^*$	$n - \pi^*$	M	Geometry	$\mu_{\text{eff}}$ BM
[L]	296	384	---	---	---
[Co <sub>2</sub> L <sub>2</sub> ]	246	371	444	Square planar	1.83
[Ni <sub>2</sub> L <sub>2</sub> ]	248	374	452	Square planar	---
[Cu <sub>2</sub> L <sub>2</sub> ]	252	370	414	Square planar	1.72

**FT-IR Studies:** The IR spectrum is used to investigate of the existence of intramolecular hydrogen bonding, the nature of the coordination mode in the metal complexes, and the presence of the tautomeric forms in the solid states. The FT-IR spectral data of the ligand and their complexes were observed in the wavenumber region at 400-4000  $\text{cm}^{-1}$ . The important vibrational frequency of the free ligand exhibits band at 1623  $\text{cm}^{-1}$ , which indicates the formation of the imine group(C=N)<sup>32</sup>. However, on complexation, this band(C=N) is shifted to lower energy range 1606-1598  $\text{cm}^{-1}$ , suggesting the participation of the azomethine nitrogen atom in coordination to the metal ion. The sharp broadband around 3345  $\text{cm}^{-1}$  in the ligand was assigned to the phenolic -OH group. The absence of  $\nu(\text{OH})$  band in the metal complexes indicates the co-ordination of the  $\nu(\text{OH})$  group after deprotonation. The  $\nu(\text{C}=\text{O})$  band at 1289  $\text{cm}^{-1}$  for ligand were lower frequency than the band at about 1700  $\text{cm}^{-1}$  for  $\nu(\text{C}=\text{O})$ , this frequency change shows the formation of azomethine group (ligand).

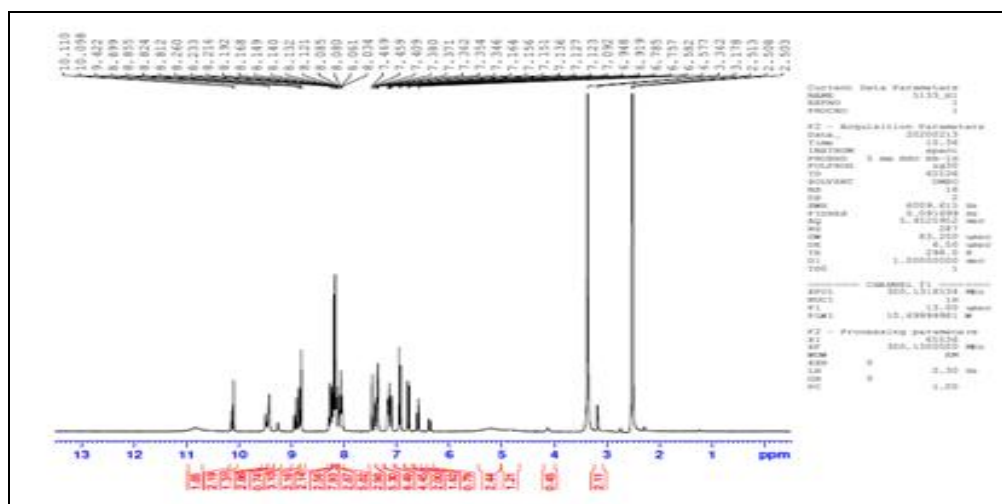
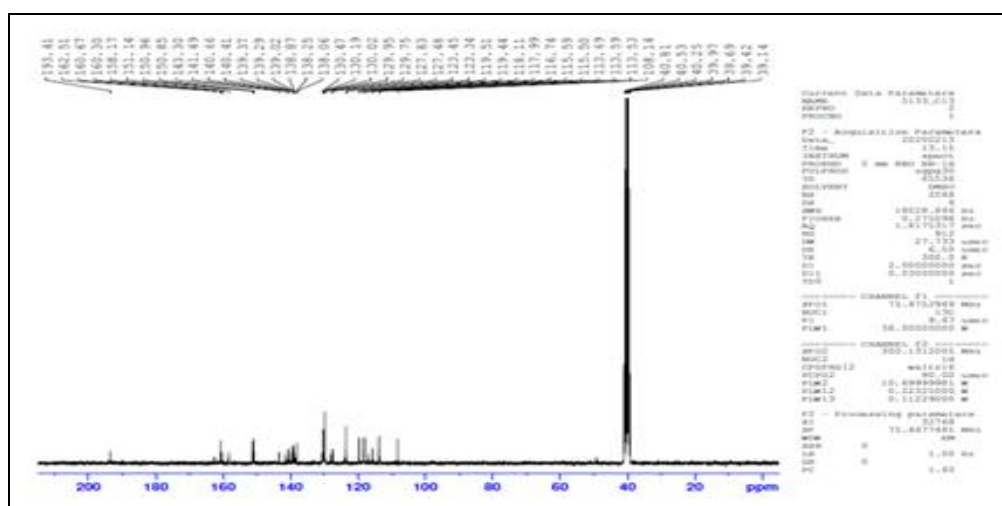
The appearance of new bands at 425-473 and 529-577  $\text{cm}^{-1}$  in the vibrational spectra of the complexes

were assigned to  $\nu_{(\text{M}-\text{N})}$  and  $\nu_{(\text{M}-\text{O})}$  frequencies respectively<sup>33</sup>. These vibrations were clearly suggesting the co-ordination of the metal ions with the azomethine nitrogen and phenolic oxygens in the complex.

#### NMR Spectral Studies:

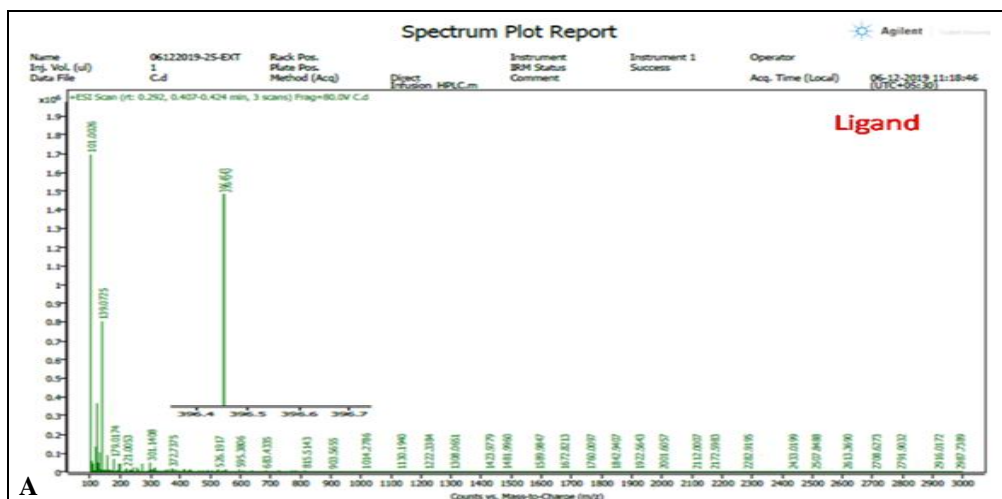
**<sup>1</sup>H-NMR Spectrum:** The <sup>1</sup>H NMR spectrum of the ligand was recorded at 25  $^{\circ}$ C (RT) in DMSO- $d_6$  solvent with TMS as an internal standard. The azomethine (CH=N) proton signal was obtained at  $\delta$ 8.85 ppm; this signal indicates the formation of mixed Schiff base ligand<sup>34, 35</sup> shown in **Fig. 1a**. The new peaks of phenolic -OH protons of the aminophenol and aromatic protons were observed at  $\delta$ 10.0, 10.1 ppm, and  $\delta$ 6.5-8.2 ppm<sup>36</sup>.

**<sup>13</sup>C-NMR Spectrum:** <sup>13</sup>C- NMR spectrum of the synthesized ligand shifted to  $\delta$  161 ppm for an azomethine carbon atom in **Fig. 1b**. The peaks showed at  $\delta$  150 ppm,  $\delta$  143 ppm, and  $\delta$  113-141 ppm were due to phenolic -OH carbon by aminophenol, substituted Cl atom by aminophenol and aromatic benzene ring carbon atoms<sup>37</sup>.

FIG. 1A: <sup>1</sup>H NMR SPECTRUM OF LIGANDFIG. 1B: <sup>13</sup>C NMR SPECTRUM OF LIGAND

**ESI-Mass Spectrum:** The electronic impact spectrum of Schiff base ligand and its copper complex showed molecular ion peaks at  $m/z$  396 ( $M+1$ )<sup>+</sup> and  $m/z$  1005 ( $M+1$ )<sup>+</sup>, which corresponds to the proposed molecular formula of ligand ( $C_{20}H_{14}ClN_3O_4$ ) and its Cu complex

( $C_{46}H_{42}Cl_2Cu_2N_6O_8$ ) respectively<sup>38, 39</sup>. The mass spectral data of Schiff base ligand and its copper (II) complex were confirmed by comparing their molecular formula weights with ( $m/z$ ) mass values, which is in good agreement for these ligand and Cu complex as shown in Fig. 2a, 2b.



A

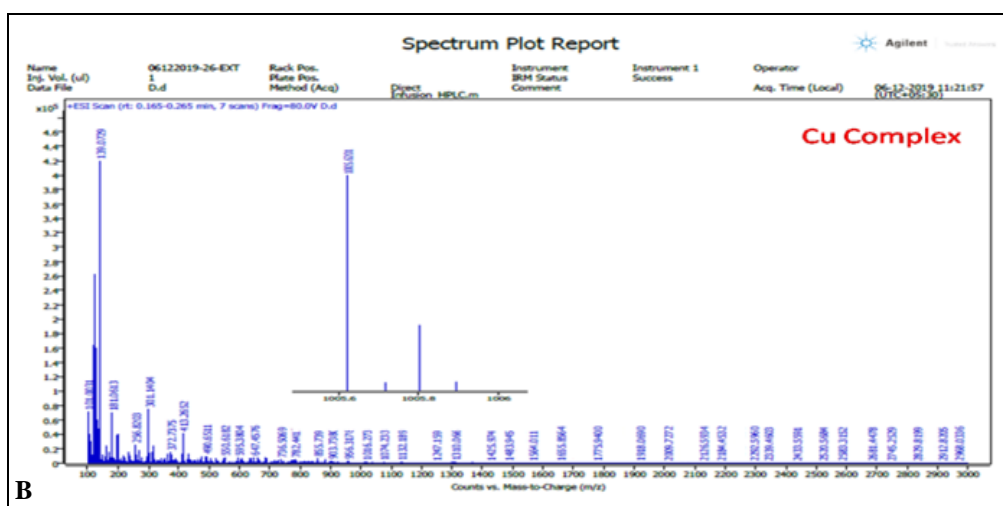


FIG. 2: A AND B: ESI-MASS SPECTRUM OF LIGAND AND CU(II) COMPLEX

**Electron Resonance Spectrum:** The electron spin resonance studies of the binuclear Cu (II) complex gives information about hyperfine and super-hyperfine structures and the nature of the bonding between the copper ion and its ligand<sup>40</sup>. The epr spectrum of the complex [Cu<sub>2</sub>L<sub>2</sub>] was displayed at room temperature and which exhibits an axial symmetry at X-band frequencies in the solid-state. The obtained g-values are  $g_{\parallel}=2.2391$  and  $g_{\perp}=2.0563$ , which are related by  $G=(g_{\parallel}-2)/(g_{\perp}-2)=4.0$ . If G value is more than 4, i.e. ( $G>4$ ), which indicates no considerable exchanging interaction between the two copper centers in the complex.

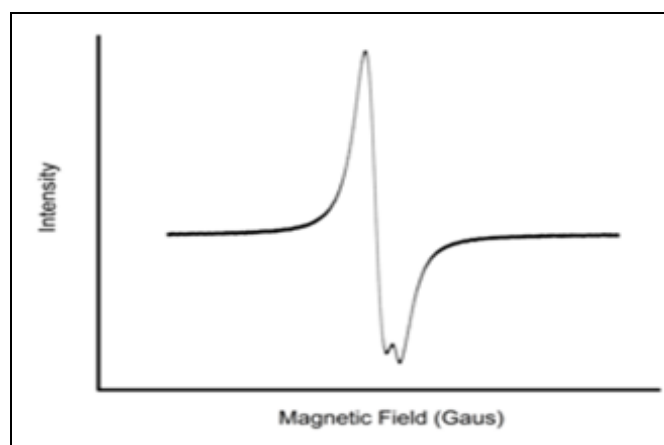


FIG. 3: ESR SPECTRUM OF CU(II) COMPLEX

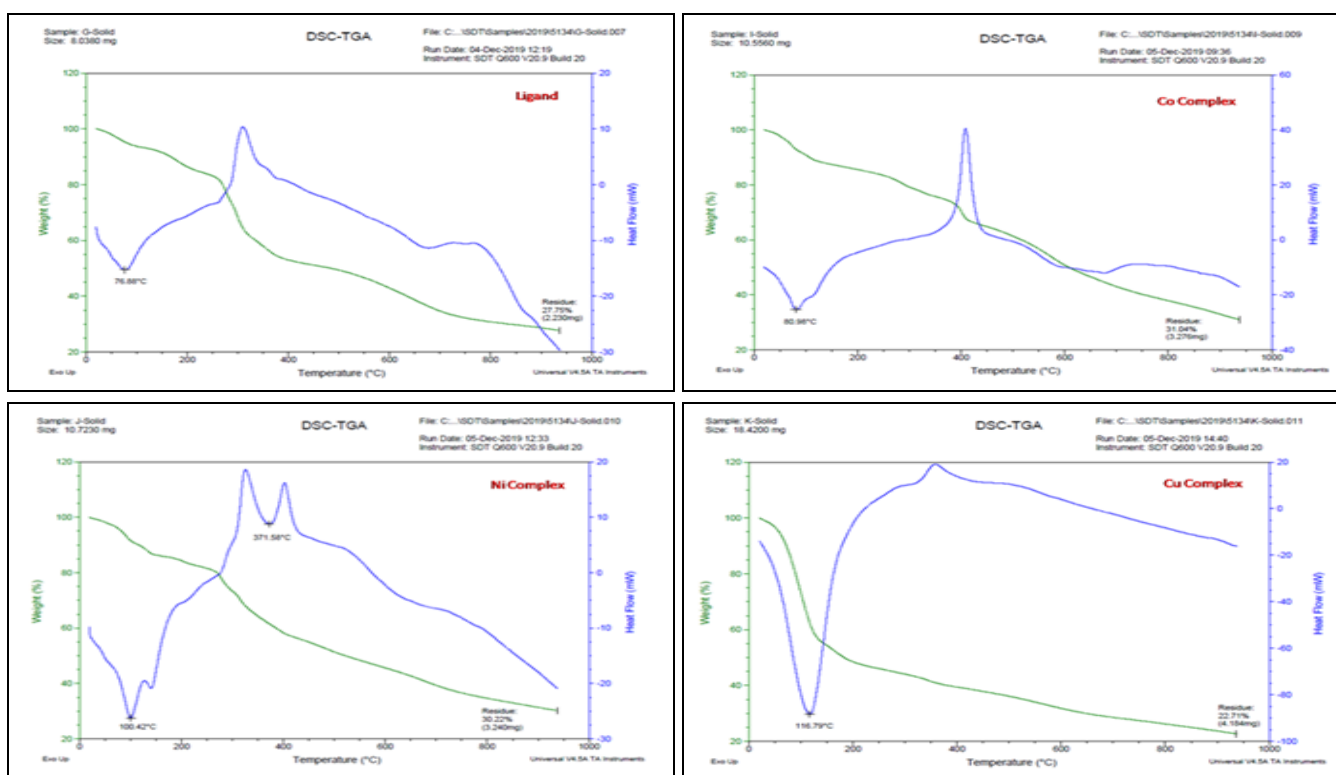
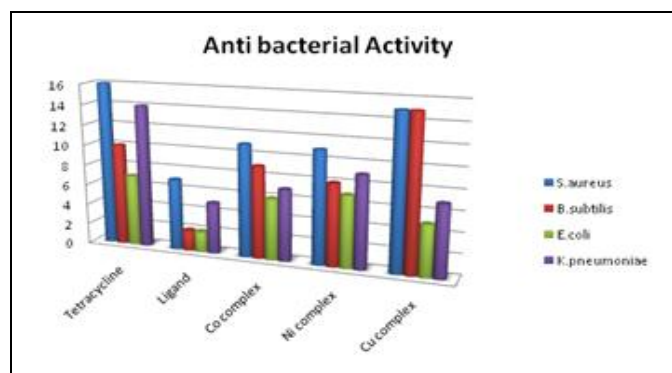


FIG. 4: THERMOGRAM CURVES OF LIGAND AND ITS METAL(II) COMPLEXES

Whereas if G value is less than 4 ( $G < 4$ ), which shows that the exchange interaction occurs in the solid-state complex. From the observed G value of Cu(II) complex is 4.24. It is clear that  $g_{\parallel} > g_{\perp} > 2.0023$ , which indicates  $d_{x^2-y^2}$  in the orbital ground state for the square planar structure of the Cu(II) complex shown in **Fig. 3**.

**Thermal Studies:** The thermal analysis (TG & DSC) was used to determine the thermal stability of compounds for ligand and its metal complexes in the presence of air atmosphere at the temperature range between 0 to 1000 °C<sup>41</sup>. The thermogram of the ligand and its complexes were shown in **Fig. 4**. Both TG and DSC analysis of compounds have three steps of decomposition process each. The first, second, and third steps correspond to the elimination of small groups of substituted Cl and methyl atom by 2-aminophenol, the removal of total ligand moiety, and the formation of metal oxide residue.

**P-XRD Analysis:** The X-ray powder diffraction analysis of the synthesized compounds of ligand and its metal complexes has been carried out to confirm whether the nature of the sample is amorphous (or) crystalline. The structure of Schiff base and its binuclear metal complexes were investigated using P-XRD analysis, which indicates the amorphous orthorhombic crystal nature of compounds within the range 10-90 °C (2 $\theta$ ) and the wavelength is 1.5406 Å<sup>42</sup>. Thus, all the compounds show crystalline nature from its observed peaks. The Powder-XRD provides the d-value, relative intensity and 2 $\theta$  value for each peak.



**FIG. 5: ANTIBACTERIAL ACTIVITY OF TESTED COMPOUNDS**

### Biological Applications:

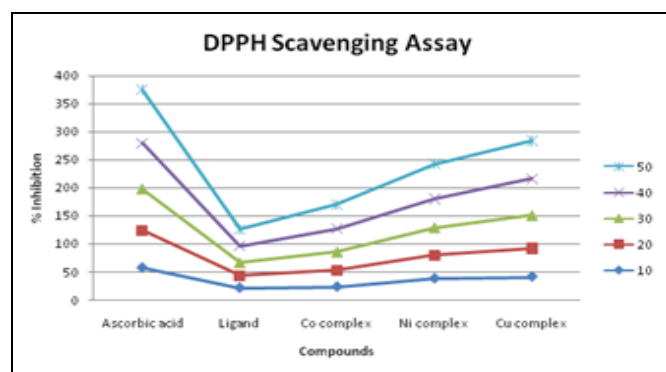
**Antibacterial Assay:** The antibacterial activity of tetra dentate Schiff base ligand and its Co(II),

Ni(II) and Cu(II) complexes were screened against *S. aureus*, *B. subtilis* (gram-positive) and *K. pneumoniae*, *E. coli* (gram-negative) bacteria and tetracycline was used as standard drug. The antibacterial results showed that the Schiff base ligand has very low (or) no inhibition activity compared to its metal complexes<sup>43, 44</sup>. Thus Cu(II) complex exhibits higher antibacterial activity than the other Co(II) and Ni(II) complexes **Fig. 5**.

### Antioxidant Assay:

**DPPH Free Radical Scavenging Activity:** The ligand and its Co(II), Ni(II), and Cu(II) complexes were screened for their DPPH free radical scavenging ability using Ascorbic acid as standard<sup>45</sup>. The ligand has very low activity when compared to all the complexes. The scavenging activity of Cu(II) complex is higher than that of other metal complexes. The IC<sub>50</sub> values were determined (shown in **Fig. 6**) for all compounds, and the IC<sub>50</sub> values of [Cu<sub>2</sub> (C<sub>46</sub>H<sub>42</sub>N<sub>6</sub>O<sub>8</sub>Cl<sub>2</sub>)] is 5.93 mg/ml showed significant activity compared to other complexes and ligand. The order of scavenging activity of all complexes according to their IC<sub>50</sub> values is given below.

Ascorbic acid > [Cu<sub>2</sub> (C<sub>46</sub>H<sub>42</sub>N<sub>6</sub>O<sub>8</sub>Cl<sub>2</sub>)] > [Ni<sub>2</sub> (C<sub>46</sub>H<sub>42</sub>N<sub>6</sub>O<sub>8</sub>Cl<sub>2</sub>)] > [Co<sub>2</sub> (C<sub>46</sub>H<sub>42</sub>N<sub>6</sub>O<sub>8</sub>Cl<sub>2</sub>)] > C<sub>20</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>4</sub>

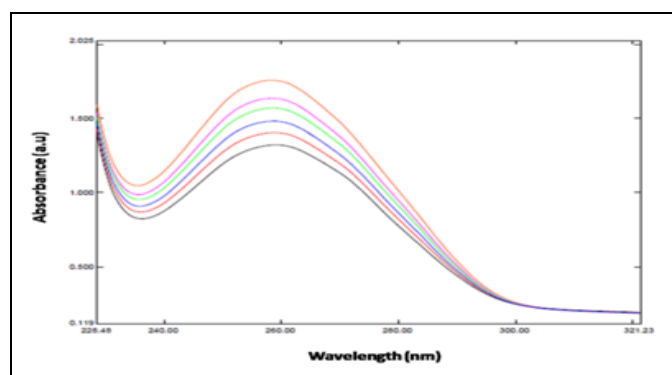


**FIG. 6: ANTIOXIDANT ASSAY OF LIGAND AND ITS METAL(II) COMPLEXES**

**DNA- Copper Complex Interaction Studies:** The interactions of CT-DNA and metal complexes were determined by the electronic absorption spectroscopy technique. From the studies, it is revealed that the intensity changes of the intraligand  $\pi \rightarrow \pi^*$  transition band occur at 250-280 nm<sup>46</sup>. The UV absorption experiments of Cu(II) complex in the presence of buffer solution are performed by using a fixed concentration of



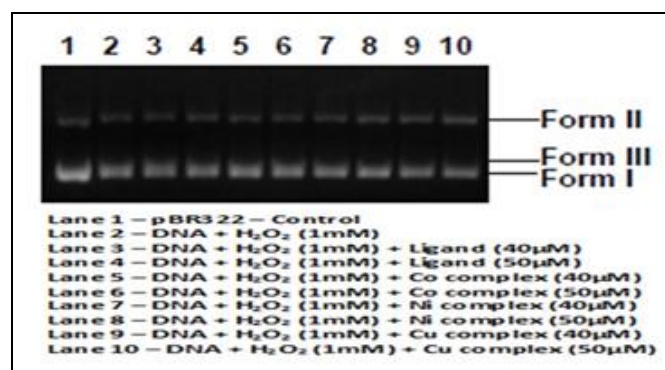
complex to which increments of the stock solution was added. The interaction of  $[\text{Cu}_2(\text{C}_{46}\text{H}_{42}\text{N}_6\text{O}_8\text{Cl}_2)]$  complex with duplex DNA led to a decrease in the intensities, and a small amount of redshift in the electronic absorption spectra and the concentration of DNA increases with the absorption bands of Cu(II) complex are affected to a considerable extent. This absorption spectra indicate clearly that the addition of CT-DNA to the Cu(II) complex yields hypochromic and redshift (shown in **Fig. 7**) to the ratio of  $[\text{DNA}]/[\text{Cu}]$  for the  $[\text{Cu}_2(\text{C}_{46}\text{H}_{42}\text{N}_6\text{O}_8\text{Cl}_2)]$  complex and the complex interact with CT-DNA most likely through a binding mode that involves  $\pi \rightarrow \pi^*$  stacking interaction between the aromatic chromophore and the base pair of DNA.



**FIG. 7: ABSORPTION SPECTRA OF Cu(II) COMPLEX WITH DIFFERENT CONCENTRATIONS (100, 120, 1140, 160, 180, 200  $\mu\text{L}$ )  $\text{H}_2\text{O}_2$  INDUCED DNA DAMAGE/ PRODUCTION ASSAY**

Gel electrophoresis activity is a method to determine different binding modes of newly synthesized complexes to supercoiled pBR322 DNA. The natural- derived plasmid pBR322 DNA has three forms of the closed-circle supercoiled form (form-I), nicked form (form-II), and linear form (form-III) <sup>47, 48</sup>. The circular plasmid DNA is conducted by electrophoresis; relatively, the fastest migration will be measured for the supercoiled form (form-I). If cleavage occurs on one strand, the supercoils will relax to generate slowed moving

open circular form (form-II). If both strands are cleaved a form-III (linear form), it will be produced that moves in between supercoiled form and open circular form. From the results (in **Fig. 8**) it indicates that the electrophoretic gel separation of plasmid pBR322 DNA, interaction with metal complexes in the presence of  $\text{H}_2\text{O}_2$ . The addition of the complexes with a mixture of form-II and form-III, which form the cleavage of supercoiled DNA. The reports show that the Co(II), Ni(II), and Cu(II) metal complexes induced the cleavage of DNA intensively in the presence of  $\text{H}_2\text{O}_2$ . These observations suggested that all dinuclear complexes effectively cleave the plasmid pBR322 DNA.



**FIG. 8: DNA CLEAVAGE STUDIES OF SCHIFF BASE AND ITS METAL(II) COMPLEXES**

**In-vitro Cytotoxicity Assay:** To determine the cytotoxicity effect, the newly synthesized homo binuclear Cu(II) complex was treated with the human breast cancer cell line (MCF-7) by MTT experiments method <sup>49</sup>. The absorbance value is lower than the control cell, which shows a reduction in the rate of cell proliferation. Contrary, a higher absorbance rate show an increase in cell proliferation cell survival is almost 50% after 24 hrs of incubation with Cu(II) complex. The anticancer results of complex revealed that Cu(II) complex exhibits significant cytotoxic effect. The  $\text{IC}_{50}$  value and percentage of inhibition of Cu(II) complex are given in **Table 2**.

**TABLE 2: ANTITUMOUR ACTIVITY OF Cu(II) COMPLEX**

Compound name	Concentrations ( $\mu\text{g}/\text{mL}$ )	Absorbance 590nm	Toxicities (%)	$\text{IC}_{50}$ ( $\mu\text{g}/\text{mL}$ )
Control (MCF-7)	0	0.858	0.00	
	10	0.810	14.06	
Cu(II) complex	20	0.719	25.57	
	40	0.504	41.21	53.7
	80	0.392	54.04	
	160	0.252	70.73	
	320	0.138	83.56	

**CONCLUSION:** Macrocyclic homo binuclear Co(II), Ni(II), and Cu(II) complexes derived from terephthalaldehyde with 2-amino 4-chlorophenol and 2-amino 4-nitrophenol. The asymmetrical tetra dentate Schiff base and its binuclear Co(II), Ni(II) and Cu(II) complexes were prepared, evaluated by physicochemical experiments and diverse spectroscopic methods.

The spectral studies indicate that all metal(II) complexes are four coordinated and possess square planar geometry around the metal ion. The biological applications of antibacterial, antioxidant, and DNA cleavage assay of the metal(II) complexes show more inhibition activity than the free ligand. The Cu(II) complex has considerable anticancer and DNA binding abilities.

**ACKNOWLEDGEMENT:** The authors are thankful to SAIF, IIT Bombay for ESR Analysis, SAIF, IIT Madras for mass analysis, St. Joseph's College, Trichy for IR analysis, Sastra Deemed University, Thanjavur for Thermal analysis. The authors are very grateful to the Guide and Principal of Kandaswami Kandar's College, P. Velur, Namakkal (Dt), for providing facilities to perform the work.

**CONFLICTS OF INTEREST:** The authors declare that there are no conflicts of interest regarding the publication of this research paper.

## REFERENCES:

1. Savithri K and Revanasiddappa HD: Synthesis and Characterization of Oxidovanadium(IV) Complexes of 2-((E)-(6-Fluorobenzo[d]Thiazol-2-ylimino)methyl)-6-methoxy phenol and Their Antimicrobial, Antioxidant and DNA Binding Studies. *Bioinorg Chem Appl* 2018; 1-12.
2. Barfeie H, Grivani G, Eigner V, Dusek M and Khalaji AD: Copper(II), Nickel(II), Zinc(II) and Vanadium(IV) schiff base complexes: synthesis, characterization, crystal structure determination and thermal studies. *Polyhedron* 2018; 1-18.
3. Arumugam AP, Guhanathan S and Elango G: Co(II), Ni(II) & Cu(II) complexes with schiff base ligand: synthesis, characterization, antimicrobial studies and molecular docking studies. *SOJ Master Sci Eng* 2017; 5(2): 1-12.
4. Hossain MS, Roy PK, Zakaria C and Kudart-E-Zahan M: Selected schiff base coordination complexes and their microbial application: a review. *Int J Chem Studies* 2018; 6(1): 19-31.
5. Sahoo J and Paidsetty SK: Antimicrobial activity of novel synthesized coumarin based transitional metal complexes. *J Taibah Univer Med Sci* 2016; 115-24.
6. Lozana V, Looseb C, Kortusb J and Kerstinga B: Coordination chemistry of robinson-type polyamine-dithiophenolate macrocycles: syntheses, structures and magnetic properties of dinuclear complexes of first-row transition metals. *Coord Chem Rev* 2009; 253: 2244-60.

7. Aidi M, Keypour H, Shooshtari A, Mahmoudabadi M, Bayat M, Ahmadvand Z, Karamian R, Asadbegy M, Tavatli S and Gable RW: Synthesis of two new symmetrical homopiperazine moiety and their mononuclear complexes: spectral, characterization, x-ray crystal structural, antibacterial activity, antioxidant effects and theoretical studies. *Polyhedron* 2019; 1-38.
8. Boghaei DM and Mohebi S: Non-symmetrical tetradentate vanadyl schiff base complexes derived from 1,2-phenylene diamine and 1,3-naphthalene diamine as catalysts for the oxidation of cyclohexene. *Tetrahedron* 2002; 58: 5357-66.
9. Malathy M and Rajavel R: Preparation, spectral characterization, antimicrobial and DNA cleavage studies of macrocyclic metal (II) complexes. *Int J Pharm Res Scholars (IJPRS)* 2014; 3: I-1.
10. Anuradha K and Rajavel R: *In-vitro* antibacterial studies of ligand and their metal complex: *Int J Res Inorg Chem* 2012; 1(1): 1-4.
11. Niraj K, Kumar YV, Stanislav Z and Lallan M: Pd(II) catalyzed transformation of schiff bases in complex of the type trans-[PdCl<sub>2</sub>(NH<sub>2</sub>Ar-X)<sub>2</sub>] (X=H, CH<sub>3</sub>, Cl): Reactivity with aldehydes and Heck coupling reaction: *Indian J Chem* 2012; 51A: 554-63.
12. Ahlawat A, Asija S and Singh N: Synthesis, characterization and antimicrobial activities of Organotin (IV) complexes of schiff bases derived from 2,3-diaminopyridine: *Asian J Chem* 2017; 29(3): 669-74.
13. Devi J, Devi S and Kumar A: Synthesis, antibacterial evaluation and QSAR analysis of schiff base complexes derived from [2,2-(ethylenedioxy)bis(ethylamine)] and aromatic aldehydes: *Med Chem Commun* 2016; 7: 932-47.
14. Baul TSAB, Singh KS, LycKa A, HolcApek M and Linden A: Synthesis of a cyclic dinuclear organotin carboxylate via simultaneous debenzoylation and decarbonylation reactions: X-Ray crystal structure of [(Phch<sub>2</sub>)<sub>2</sub>{O<sub>2</sub>C<sub>6</sub>H<sub>4</sub>{N(H)-N=(C<sub>6</sub>H<sub>3</sub>-4(=O)-5-O)-O}Sn]<sub>2</sub>. *J Organomet Chem* 2005; 690: 1581-87.
15. Berhanu AL, Gaurav, Mohiuddin I, Malik AK, Aulakh JS, Kumar V and Kim KH: A review of the applications of schiff bases as optical chemical sensors. *Trends in Analytical Chem* 2019; 166: 74-91.
16. Zoubi WA, Mohamed SG, Al-Hamdani AAS, Mahendradhany AP and Ko YG: Acyclic and cyclic imines and their metal complexes: recent progress in biomaterials and corrosion applications: *RSC Adv* 2018; 8: 23294-18.
17. Balskue PE and Walsh CT: The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. *Science* 2010; 329: 1653-56.
18. Yousefia M, Safari M, Torbati MB, Kazemiha VM, Sanatic H and Amanzadeh A: New Mononuclear Diorganotin(IV) Dithiocarboxylates: synthesis, characterization and study of their cytotoxic activities. *Appl Organomet Chem* 2012; 26: 438-44.
19. Hossain MS, Banu LA, E-Zahan MK and Haque MM: Synthesis, characterization and biological activity studies of mixed ligand complexes with schiff base and 2,2'-bipyridine. *Appl Sci Res Rev* 2019; 6(1): 1-7.
20. Palanimurugan A, Dhanalakshmi A, Selvapandian P and Kulandaisamy A: Electrochemical behavior, Structural, Morphological, Calf Thymus-DNA interaction and in-vitro antimicrobial studies of synthesized Schiff Base Transition Metal Complexes: *Heliyon* 2019; 1-10.
21. Verasantharam M, Manikandamathavan, Parameswari RP, Weyhermüller T, Vasanthi HR and Nair BU: Cytotoxic Copper (II) mixed ligand complexes: crystal structure and DNA cleavage activity. *Eur J Med Chem* 2011; 46: 4537-47.
22. Naeimi H, Rabiei K and Salimi F: Template synthesis of some double Schiff-base metal(II) complexes through one pot four component reactions under mild and convenient conditions: *J Coord Chem* 2009; 62(7): 1199-05.
23. Jayaseelan P, Prasad S, Vedanayaki S and Rajavel R: Synthesis, spectral characterization, electrochemical and

- anti-microbial activities of new binuclear Schiff base metal complexes derived from 3,3' diaminobenzidine. *Eur J Chem* 2011; 2(4): 480-84.
24. Kavitha P, Saritha M and Reddy KL: Synthesis, structural characterization, fluorescence, antimicrobial, antioxidant and DNA cleavage studies of Cu(II) complexes of formyl chromone Schiff bases: *Spectrochimica Acta Part A* 2012; 102: 159-68. Srinivasulu K, Reddy KH, Anuja K, Dhanalakshmi D and Ramesh G: DNA binding properties and antibacterial activity of heterolytic transition metal complexes with 2,2-bipyridyl and 2-acetylthiophene thiosemicarbazone. *Asian J Chem* 2019; 31(9): 1905-12.
  25. Jayaseelan P, Akila E, Rani MU and Rajavel R: Synthesis, spectral characterization, electrochemical: antimicrobial, DNA binding and cleavage studies of new binuclear schiff base Metal(II) complexes derived from o-hydroxy-acetophenone: *Journal of Saudi Chemical Society* 2016; 20: 625-34.
  26. Domotor O, de Almeida RFM, Corte-Real L, Matos CP, Marques F, Matos A, Real C, Kiss T, Enyedy EA, Garcia MH and Tomaz AI: Studies on the mechanism of action of antitumor bis(aminophenolate) ruthenium(III) complexes: *J Inorg Biochem* 2016; 168: 27.
  27. Khedr AM and Marwani HM: Synthesis, spectral, thermal analyses and molecular modeling of bioactive Cu(II)-complexes with 1,3,4-thiadiazole schiff base derivatives. their catalytic effect on the cathodic reduction of oxygen. *Int J Electrochem Sci* 2012; 7(10): 10074-93.
  28. Pallikkavil R, Ummathur MB and Krishnankutty K: Schiff bases of terephthalaldehyde with 2-aminophenol and 2-aminothiophenol and their metal complexes. *Arch Appl Sci Rese* 2012; 4(5): 2223-27.
  29. Singh NP, Tyagi VP and Ratnam B: Synthesis and spectroscopic studies of tetradentate schiff base complexes of Cu(II), Ni(II), Mn(II) and Co(II). *J Chem Pharm Res* 2010; 2(1): 473-77.
  30. Abdalrazaq EA, Al-Ramadane OM and Al-Numa KS: Synthesis and characterization of dinuclear metal complexes stabilized by tetradentate schiff base ligands: *Am J Appl Sci* 2010; 7(5): 628-33.
  31. Thakor YJ, Patel SG and Patel KN: Synthesis, characterization and biocidal studies of some transition metal complexes containing tetra dentate and neutral bi dentate schiff base. *Journal of Chemical and Pharmaceutical Research* 2010; 2(5): 518-25.
  32. Halli MB, Reddy PV, Sumathi RB and Basavaraja A: Synthesis, spectral characterization and Antimicrobial studies of Metal (II) complexes with (12E)-N'-[1-(2-oxo-2H-chromen-3-yl)ethylidene]benzofuran carbohydrazide: *Der Pharma Chemica* 2012; 4(3): 1214-25.
  33. Shebl M, Khalil SME, Ahmed SA and Medien HAA: Synthesis, spectroscopic characterization and antimicrobial activity of mono-, bi- and tri-nuclear metal complexes of a new Schiff base ligand: *J Mol Struct* 2010; 980: 39-50.
  34. Patil AR, Donde KJ, Raut SS, Patil VR and Cokhande RS: Synthesis, spectral and antimicrobial studies on mixed ligand Cu(II) complex of schiff base 2-amino-4-nitrophenol-n-allylidene and some amino acids. *J Pharm Res* 2011; 4(7): 2256-60.
  35. Pawar RK, Sakhare MA and Arbad BR: Synthesis, spectroscopic characterization and thermal studies of some transition metal complexes of novel schiff base ligand. *Int J Chem Sci* 2016; 14(4): 2575-84.
  36. Kumaran JS, Priya S, Jayachandramani N and Mahalakshmi S: synthesis, spectroscopic characterization and biological activities of transition metal complexes derived from a tridentate Schiff base. *Journal Chem* 2013: 1-10.
  37. Sakthi M and Ramu A: Synthesis, structure, DNA/BSA binding and antibacterial studies of NNO tridentate Schiff base metal complexes: *J Mole Struct* 2017; 1149: 727-35.
  38. Patil AR, Donde KJ, Raut SS, Patil VR and Cokhande RS: Synthesis, characterization and Biological Activity of Mixed Ligand Co(II) Complexes of Schiff Base 2-Amino-4-Nitrophenol-N-allylidene with Animo Acids: *J Chem Pharm Res* 2012; 4(2): 1413-25.
  39. Vamsikrishna N, Kumar MP, Ramesh G, Ganji N, Daravath S and Shivaraj: DNA interactions and biocidal activity of metal complexes of benzothiazole Schiff bases: synthesis, characterization and validation. *J Chem Sci* 2017; 129(5): 609-22.
  40. Prashanthi Y and Raj S: Synthesis and characterization of transition metal complexes with N,O; N,N and S,N-donor schiff base ligands. *J Sci Res* 2010; 2 (1): 114-126.
  41. Biradar VD and Mruthyunjayaswamy BHM: Synthesis characterization and antimicrobial activity studies of some transition metal complexes derived from 3-chloro-n'-(1e)-(2-hydroxy phenyl)methylene]-6-methoxy-1-benzothio-phene-2-carbohydrazide. *Scientific World Journal* 2013; 1-13.
  42. Raman N, Sobha S and Mitu L: Design, synthesis, DNA binding ability, chemical nuclease activity and antimicrobial evaluation of Cu(II), Co(II), Ni(II) and Zn(II) metal complexes containing tridentate Schiff base: *J Saudi Chem Soc* 2013; 17(2): 151-59.
  43. Osowole AA, Ekennia AC and Achugbu BO: Synthesis, spectroscopic characterization and antibacterial properties of some metal(II) complexes of 2-(6-methoxy-benzothiazol-2-ylimino)methyl)-4-nitrophenol: *Res Rev J Phar Analysis* 2013; 2(2): 1-5.
  44. Colak, Terzi U, Col M, Karaoglu SA, Karabocek S, Kuçukdumlu A and Ayaz FA: DNA binding, antioxidant and antimicrobial activities of homo- and heteronuclear copper(II) and nickel(II) complexes with new oxime-type ligands: *Eur J Med Chem* 2010; 45(11): 5169-75.
  45. Hu K, Liu C, Li J and Liang F: Copper(II) complexes based on quinoline derived Schiff-base ligands: synthesis, characterization, HSA/DNA binding ability, and anticancer activity: *Med Chem Commun* 2018; 9(10): 1663-72.
  46. Akila E, Usharani M and Rajavel R: Inspired research on the DNA binding and cleavage ability: design, synthesis, characterization and properties of their dinuclear Metal (II) complexes. *Int J Bio-Tech Res* 2013; 3(2): 61-70.
  47. Raman N, Raja JD and Sakthivel A: Synthesis, spectral characterization of schiff base transition metal complexes: DNA cleavage and antimicrobial activity studies: *J Chem Sci* 2007; 119(4): 303-10.
  48. Marques MPM, Girao T, Pedrosa De Lima MC, Gameiro A, Pereira E and Garcia P: Cytotoxic effects of metal complexes of biogenic polyamines. I. Platinum(II) spermidine compounds : prediction of their antitumour activity: *Biochem Biophys Acta* 2002; 1589: 63-70.

**How to cite this article:**

Savitha K and Vedanayaki S: Synthesis, spectral elucidation, antibacterial, antioxidant and DNA studies of onno tetradentate schiff base metal(II) complexes derived from 2-aminophenol derivatives. *Int J Pharm Sci & Res* 2020; 11(9): 4513-23. doi: 10.13040/IJPSR.0975-8232.11(9).4513-23.

All © 2013 are reserved by the International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)