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Synthesis, Characterization, Antioxidant and Antimicrobial Evaluation of a Series of Co(II), Ni(II), Cu(II) and Zn(II) Metal Complexes with Bidentate Schiff base : 4-[{3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene}-amino]-3-mercapto-6-methyl-5-oxo 1,2,4-triazine

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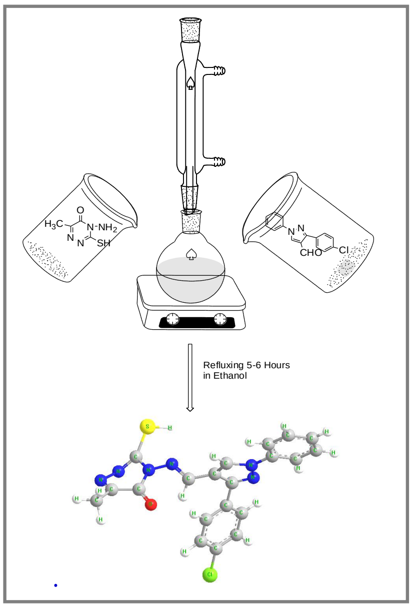
ABSTRACT

Some new metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Schiff base derived from 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine with 3-(p-chlorophenyl)-1-phenyl-1H-pyrazole carboxaldehyde were synthesized and characterized by elemental analysis, spectroscopic techniques (IR, ^{1}H-NMR, Electronic, Fluorescence, ESR), cyclic voltammetry, magnetic moment measurements, thermal studies and kinetic calculations. With the help of above mentioned techniques an octahedral geometry for Co(II), Ni(II) and Zn(II) complexes and a square planar geometry of Cu(II) complexes have been proposed. Conductance measurements suggest non-electrolytic nature of metal complexes. The ligand and its metal complexes were screened in vitro for antibacterial and antifungal activities. Also, in vitro antioxidant activity of ligand and its metal complexes was assayed by radical scavenging activity (DPPH).

Graphical Abstract



Keywords: Metal complexes, Antifungal, Antibacterial, Fluorescent, Antioxidant.

INTRODUCTION

The pyrazole motif makes up the middle shape of several biologically energetic compounds. Pyrazoles are significant medicinal scaffolds and show a full spectrum of biological importance [1, 2]. Many substituted pyrazole derivatives are recognized to possess a wide range of biological activities like antimicrobial, anti-inflammatory, antitubercular etc [3-5]. There are many pyrazole derivatives which are evolved through linking pyrimidine, carboxyhydrazide molecules with pyrazole moiety and are especially effective towards lungs cellular carcinoma [6, 7].

On the other hand, triazine derivatives have obtained full attention because of their mighty organic interest which includes anticancer [8], antivirals [9]. It has been suggested that triazine derivatives possess strong antimicrobial activity [10]. There are many N, S donor triazines whose activity may be greatly enhanced by using the presence of extra donor sites and a diffusion of coordination modes can be proven via these systems. Potential ligands can be formed by attaching different triazines with aldehyde compounds having a heterocyclic moiety. When such bioinorganic molecules are bonded to metal ions, it has been observed that there is drastic modification in their pharmacological properties such as 3d metal complexes of some Schiff bases play important role in liquid crystal study [11], in biological field like antimicrobial, antifungal and antibacterial agents [12, 13]. Many Zn(II) metal complexes of Schiff bases are known as fluorescent materials, sensors and also possess biological importance [14]. Keeping in mind the biological and medicinal importance of triazines, pyrazoles and their compatibility with metal ions, we consider it essential to join the properties of both moieties with metals, in order to design new Schiff base metal complexes. Considering the forgoing facts and our continuous work on triazine based metal complexes, herein we report synthesis, spectral characterization, thermal and magnetic studies, antioxidant and biological importance of 4-[{3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-ylmethylene}-amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine and its metal complexes with cobalt, nickel, copper and zinc metal ions.

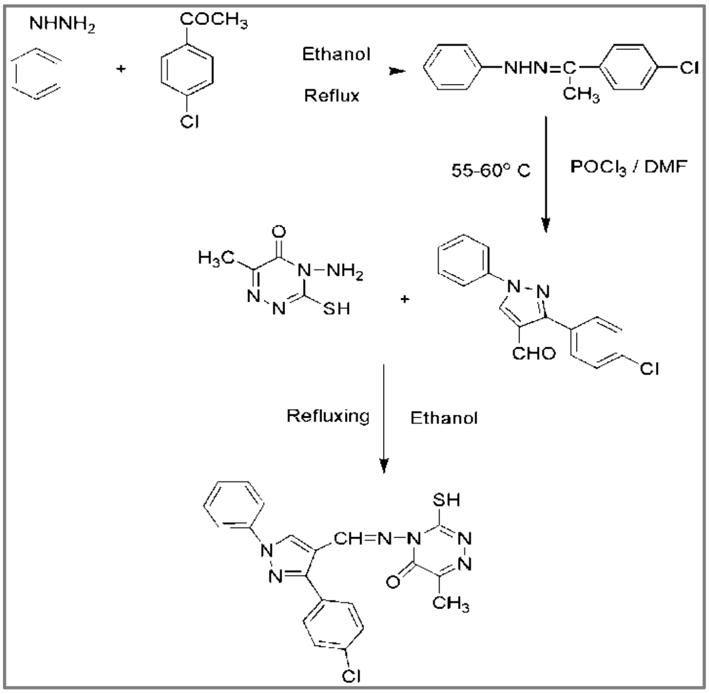
MATERIALS AND METHODS

All chemicals were of analytical grade and were used without any further purification. Elemental analyses (C, H and N) were performed on a Thermo Scientific (Flash 2000) CHN Elemental Analyzer. IR spectra of the ligand and its metal complexes were recorded on a MB 3000 ABB Spectrophotometer in KBr pellets. Far IR (700-30 cm^{-1}) spectra of the metal complexes were recorded on a Perkin Elmer Spectrophotometer at SAIF PU, Chandigarh. The molar conductance measurements were made using Alico conductivity meter with a dip type cell (cell constant = 1 cm^{-1}) using approximately 10^{-3} M solutions of the complexes in DMF. ^{1}H-NMR spectra of ligand and its Zn(II) chelates were recorded in DMSO on a BRUKER 400 MHz spectrometer using “tetramethylsilane” as internal reference. Fluorescence spectra were obtained on SHIMADZU RF-5301 PC spectrofluorometer. The UV-Visible absorption spectra of metal complexes were recorded in DMSO on T 90 (PG Instruments Ltd.) UV-Vis spectrometer in the region 900-190 nm. Magnetic moments were measured using mercury (tetrathiocyanato) cobaltate as the standard on Vibrating Sample Magnetometer at the Institute Instrumentation Centre, IIT Roorkee. Thermal analysis of metal complexes were carried out in nitrogen atmosphere over the temperature range 40-800°C on the Perkin Elmer (Pyris Diamond) instrument at a heating rate of 10°C min^{-1} by using alumina as reference. X-Band ESR spectra of Cu complexes were obtained under the magnetic field 0.3 Tesla at frequency 9.1 GHz by using Varian E-112 ESR spectrometer at SAIF, IIT Bombay. Ivium Stat Electrochemical Analyzer was used to record the cyclic voltammetry measurements of Cu(II) complexes using TBAP as a supporting electrolyte. Gravimetric analyses were performed to find the metal contents in metal complexes; cobalt was estimated as cobalt pyridine thiocyanate, nickel as nickel dimethylglyoximate, copper as cuprous thiocyanate and zinc as zinc ammonium phosphate [15].

Synthesis

Synthesis of Ligand: 3-(p-chlorophenyl)-1-phenyl-1H-pyrazolecarboxaldehyde [16] and 4-amino-3-mercapto-6-methyl-5-oxo-1, 2, 4-triazine (AMMOT) were synthesized using reported method [17].

(4-[{3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene}-amino]-3-mercapto-6-methyl-5-oxo -1,2,4-triazine (HL) (Scheme 1): To 20 ml hot ethanolic solution of 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (0.57 g, 3.6 mmol), 20 ml ethanolic solution of 3-(p-chlorophenyl)-1-phenyl-1H-pyrazolecarboxaldehyde (1.02 g, 3.6 mmol) was added with constant stirring and then refluxed for 5 hours. After cooling, cream color product was separated out. It was washed with cooled ethanol, recrystallized from same solvent and then dried. $C_{20}H_{15}ClN_6OS$, Calcd. C; 56.80% (found 56.34%), H; 3.57% (found 3.19%), N; 19.89% (found 19.90%). Color: Cream, Yield: 90%.



Scheme 1. Synthesis of Schiff Base i.e. (4-[{3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene}-amino]-3-mercapto-6-methyl-5-oxo-1, 2, 4-triazine (HL).

Synthesis of Metal Complexes

Synthesis of 1:1 metal complexes of HL: To a solution of Schiff base (HL) (0.2 g, 0.47 mmol) in ethanol (20 ml), ethanolic solutions of acetates of Co(II) (0.118 g, 0.47 mmol), Ni(II) (0.118 g, 0.47 mmol), Cu(II) (0.094 g, 0.47 mmol) and Zn(II) (0.104 g, 0.47 mmol) were added separately and refluxed for an hour on a magnetic stirrer with heating. The colored amorphous compounds were filtered off, washed with ethanol, followed by acetone and dried in desiccator over anhydrous calcium chloride.

$Co(L)OAc.3H_2O$: $C_{22}H_{23}ClN_6O_6SCo$, Calcd. C; 44.49% (found 44.31%), H; 3.91% (found 3.58%), N; 14.15% (found 14.10%), Co; 9.93% (found 9.18%). Color: Brown, Yield: 76%, Λ_m (Scm^2mol^{-1}): 45.34.

$Ni(L)OAc.3H_2O$: $C_{22}H_{23}ClN_6O_6SNi$, Calcd. C; 44.50% (found 44.36%), H; 3.88% (found 3.58%), N; 14.16% (found 14.11%), Ni; 9.90% (found 9.14%). Color: Yelloish green, Yield: 88%, Λ_m (Scm^2mol^{-1}): 53.68.

Cu(L)OAc.H_2O: $C_{22}H_{19}ClN_6O_4SCu$, Calcd. C; 46.98% (found 46.48%), H; 3.38% (found 3.09%), N; 14.94% (found 14.77%), Cu; 11.31% (found 11.09%). Color: Olive green, Yield: 83%, Λ_m (Scm^2mol^{-1}): 50.64.

Zn(L)OAc.3H_2O: $C_{22}H_{23}ClN_6O_6SZn$, Calcd. C; 44.10% (found 44.90%), H; 3.83% (found 3.53%), N; 14.00% (found 13.99%), Zn; 10.90% (found 10.07%). Color: Pale yellow, Yield: 71%, Λ_m (Scm^2mol^{-1}): 68.96.

Synthesis of 1:2 metal complexes of HL: 1:2 metal complexes of ligand (HL) were synthesized by refluxing hot ethanolic solutions of acetates of Co(II) (0.118 g, 0.47 mmol), Ni(II) (0.118 g, 0.47 mmol), Cu(II) (0.094 g, 0.47 mmol) and Zn(II) (0.104 g, 0.47 mmol) with hot ethanolic solutions of HL (0.4 g, 0.94 mmol) for an hour on a magnetic stirrer. The colored amorphous compounds were filtered off, washed with ethanol, followed by acetone and dried in desiccator over anhydrous calcium chloride.

$Co(L)_2$.2H_2O: $C_{40}H_{32}Cl_2N_{12}O_4S_2Co$, Calcd. C; 51.18% (found 51.69%), H; 3.44% (found 3.10%), N; 17.91% (found 17.29%), Co; 6.28% (found 6.18%). Color: Brown, Yield: 82%, Λ_m (Scm^2mol^{-1}): 14.13

$Ni(L)_2$.2H_2O: $C_{40}H_{32}Cl_2N_{12}O_4S_2Ni$, Calcd. C; 51.19% (found 51.70%), H; 3.41% (found 3.08%), N; 17.92% (found 17.28%),Ni; 6.26% (found 6.11%). Color: Yelloish green, Yield: 86%, Λ_m (Scm^2mol^{-1}): 16.57

$Cu(L)_2$: $C_{40}H_{28}Cl_2N_{12}O_2S_2Cu$, Calcd. C; 52.95% (found 52.21%), H; 3.09% (found 2.98%), N; 18.53% (found 17.97%), Cu; 7.00% (found 6.91%). Color: Olive green, Yield: 84%, Λ_m (Scm^2mol^{-1}): 14.76

$Zn(L)_2$.2H_2O: $C_{40}H_{32}Cl_2N_{12}O_4S_2Zn$, Calcd. C; 50.83% (found 50.40%), H; 3.39% (found 3.05%), N; 17.79% (found 17.19%), Zn; 6.92% (found 6.27%). Color: Pale yellow, Yield: 72%, Λ_m (Scm^2mol^{-1}): 10.83

Antimicrobial Assay: All newly synthesized compounds were checked in vitro for their antimicrobial activity against some bacteria and fungi. The microorganisms involved were *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative) and *Candida albicans*, *Saccharomyces cerevisiae* (Fungi) by using the agar diffusion method [18]. The potential of the tested compounds was compared to that of Ciprofloxacin and Amphotericin-B.

In Vitro Antimicrobial Activity: Agar well diffusion method was used to screen newly synthesized compounds, 0.1 mL of dilute inoculum (10^5 CFU mL^{-1}) of test microorganism was spread on Sabouraud Dextrose (SD) agar plates. Wells of 8 mm were punched into the agar medium using sterile cork borer and then filled with 100 µL volume with concentration of 4.0 mg mL^{-1} of each compound reconstituted in dimethyl sulphoxide (DMSO). The plates were kept for incubation for 18 h at 37°C. Antimicrobial activity was checked by measuring the zone of inhibition against the test microorganism. DMSO was used as negative control and Ciprofloxacin and Amphotericin-B were used as positive control.

Minimum Inhibitory Concentration (MIC): The lowest concentration of an antimicrobial agent which will inhibit the visible growth of a microorganism is known as MIC. Most appropriate methods for determining MIC are dilution methods. Herein, MIC of all the compounds against microorganism strains was tested through agar well diffusion technique [19]. In this technique, a twofold serial dilution of every compound was prepared by initial reconstituting the compound in DMSO followed by dilution in sterile water to attain a decreasing concentration vary of 256 to 0.5 µg mL^{-1}. A 100 mL volume of every dilution was introduced into wells (in triplicate) within the agar plates already seeded with a 100 µL of standardized inoculums (10^6 CFU mL^{-1}) of the test microbial strain. All plates were incubated aerobically at 37°C for18 h and determined for the inhibition zones.

Antioxidant Activity: Scavenging activity of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Radical is a rapid technique for studying antioxidant properties of synthesized compounds [20]. DPPH radical absorbs visible radiations strongly at 517 nm and on reacting with antioxidants, absorbance gets decreasing. Solutions of Schiff base and its 1:2 metal complexes (Co(II), Ni(II), Cu(II), Zn(II)) of various concentration (100, 200, 300, 400, 500 µg mL^{-1}) were prepared in DMF and then added to 0.4 mM methanolic solution of DPPH (3 mL) separately. All mixed solutions were stirred thoroughly and kept at room temperature for about 30 min in dark place to complete the reaction and then checked the scavenging activity of all compounds by measuring the decrease in absorbance of DPPH at 517 nm. Percentage of scavenging ability of samples was also calculated using equation (1) by observing the absorbance of blank DPPH solution. The experiment for each concentration was carried out in triplicate and average values were calculated.

$$\% \text{ of scavenging ability} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \qquad ..(1)$$

Abs$_{\text{control}}$ -absorbance of DPPH solution without compounds, Abs$_{\text{sample}}$ -absorbance of DPPH solution with compounds.

RESULTS AND DISCUSSION

New synthesized metal complexes are found to be soluble in N,N-dimethylformamide, dimethyl, sulfoxide and acetonitrile but partially soluble in common organic solvents like ethanol, methanol and chloroform. The molar conductance values in DMF (10^{-3} M) have been observed in the range (10.83-68.96) Scm^2 mol^{-1} indicating the non-electrolytic nature of metal complexes. All the complexes are colored and get decomposed at high temperature.

IR Spectral Studies: The characteristic IR frequencies for the Schiff base and its metal complexes were obtained over a spectral range 4000-400 cm^{-1} and are tabulated in table 1. The Schiff base shows ν (-CH=N) azomethine band at 1605 cm^{-1}. On complexation, this band got shifted to lower frequency values in metal complexes as shown in table 1, which is indication of coordination of azomethine nitrogen to metal ions [21]. A broad band in the region 3300-3770 cm^{-1} in metal complexes is attributed to presence of coordinated water molecules. A new band in spectra of metal complexes appeared at 753-759 cm^{-1} due to ν (C-S) [22]. In 1:1 metal complexes, a characteristic band observed in the region 1740-1744 cm^{-1} may be assigned to ν (OCOCH$_3$). In far infrared region, all complexes exhibit bands at 490-499 cm^{-1} and 318-340 cm^{-1} due to ν (M-N) and ν (M-S) modes respectively [23]. Free ligand also exhibits a weak band at ~2860 cm^{-1} due to ν (SH) vibrations, disappeared in metal complexes on coordination through sulphur indicating deprotonation [12].

Table 1. IR frequencies (cm^{-1}) of Ligand and its metal complexes

Compounds	ν(N=CH)	ν(C-S)	ν(S-H)	ν(OCOCH$_3$)	ν(H$_2$O/OH)	ν(M-S)	ν(M-N)
HL	1605	--	2860	--	--	--	--
Co(L)(OAc).3H$_2$O	1595	759	--	1744	3618	340	490
Co(L)$_2$.2H$_2$O	1590	756	--	--	3652	341	495
Ni(L)(OAc).3H$_2$O	1598	756	--	1740	3495	342	499
Ni(L)$_2$.2H$_2$O	1597	756	--	--	3549	341	490
Cu(L)(OAc).H$_2$O	1591	753	--	1744	3770	325	495
Cu(L)$_2$	1597	756	--	--	--	323	495
Zn(L)(OAc).3H$_2$O	1559	756	--	1740	3610	342	497
Zn(L)$_2$.2H$_2$O	1561	758	--	--	3300	342	499

^{1}H-NMR spectral data of Schiff base and its Zn(II) metal complexes: ^{1}H-NMR spectra of ligand and its zinc metal complexes were recorded in DMSO-d_6. The signal observed at δ 13.67 ppm is assigned to (–SH) proton in ligand, disappeared in Zn(II) complexes which indicates coordination of the sulphur atom of thiol (-SH) group to zinc metal [24]. The spectrum of the ligand shows a signal at δ 9.31 ppm which is attributed to the azomethine proton (-CH=N-). The azomethine proton peak of the 1:1 and 1:2 Zn (II) complexes got shifted to downfield region as compared to the free ligand, suggesting the coordination of azomethine nitrogen with the metal ion [25].

^{1}H-NMR spectrum of ligand (HL) exhibits signals at δ8.67 (s, 1H, -C**H**=N-), 9.31 (s, 1H, pyrazole-**H**), 8.03-8.05 (d, 2H, Ar-**H**), 7.43-7.46 (t, 1H, Ar-**H**), 7.54-7.58 (m, 4H, Ar-**H**), 7.86-7.88 (d, 2H, Ar-**H**), 2.18 (s, 3H, -C**H**$_3$), 13.67 (br-s, 1H, -S**H**).

^{1}H-NMR spectrum of Zn(L)(OAc).3H$_2$O exhibits signals at δ8.70 (s, 1H, -C**H**=N-), 9.30 (s, 1H, pyrazole-**H**), 8.03-8.05 (d,2H, Ar-**H**), 7.42-7.46 (t, 1H, Ar-**H**), 7.54-7.60 (m, 4H, Ar-**H**), 7.88-7.90 (d, 2H,Ar-**H**), 1.92 (s, 3H, -C**H**$_3$), 1.28 (s, 3H,-OCOC**H**$_3$), 4.00 (s, 4H, **H**$_2$O).

^{1}H-NMR spectrum of Zn(L)$_2$.2H$_2$O exhibits signals at δ8.71 (s, 2H, -C**H**=N-), 9.31 (s, 2H, pyrazole-**H**), 8.03-8.05 (d, 4H, Ar-**H**), 7.86-7.90 (m, 4H,Ar-**H**), 7.42-7.46 (t, 2H, Ar-**H**), 7.54-7.58 (m, 8H, Ar-**H**), 2.12 (s, 6H, -C**H**$_3$), 4.00 (s, 4H, **H**$_2$O).

Visible Absorption Spectral Studies: Visible absorption spectra of synthesized metal complexes were recorded in DMSO (10^{-3} M) as solvent at 25°C. Electronic spectral frequencies and ligand field parameters of cobalt and nickel metal complexes are shown in table 2.

Table 2. Electronic spectral data of octahedral Co(II) and Ni(II) Complexes in DMSO solution

Compounds	ν(cm^{-1})	Band Assignment	D$_q$ (cm^{-1})	B(cm^{-1})	ν$_2$/ν$_1$	β	β%	μ$_{eff}$(B.M.)
Co(L)(OAc).3H$_2$ O	14,265 29,835^a 23,255	$^4T_{1g}$ (F) → $^4T_{2g}$ (F) (υ_1) $^4T_{1g}$ (F) → $^4T_{1g}$ (P) (υ_3)	1557.0	686.0	2.090	0.706	29.4	4.5
Co(L)$_2$.2H$_2$ O	14,084 29,380^a 22,123	$^4T_{1g}$ (F) → $^4T_{2g}$ (F) (υ_1) $^4T_{1g}$(F) → $^4T_{1g}$ (P) (υ_3)	1529.6	616.7	2.086	0.635	36.5	4.8
Ni(L)(OAc).3H$_2$O	11,210 16,510 24,015	$^3A_{2g}$ (F) → $^3T_{2g}$ (F) (υ_1) $^3A_{2g}$(F) → $^3T_{1g}$ (F) (υ_2) $^3A_{2g}$ (F) → $^3T_{1g}$ (P) (υ_3)	1121.0	460.0	1.473	0.442	55.8	3.2
Ni(L)$_2$.2H$_2$O	11,148 16,487 24,010	$^3A_{2g}$ (F) → $^3T_{2g}$ (F) (υ_1) $^3A_{2g}$ (F) → $^3T_{1g}$ (F) (υ_2) $^3A_{2g}$ (F) → $^3T_{1g}$ (P) (υ_3)	1114.8	470.0	1.479	0.451	54.9	3.1

^{a}calculated values.

The electronic spectra of 1:1 and 1:2 Co(II) complexes exhibit absorption bands in the region (14, 084-14, 265 cm^{-1}) and (22,123 - 23,255 cm^{-1}) corresponding to ν_1 and ν_3 transitions respectively, these can be attributed to the transition $^4T_{1g}$(F) → $^4T_{2g}$(F) (ν_1) ; $^4T_{1g}$(F) → $^4T_{1g}$(P) (ν_3) [26], ν_2 is calculated using equation ($\nu_2 = \nu_1 + 10$ Dq) and other ligand field parameters (Dq, B, β, β%) have also been calculated using Band-Fitting Equation [27], The crystal field splitting energy (Dq) is found to be 1529.6 and 1557.0 cm^{-1} for 1:1 and 1:2 Co(II) complexes respectively, which is in accordance with octahedral complexes. The Racha parameter values B (686.0 and 616.7 cm^{-1}), which are less than that of free cobalt metal ion (971 cm$^{-1)}$, indicate orbital overlapping and delocalization of electrons on metal ions. Also the values of β% are less than one, which favor the partial covalent character of M-L bond. 1:1 and 1:2 Ni(II) complexes exhibit three absorption bands in the region (11,148-11,210 cm^{-1}), (16,487-16,510 cm^{-1}) and (24,010-24,015 cm^{-1}), which are attributed to $^3A_{2g}$ (F) → $^3T_{2g}$ (F) (ν_1), $^3A_{2g}$ (F) → $^3T_{1g}$ (F) (ν_2) and $^3A_{2g}$ (F) → $^3T_{1g}$ (P) (ν_3) respectively. Existence of three bands is consistent with observed octahedral geometry around the Ni(II) centers [28]. The ligand field parameters (Dq, B, β, β%) are also calculated for Ni(II) complexes using Band-Fitting equation and are displayed in table 2 [27].

In electronic spectra of 1:1 and 1:2 Cu(II) complexes, one band is observed in the region (21,691-21,881 cm^{-1}) , which is assigned to $^2B_{1g}\rightarrow{}^2A_{1g}$ (ν_1) transition and suggesting square planar geometry around the Cu(II) metal ion [29]. The geometry was further confirmed by magnetic moment measurements i.e. (1.8-1.9 BM) [30].

Fluorescence Spectral Studies: Many research papers have been reported on the prospective use of fluorescence studies on transition metal complexes of Schiff bases [31]. In this work, fluorescence characteristics of the ligand and its metal complexes have been studied in DMF (10^{-3} M) solutions with excitation wavelength 260 nm, at room temperature. Schiff base shows weak emission band at 456 nm whereas its 1:1 and 1:2 metal complexes with Co(II), Ni(II), Cu(II) and Zn(II) show fluorescence with higher intensity. The increase in fluorescent intensity has been observed in 1:1 metal complexes, with emission bands at 535 nm for Co(II), 537 nm for Ni(II), 538 nm for Cu(II) and 499 nm for Zn(II) complexes with a shoulder at 399 nm figure 1a.1:2 metal complexes exhibit strong emission bands at 541 nm, 528 nm, 542 nm and 542 nm for Co(II), Ni(II), Cu(II) and Zn(II) complexes respectively figure 1 b. This enhancement of fluorescent intensity of metal complexes can be due to the coordination of ligand to metal ions, which effectively increases the rigidity of ligand and decreases the loss of energy via non-radiative thermal vibrations [32]. Fluorescence properties of zinc metal complexes indicate that they can be used as potential photoactive material [33].

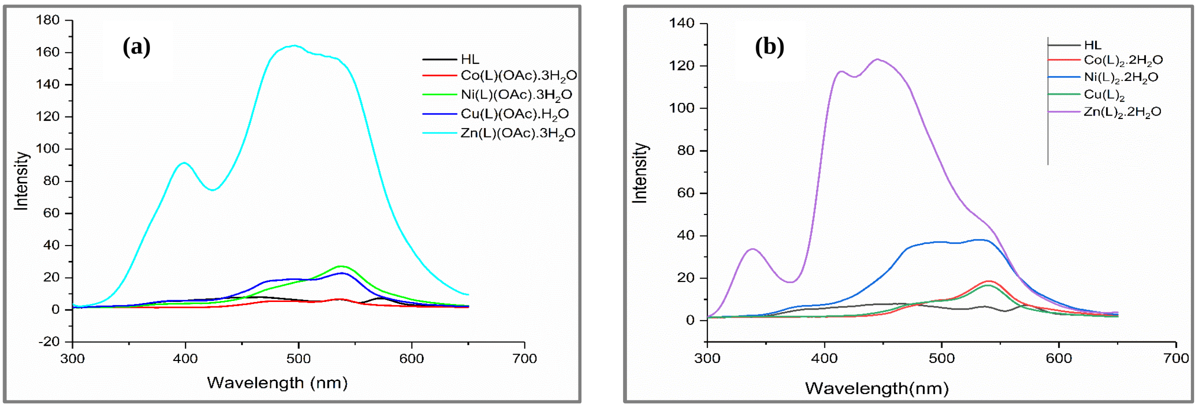


Figure 1(a). Fluorescence spectra of HL and its 1:1 and **(b)** 1:2 metal complexes.

Electrochemical Studies: Electrochemical redox properties of 1:1 and 1:2 Copper metal complexes have been investigated by cyclic voltammetry in potential ranging from -1.0 to +1.0 V at room temperature in DMF (10^{-3} M), containing tetrabutylammonium perchlorate as the background electrolyte. The cyclic voltammograms are shown in figure 2 a, b. $Cu(L)(OAc).H_2O$ complex exhibits reduction peak at E_{pc} = 0.330 V and corresponding oxidation peak at E_{pa} = 0.415 V for Cu(II) to Cu(I) and Cu(I) to Cu(II) respectively. 1:2 Copper complex i.e. $Cu(L)_2$ shows reduction peak at E_{pc} = 0.270 V and corresponding oxidation peak at E_{pa} = 0.400 V for Cu(II) to Cu(I) and Cu(I) to Cu(II) respectively. ΔE for these processes were in range (85-130) mV which is greater than 60 mV and ratio of cathodic to anodic peak currents (I_c/I_a = 0.5 and 0.375) for 1:1 and 1:2 copper complexes respectively are found to be less than one [34], therefore redox couples in voltammograms for both complexes are attributable to quasi-reversible one electron transfer process [35].

Electronic Spin Resonance Spectral Studies: ESR studies of copper complexes of HL provide significant information related to the distribution of unpaired electron and nature of bonding between metal ion and ligand. ESR spectra of 1:1 and 1:2 Cu(II) complexes are recorded in powder form at room temperature. The $g_{||}$ and $g_{\perp}$values are calculated from the spectra using tetracyanoethylene (TCNE) free radical as (g) marker. For $Cu(L)(OAc).H_2O$, observed g values are $g_{||}$ = 2.274, $g_{\perp}$=2.212, g_{av} = 2.232 and G = 1.296 at room temperature and for $Cu(L)_2$, $g_{||}$ = 2.336, $g_{\perp}$= 2.197, g_{av} = 2.243 and G = 1.714. ESR spectra of $Cu(L)(OAc).H_2O$ is displayed in figure 3. The trend $g_{||}>g_{\perp}>$

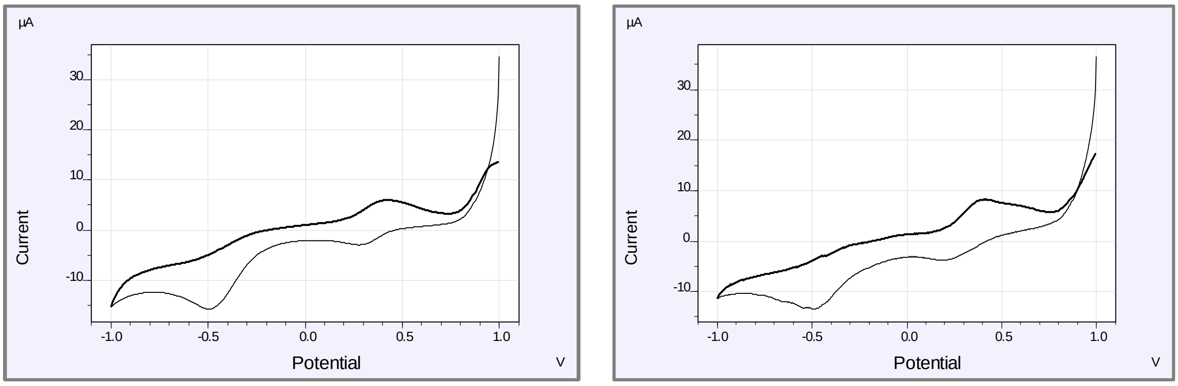


Figure 2. Cyclic voltammograms of Cu(L)(OAc).H_2O and Cu$(L)_2$.

2.0023 observed for both the complexes indicate that unpaired e^- is localized in $d_{x^2-y^2}$orbital of metal ion and suggests square planar geometry of complexes [36, 37]. The axial symmetry parameter G is defined as G = $(g_{||} - 2.0023)/(g_{\perp} - 2.0023)$. The calculated G (1.714, 1.296) values for copper complexes, which are less than 4.0, indicate that there is an exchange interaction of Cu-Cu in the complexes [38].

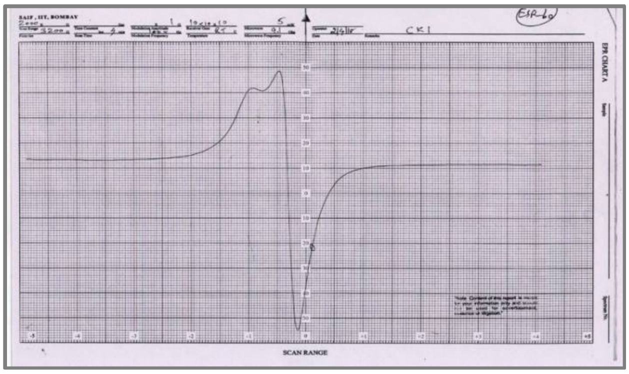


Figure 3. X-Band EPR Spectrum of Cu(L)(OAc).H_2O.

Thermogravimetric analysis: Thermal stability of synthesized metal complexes has been evaluated by thermogravimetric analysis. TG analysis of Co$(L)_2$.2H_2O, Ni$(L)_2$.2H_2O, Cu$(L)_2$ and Zn$(L)_2$.2H_2O are carried out in nitrogen atmosphere at a heating rate of 10ºC min^{-1} from 50º to 750ºC. TG curves of Co$(L)_2$.2H_2O, Ni$(L)_2$.2H_2O, Cu$(L)_2$ and Zn$(L)_2$.2H_2O are shown in figure 4 and their corresponding TG data having determined temperature ranges with % mass loss are given in table 3.

Table 3. Thermogravimetric data of Co$(L)_2$.2H_2O and Ni$(L)_2$.2H_2O metal complexes

Compounds	Temp.(°C)	% Weight loss from TG (Theoretical)	Decomposed Moieties	Metallic Residue
Co$(L)_2$.2H_2O	50-114	3.89 (3.83)	H_2O	
	218-363	58.54 (56.12)	Organic Moiety	CoO
	364-746	34.80 (33.05)	Triazine Moiety	
Ni$(L)_2$.2H_2O	53-168	3.90 (3.84)	H_2O	
	170-413	54.50 (56.01)	Organic Moiety	NiO
	414-768	30.10 (33.06)	Triazine Moiety	

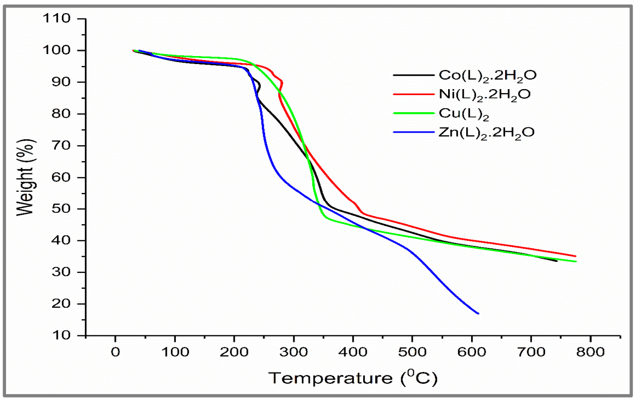


Figure 4. Thermogravimetric data of 1:2 metal complexes.

For $Co(L)_2.2H_2O$, the first stage of decomposition is in range 50-114°C, can be attributed to loss of two coordinated water molecules with weight loss 3.89 % (calculated 3.83 %). The second stage of decomposition corresponds to loss of organic moiety within temperature range 218-363° C with weight loss of 58.54 % (calculated 56.12 %). Third step results in loss of triazine ring with a weight loss of 34.80 % (calculated 33.05 %) in temperature range 364-746°C leaving metal oxide as residue. TG curve of $Ni(L)_2.2H_2O$ shows three steps of decomposition process. Elimination of two water molecules was observed within temperature range 53-168°C with weight loss 3.90 % (calculated 3.84 %). Another weight loss 54.50 % (calculated 56.01 %) corresponds to organic moiety in temperature range of 170-413°C. In third step, 30.10 % (calculated 33.06 %) loss in weight was observed corresponding to loss of triazine moiety in temperature range 414-768°C. The remaining final product was nickel oxide residue [39]. The TG curve of $Cu(L)_2$ exhibited two steps of thermal decomposition within temperature range 58-355°C and 360-770°C respectively, corresponding to loss of organic moiety with weight loss of 53.00 % (calculated 58.70 %) and triazine ring with weight loss of 30.11% (calculated 34.00 %). The $Zn(L)_2.2H_2O$ complex showed three decomposition steps in range 53-114°C, 115-415°C and 416-748°C corresponding to loss of water molecules, organic moiety and triazine ring respectively [40].

Kinetic Calculations: To evaluate thermal degradation activation energy and mechanism of metal complexes, Horowitz-Metzger method was used [41]. The equation used for calculating energy of activation (E_a) is

$$\ln\ln\left[\frac{1}{1-\alpha}\right] = \frac{E\,\theta}{R\,T_{s2}}$$

Where θ is difference between the peak temperature and the temperature at particular weight loss $[\theta = T - T_s]$,

$$\alpha = \frac{W_t - W_\infty}{W_0 - W_\infty} ,$$

W_0 is the initial weight, W_t is weight at any time t, T_s is peak temperature, T is temperature at particular weight loss. A plot of lnln $[\frac{1}{1-\alpha}]$ vs θ gives a good approximation to a straight line and from the slope, E_a activation energy can be calculated. The kinetic parameters involve temperature range of different stages of decomposition, activation energy and regression coefficient values (R^2). According to Horowitz-Metzger Method, Activation energy values for $Co(L)_2.2H_2O$, $Ni(L)_2.2H_2O$, $Cu(L)_2$ and $Zn(L)_2.2H_2O$, for second and third stages are summarized in table 4 and figure 5(a-d) [42]. Based on activation energy values (E_2), as deduced by Horowitz-Metzger method, following stability order of the complexes is suggested $Co(L)_2.2H_2O > Cu(L)_2 > Ni(L)_2.2H_2O > Zn(L)_2.2H_2O$.

Table 4. Calculated activation energies (KJ mol^{-1}) for different stages in degradation of metal complexes

Compounds	**Heating Rate (°Cmin^{-1})**	**Energy* (Regression Coefficient)**	
Co(L)$_2$.2H$_2$O	10	**E_2 (R^2)** = 69.66 (0.92)	**E_3(R^2)** = 27.50 (0.95)
Ni(L)$_2$.2H$_2$O	10	**E_2(R^2)** = 54.80 (0.86)	**E_3(R^2)** = 24.84 (0.98)
Cu(L)$_2$	10	**E_1 (R^2)** = 57.33 (0.97)	**E_2 (R^2)** = 05.84 (0.99)
Zn(L)$_2$.2H$_2$O	10	**E_2 (R^2)** = 05.25 (0.99)	**E_3(R^2)** = 02.74 (0.96)

***1, 2, 3** represents first, second and third stage of decompositions

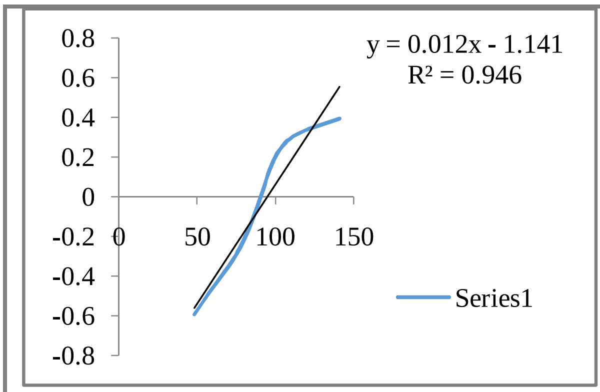


Figure 5(a). Horowitz-Metzger plot of ln[1/1-α] against θ for third stage decomposition of Co(L)$_2$.2H$_2$O.

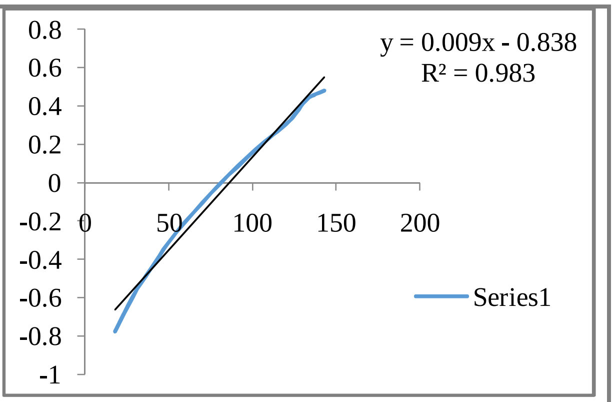


Figure 5(b). Horowitz-Metzger plot of ln[1/1-α] against θ for third stage decomposition of Ni(L)$_2$.2H$_2$O.

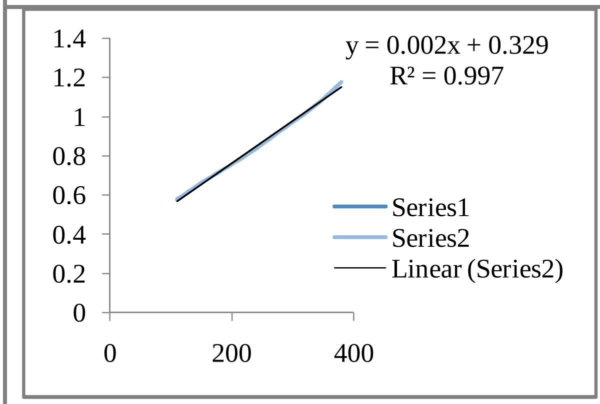


Figure 5 (c). Horowitz-Metzger plot of ln[1/1-α] against θ for second stage decomposition of Cu(L)$_2$.

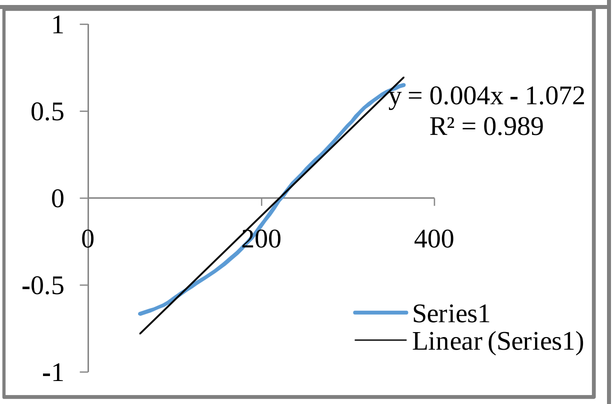


Figure 5(d). Horowitz-Metzger plot of ln[1/1-α] against θ for second stage decomposition of Zn(L)$_2$.2H$_2$O.

APPLICATION

Antimicrobial Screening: Agar well diffusion technique was used to check out biological properties of synthesized compounds. The investigations were made against some gram positive bacteria, gram negative bacteria and two fungi i.e. *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *S. cerevisiae.* Zone of inhibition in mm for the ligand and its metal complexes are listed in table 5 and also shown in figure 6. From the data, it is observed that metal complexes exhibit greater antimicrobial activity than that of free ligand [43]. Also, it has been observed that the metal complexes are more susceptible toward bacterial strains than the fungicidal strains. MIC values (μg mL^{-1}) of compounds are also shown in figure 7 along with that of standard drugs.

Table 5. *In vitro* antimicrobial activity of synthetic chemical compounds through agar well diffusion method

Compounds		**Diameter of growth of inhibition zone (mm)**[a]					
		Gram Positive Bacteria		**Gram Negative Bacteria**		**Fungi**	
S. No	**Name**	***Bacillus subtilis***	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***	***Saccharomyces cerevisiae***
1	Schiff Base	23	15	13	-	10	-
2	Co(L)(OAc).3H_2O	19	18	15	-	15	-
3	Ni(L)(OAc).3H_2O	18	19	18	-	18	-
4	Cu(L)(OAc).H_2O	17	17	23	-	13	-
5	Zn(L)(OAc).3H_2O	14	21	14	-	14	-
6	$Co(L)_2$.2H_2O	8	15	24	-	16	-
7	$Ni(L)_2$.H_2O	23	23	18	-	18	-
8	$Cu(L)_2$	18	20	18	-	26	-
9	$Zn(L)_2$ .2H_2O	16	23	23	-	23	-
10	Amphotericin-B	-	-	-	-	16.6	19.3
11	Ciprofloxacin	24	26	25	22	-	-

- No activity, [a] Values, not including diameters of well (8mm), are means of three replicates.

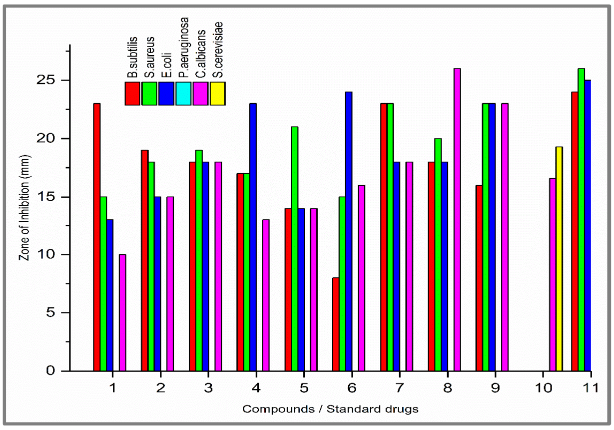


Figure 6. Antimicrobial activity of compounds/ Standard Drugs showing zone of inhibition in mm.

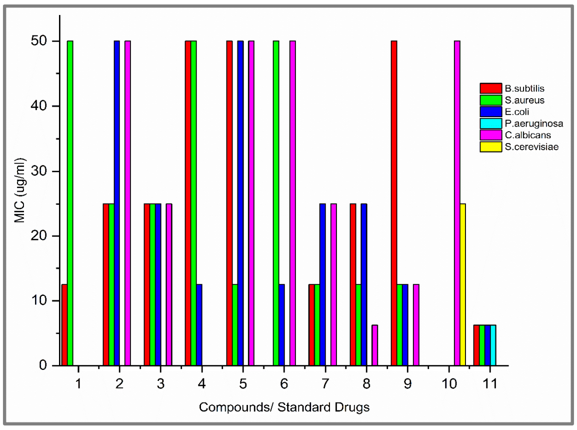


Figure 7. MIC values (µg mL^{-1}) of compounds/ Standard Drugs.

Probably this could attributable to greater lipophilic nature of complexes. Such accumulated activity of metal chelates can be explained on the premise of Overtone's idea and chelation theory [44]. In line with Overtone's idea of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble things or in other words we can say that it favors the antimicrobial activity. On chelation, the polarity of metal ions is reduced to a larger extent attributable to the increases of delocalization of pi-electrons over the whole chelation area and increases the lipophilicity of the metal complexes. This enhanced lipophilicity increases the penetration of complexes into the lipid membrane and block the metal binding sites [45].

Antioxidant Assay: Reactive oxygen species (ROS) gets formed in our body system during various biochemical processes. The oxidative processes caused by ROS on proteins, nucleic acids and lipids can cause chronic diseases like cancer, atherosclerosis, hypertension and heart diseases etc [46]. So, it is important to administer the drugs having rich antioxidant properties. Thus, in vitro antioxidant activity of Schiff base and its 1:2 metal (Co(II), Ni(II), Cu(II), Zn(II)) complexes was evaluated by using DPPH radical scavenging method at different concentrations (100, 200, 300, 400, 500 µg mL^{-1}) [47].

Freshly prepared deep purple solution of DPPH exhibits maximum absorbance at 517 nm due to free radical, its color gets faded in presence of an antioxidant medium. It was also found that antioxidant activity of tested compounds and standards is dose dependent and gets increased with increasing concentration. Antioxidant activity of ligand and metal complexes together with standards (vitamin C) along with correlation coefficients (R^2) is displayed in figure 8. Ligand showed moderate antioxidant activity. Copper and Zinc metal complexes possess higher antioxidant potential than other metal complexes as well as ligand but lower than vitamin C. Antioxidant activity of compounds is associated with their actions of breaking the free radical chain via hydrogen atom donation [48].Hence, the results of this study open up a way for using synthesized compounds in the treatment of pathological diseases arising from oxidative processes.

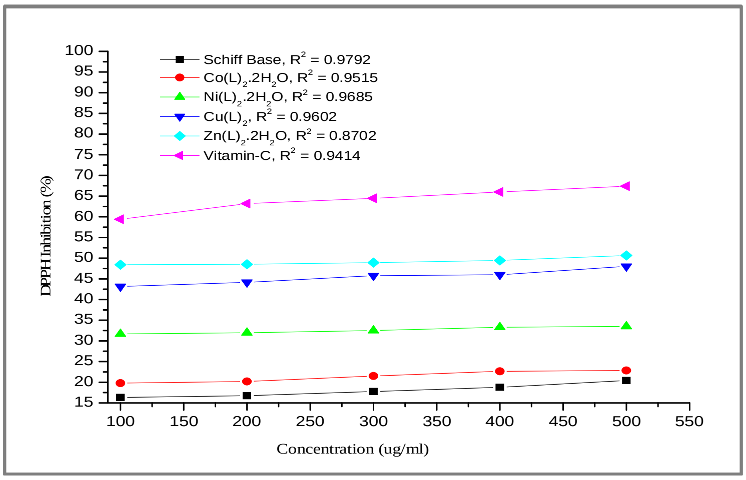


Figure 8. DPPH scavenging activity of Schiff base and metal complexes, also compared with standard.

Optimized structure of ligand and its metal complexes are depicted in figure 9.

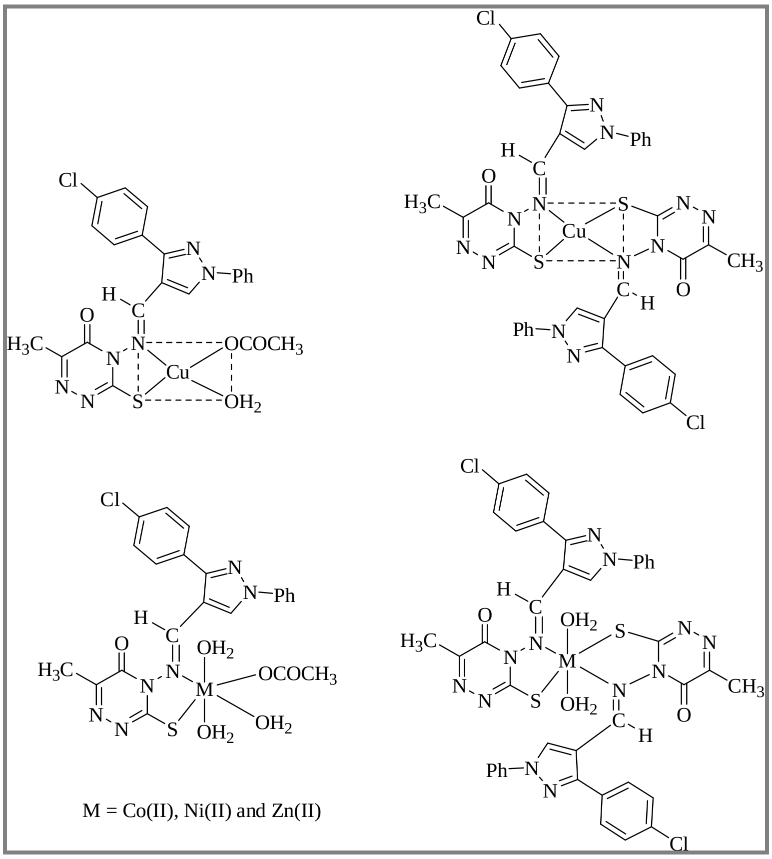


Figure 9. Proposed structure of metal complexes.

CONCLUSION

Co(II), Ni(II), Cu(II) and Zn(II) complexes of (4-[{3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-ylmethylene}-amino]-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (HL) are insoluble in water and in common organic solvents but soluble in DMF and DMSO. The proposed structures of all metal complexes are based on elemental analysis, IR, ^{1}H-NMR, thermal analysis, electronic and magnetic measurements. Potent fluorescence properties shown by Zn(II) metal complexes can offer wide applicability as sensor devices and fluorescent markers with high resistance. Also Metal complexes were found to be more active against microbial strains than the parent ligand. Additionally, the metal complexes exhibited stronger scavenging effects than their Schiff base in a dose dependent manner, which reveal their capability of donating electron or hydrogen atom and terminating some reactions.

Further, efforts are under progress to synthesize, characterize, evaluate biological properties of different metal complexes of Schiff bases derived from condensation of substituted 1,2,4-triazine with aromatic aldehydes containing heteroatom moieties and finding their applicability into various analytical and spectroscopic processes.

Conflict of Interest: Authors declare no conflict of interests.

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