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ORIGINAL ARTICLE

Synthesis, physico-chemical investigations and biological screening of metal (II) complexes with Schiff base derived from naphthofuran-2-carbohydrazide and citral

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KEYWORDS

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DNA cleavage

Abstract A series of Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes of the type $CuLCl_2$. $2H_2O$ and ML_2Cl_2 [M = Co(II), Ni(II), Cd(II), Zn(II) and Hg(II)], respectively, where L = Schiff's base derived from condensation of citral and naphthofuran-2-carbohydrazide have been synthesized. The proposed structures of the obtained complexes have been established from elemental analyses, IR, Electronic, Mass, ^{1}H NMR, ESR spectral data, magnetic and thermal studies. From the above spectral studies it is concluded that the ligand acts as a bidentate coordinating through azomethine nitrogen and amide oxygen. The measured low molar conductance values in DMF indicate that the complexes are non-electrolytic in nature. The electron transfer mechanism of the Cu(II) complex is investigated by the aid of cyclic voltammetry. The free ligand and its metal complexes have been screened for their antioxidant activity by the DPPH method and *in vitro* antibacterial (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*) and antifungal (*Aspergillus niger, Aspergillus flavus, Cladosporium oxysporum* and *Candida albicans*) activities by the minimum inhibitory concentration (MIC) method. The DNA cleavage studies of all the complexes were studied by agarose gel electrophoresis method. The results indicate that the biological activity increases on complexation.
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1. Introduction

Naphthofuran nuclei are key structural moieties found in a large number of biologically important natural products. Many of the natural naphthofurans, such as (±)-laevigatin, (+)-heritol and balsaminone A possess interesting pharmacological and cytotoxic properties (Cumhur kirilmis et al., 2009). A large number of naphthofuran derivatives possess various biological activities like anthelmentic, anticonvulsant and

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antipyretic (Basavaraj Padmashali et al., 2005). They also act as florescent dyes and probes as well as photosensitizers. Naphthofurans when condensed with various heterocycles exhibit a wide spectrum of activities (Kumaraswamy and Vaidya, 2005; Kumaraswamy et al., 2008; Nagendra Prasad et al., 2010). Many heterocycles and metal containing compounds (Bukhari et al., 2008) have exhibited various antioxidant activities, more importantly naphthofuran derivatives have been proven to be potent antioxidant agents (Basavaraj Padmashali and Vaidya, 2002). Citral is an acyclic monoterpene and an important constituent of lemon grass oil.

Although a large number of Schiff base ligands have been investigated, similar studies on coordinated ligands are relatively scarce. Schiff base ligands are extensively important and studied widely because they can readily form stable complexes with most of the metal ions (Yu et al., 2005; Lin and Feng, 2005). In addition they possess interesting antibacterial, antifungal and antitumor activities (El-Gammal, 2010; Chohan et al., 2005). Metal ions when bonded to biologically active molecules may modify and enhance their activity (Al-Shaalan, 2007). Schiff base complexes involving oxygen and nitrogen donor ligands are well known (Abdallah et al., 2010) and have excited great interest among chemists due to their applications in catalysis and their relevance to bioinorganic systems (Raman and Sobha, 2010). During the last decades there has been curiosity owing to the interaction of small molecules with DNA (Tan et al., 2008). The reaction of metal complexes with DNA has been extensively studied in relation to the progress of development of new reagents in the field of medicine and biotechnology.

In this communication, we describe the chelation behavior of Schiff base derived from the condensation of naphthofuran-2-carbohydrazide and citral. Further the metal complexes were obtained employing different metal ions like Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) in order to obtain more potent biologically active compounds and to know the geometry of the compounds. All the synthesized compounds were studied by analytical, thermal and various spectral techniques. Biocidal activity was carried out for all the compounds.

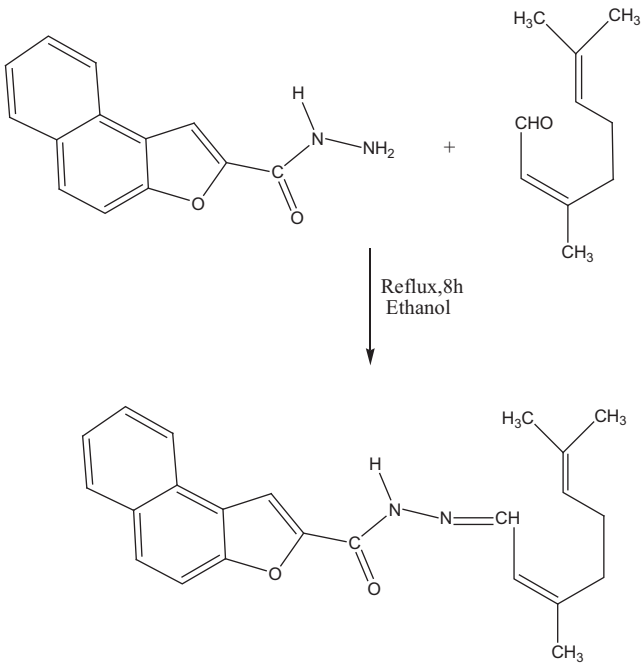
2. Experimental

All chemicals and reagents were of reagent grade and are used without further purification. Melting points of the compounds are determined in open capillaries and are uncorrected. The metal salts were used as their chlorides. Naphthofuran-2-carbohydrazide was prepared as reported (Kawas et al., 1962). The metal and chloride contents were determined as described in the literature (Vogel, 1968).

2.1. Synthesis of Schiff's base ligand

The synthesis of Schiff base is schematically represented in Scheme 1. A mixture of naphthofuran-2-carbohydrazide (0.01 mol, 2.26 g) and citral (0.01 mol, 1.71 mL) in 20 mL hot ethanol was boiled under reflux for 8 h on a water bath during which a pale yellowish solid separated. It was filtered, dried and recrystallized from hot ethanol. The purity of Schiff's base was checked by TLC.

$$M.F = C_{23}H_{25}O_2N_2, Mol.wt = 361, m.p = 110^{\circ}C, Yield = 75\%$$



Scheme 1 Synthesis of Schiff's base ligand.

2.1.1. General method for the synthesis of Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes

An ethanolic solution (30 mL) of Schiff base and metal salts in the ratio 1:1 for Cu(II) complex and 2:1 for Co(II), Ni(II), Cd(II), Zn(II) and Hg(II) complexes was refluxed with an ethanolic solution (10 mL) of metal salts on water bath for about 3 h. Then, to the reaction mixture an aqueous alcoholic solution of sodium acetate was added to adjust the pH to 6.0–7.0. The precipitated complexes were further refluxed for about an hour. Later they were filtered off, washed thoroughly with water and little warm ethanol to remove any traces of unreacted starting materials and finally dried in a vacuum desiccator over fused $CaCl_2$. (Yield: 50–55%).

2.2. Analysis and physical measurements

Elemental analyses (C, H and N) were performed on Perkin Elmer 240C model elemental analyzer at the Central Drug Research Institute (CDRI), Lucknow. The IR spectra of all the compounds were recorded on a Perkin Elmer 783 FT-IR spectrophotometer in the 4000–350 cm^{-1} region in KBr pellets. The ^1H NMR spectra were recorded in DMSO-d_6 on a BRUKER 400 MHz spectrophotometer using TMS as an internal reference. The electronic spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded on an ELICO SL-164 double beam UV–Visible spectrophotometer in the range of 200–1100 nm in DMF (10^{-3} M) solution. At room temperature the ESR spectrum of the Cu(II) complex in the polycrystalline state was recorded on a Varian-E-4X band EPR spectrophotometer using TCNE as the 'g' marker (g = 2.00277). The LC-MS was recorded on a TOF MS ES + mass spectrophotometer. The DART-MS was recorded on a JEOL-Accu TOF JMS-T100LC mass spectrometer having a DART source. Thermal analyses were measured from room temperature to

1000 °C in N_2 on a Perkin Elmer, Diamond TG/DTA model thermal analyzer at STIC, Cochin, with a heating rate of 10 °C min^{-1}. Electrochemistry of the Cu(II) complex was recorded on a 600D series model electrochemical analyzer in DMF (10^{-3} M). Molar conductivity measurements were recorded on an ELICO CM-180 conductivity bridge in DMF (10^{-3} M) solution using a dip-type conductivity cell fitted with a platinum electrode and the magnetic susceptibility measurements were made at room temperature on a Gouy balance using $Hg[Co(NCS)_4]$ as the calibrant.

3. Pharmacology

3.1. Antimicrobial assay

Schiff's base and its Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes were screened for their antibacterial and antifungal activities by the agar and potato dextrose agar diffusion method respectively (Threlfall et al., 1999; Prescott et al., 2000). The antibacterial and antifungal activities were done at 100, 200 and 500 μgmL^{-1} concentrations in DMSO solvent by using four bacteria *Escherichia coli* (MTCC 723), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 736), and *Pseudomonas aeruginosa* (MTCC 7837) and four fungi, *Aspergillius flavus* (MTCC 1883), *Aspergillius niger* (MTCC 1881), *Cladosporium oxysporum* (MTCC 1777) and *Candida albicans* (MTCC 3958) by the minimum inhibitory concentration (MIC) method. Separate studies carried out with solutions of DMSO alone showed no activity against any of the bacterial and fungal strains.

3.2. Antibacterial screening

Media Used: Peptone 10 g, NaCl 10 g and Yeast extract 5 g, Agar 20 g in 1000 mL of distilled water.

Initially, the stock cultures of *E. coli, S. aureus, B. subtilis and P. aeruginosa* were revived by inoculating in broth media and grown at 37 °C for 18 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures and spread evenly on the plate. After 20 min, the wells were filled with test solutions. Gentamycine was used as the standard antibacterial drug. All the plates were incubated at 37 °C for 24 h and the diameter of inhibition zones was noted.

3.3. Antifungal screening

Media used: potato dextrose agar (PDA). 250 g of peeled potato was boiled for 20 min and squeezed and filtered. To this filtrate 20 g of dextrose was added and the volume was made up to 1000 mL by distilled water.

Initially, the stock cultures of *A. flavus*, *A. niger, C. oxysporum* and *C. albicans* were revived by inoculating in broth media and grown at 37 °C for 48 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures and spread evenly on the plate. After 20 min, the wells were filled with test solutions. Fluconazole was used as the standard antifungal drug. All the plates were incubated at 37 °C for 48 h and the diameter of inhibition zones was noted.

3.4. Antioxidant assay (free radical scavenging activity)

Antioxidant activity was done by the DPPH method (Singh et al., 2002). Different concentrations (10, 50 and 100 μg) of samples and butylated hydroxy anisole (BHA) were taken in different test tubes. The volume was adjusted to 100 μL by adding DMF. Five milliliters of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract. The absorbance of samples was measured at 517 nm. Radical scavenging activity was calculated using the following formula:

$$\%\text{Radical scavenging activity} = [(\text{Control OD} - \text{Sample OD})/(\text{Control OD})] \times 100.$$

3.5. DNA cleavage experiment

3.5.1. Preparation of culture media

DNA cleavage experiment was done according to the literature (Sambrook et al., 1989). Nutrient broth was used as the medium. Composition of the medium (g L^{-1}): Peptone 10, Yeast extract 5, NaCl 10. Calf thymus DNA was used for the experiment. The compounds were screened at 100 μg concentration.

3.6. Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method (Sambrook et al., 1989). 250 mg of agarose was weighed and dissolved in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 l) by boiling. When the gel attained ~55 °C, it was poured into the gel cassette fitted with a comb and left to get it solidified. The comb was carefully removed and the gel was placed in the electrophoresis chamber flooded with TAE buffer. 20 μL of DNA sample was loaded (mixed with bromophenol blue dye @ 1:1 ratio), carefully into the wells, along with a standard DNA marker and a constant 50 V of electricity was passed for around 45 min. The gel was removed and carefully stained with ETBR solution (10 μg mL^{-1}) for 10–15 min and the bands were observed under UV transilluminator. The results were then compared with the standard DNA marker.

4. Results and discussion

All the synthesized new complexes were non-hygroscopic, light in color and stable at room temperature. These complexes are insoluble in water and many common organic solvents but are remarkably soluble in solvents such as DMF and DMSO. The analytical data (Table 1) showed that the complexes had stoichiometry of the type $CuLCl_2{\cdot}2H_2O$ and ML_2Cl_2 [M = Co(II), Ni(II), Cd(II), Zn(II) and Hg(II)], respectively, where L = Schiff base ligand. The values of molar conductance of the solutions of the compounds are lower than those expected for an electrolyte (Geary, 1971) indicating their non-electrolytic nature.

4.1. IR spectral studies

In order to study the binding mode of the Schiff base to the metal in the complexes, the IR spectrum of the free ligand

Table 1 Elemental analysis, molar conductance and magnetic susceptibility data of Schiff base and its metal complexes.

Molecular formula of Schiff base/ complexes	Mol. weight	m.p. (°C)	C (%)		H (%)		N (%)		M (%)		Cl (%)		Λ_M*	μ_{eff} (BM)
			Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.		
$C_{23}H_{25}O_2N_2$	361	110	76.18	76.44	6.65	6.91	7.48	7.75	–	–	–	–	–	–
$[Co(C_{23}H_{25}O_2N_2)_2Cl_2]$	851.93	> 300	64.43	64.79	5.56	5.85	6.21	6.56	6.79	6.90	8.21	8.33	6.12	4.86
$[Ni(C_{23}H_{25}O_2N_2)_2Cl_2]$	851.69	> 300	64.51	64.80	5.62	5.86	6.29	6.57	6.75	6.88	8.20	8.32	5.45	2.80
$[Cu(C_{23}H_{25}O_2N_2)Cl_2{\cdot}2H_2O]$	531.50	> 300	51.62	51.93	5.13	5.44	4.97	5.25	11.81	11.93	13.20	13.34	5.34	1.91
$[Cd(C_{23}H_{25}O_2N_2)_2Cl_2]$	905.41	290	60.68	60.95	5.24	5.51	5.88	6.17	12.29	12.41	7.71	7.83	7.86	–
$[Zn(C_{23}H_{25}O_2N_2)_2Cl_2]$	858.39	295	64.11	64.30	5.58	5.81	6.24	6.52	7.48	7.62	8.15	8.26	8.23	–
$[Hg(C_{23}H_{25}O_2N_2)_2Cl_2]$	993.59	286	55.36	55.54	4.73	5.02	5.36	5.62	20.02	20.17	7.01	7.14	6.59	–

* Molar conductance values in ohm-1cm2mole-1.

was compared with the spectra of metal complexes which exhibited characteristic frequencies of the expected functional groups and are tabulated in Table 2.

The IR spectrum of the free Schiff's base ligand shows a broad medium band at 3419 cm^{-1} due to ν(NH) stretch of the CONH group. This band shifts slightly to a higher wave number side in all the complexes indicating the non-involvement of 'N' of the CONH group in bonding. A strong band observed at 1634 cm^{-1} in the free ligand is attributed to *ν*(C═O) of carbonyl group. This band shifted to a lower wave number side in all the complexes indicating the participation of the carbonyl oxygen in bonding with metal ions. A medium-to-strong intensity band at 1593 cm^{-1} in the free ligand was attributed to *ν*(C═N) stretch of the azomethine group. Coordination of Schiff's base to the metal ions through the nitrogen atom is expected to reduce electron density in the azomethine link and lower the *ν*(C═N) absorption frequency. This band shifted to a lower wave number side in all the complexes indicating the participation of the azomethine nitrogen in coordination with metal ions. The medium intensity band at 974 cm^{-1} is assigned to *ν*(N–N) stretching vibration of hydrazine residue. This band in the complexes shifts slightly to a higher wave number side conforming the involvement of one of nitrogen's of –N–N– in bonding with the metal ions. Furthermore, the presence of a coordinated water molecule in the $[Cu(C_{23}H_{25}O_2N_2)Cl_2{\cdot}2H_2O]$ complex is evidenced by a broad band appearing at 3444 cm^{-1} due to O–H stretching vibration as supported from the thermal analysis (Mohamed et al., 2005). Many researchers (Singh et al., 1996; Ahuja and Singh, 2001) have reported *ν*(C–O–C) stretching vibrations of the furan ring in the region 1020–1250 cm^{-1}. In the present work, the *ν*(C–O–C) stretch is observed at 1077 cm^{-1}, remains unaltered in the metal complexes, indicating non-participation of the furan ring oxygen atom in the bonding with metal ions.

The new weak non-ligand bands in the region 468–482 cm^{-1} and 378–410 cm^{-1} in the spectra of the complexes are assigned to frequencies of *ν*(M–O) and *ν*(M–N) stretching vibrations, respectively (Nakamoto, 1986).

4.2. ^1H NMR spectral studies

The ^1H NMR spectra of the ligand (Fig. 1) and its Cd(II) complex were recorded in DMSO-d_6. The signals at *δ* (11.82) (s, 1H) and *δ* (8.50) (s, 1H) in the free ligand are assigned to the protons of (–CONH–) and (–CH═N) groups, respectively. In the Cd(II) complex both the signals shifted downfield in the region *δ* (11.93) (s, 1H) and *δ* (8.60) (s, 1H) confirming the coordination of the oxygen atom and azomethine nitrogen in bonding with the metal ions. The aromatic protons at *δ* (7.56–8.48) (m, 7H) shifted downfield in the complex. The signals at *δ* (2.490) (s, 3H, $–CH_3$), *δ* (2.494) (s, 3H, $–CH_3$), *δ* (2.480) (s, 3H, $–CH_3$) are due to protons of three $–CH_3$ groups. Thus, the ^1H NMR results further supports the IR inferences.

4.3. Mass spectral studies

Mass spectroscopy has been increasingly used as a powerful structural characterization technique in coordination chemistry. The LC–MS spectrum of Schiff's base (Fig. 2) showed a molecular ion peak at *m*/*z* 361.2, which is equivalent to its molecular weight. The DART-mass spectrum of Ni(II)

Table 2 Important IR spectral bands (cm^{-1}) of the ligand and its metal complexes.

Schiff base/complexes	ν (NH)	ν (C═O)	ν (C═N)	ν (N–N)	ν (M–O)	ν (M–N)
$C_{23}H_{25}O_2N_2$	3419	1634	1593	974	–	–
$[Co(C_{23}H_{25}O_2N_2)_2Cl_2]$	3434	1612	1575	985	480	397
$[Ni(C_{23}H_{25}O_2N_2)_2Cl_2]$	3436	1612	1578	987	481	397
$[Cu(C_{23}H_{25}O_2N_2)Cl_2{\cdot}2H_2O]$	3439	1629	1588	978	468	378
$[Cd(C_{23}H_{25}O_2N_2)_2Cl_2]$	3448	1622	1574	978	476	410
$[Zn(C_{23}H_{25}O_2N_2)_2Cl_2]$	3446	1595	1578	984	473	378
$[Hg(C_{23}H_{25}O_2N_2)_2Cl_2]$	3448	1610	1571	978	482	412

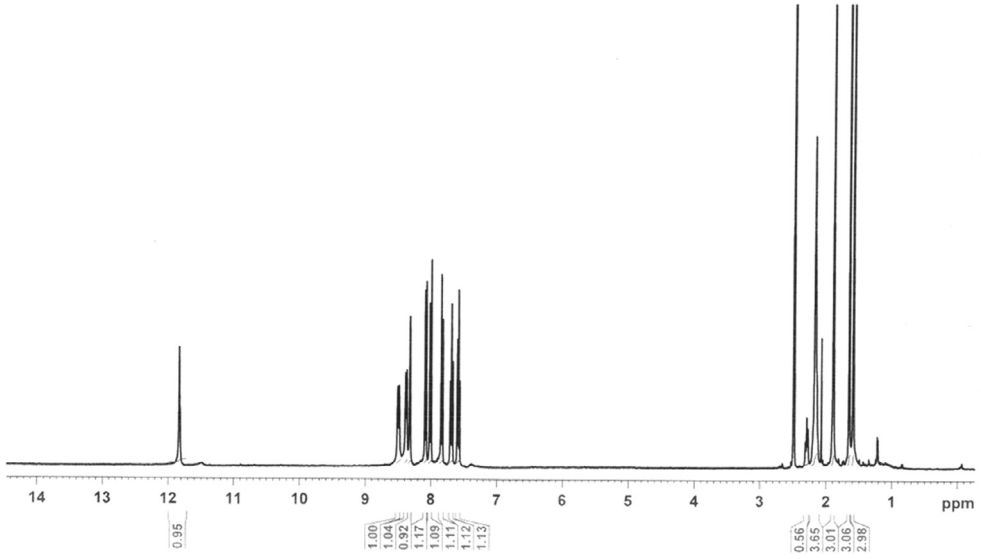


Figure 1 ^{1}H NMR spectrum of Schiff's base ligand.

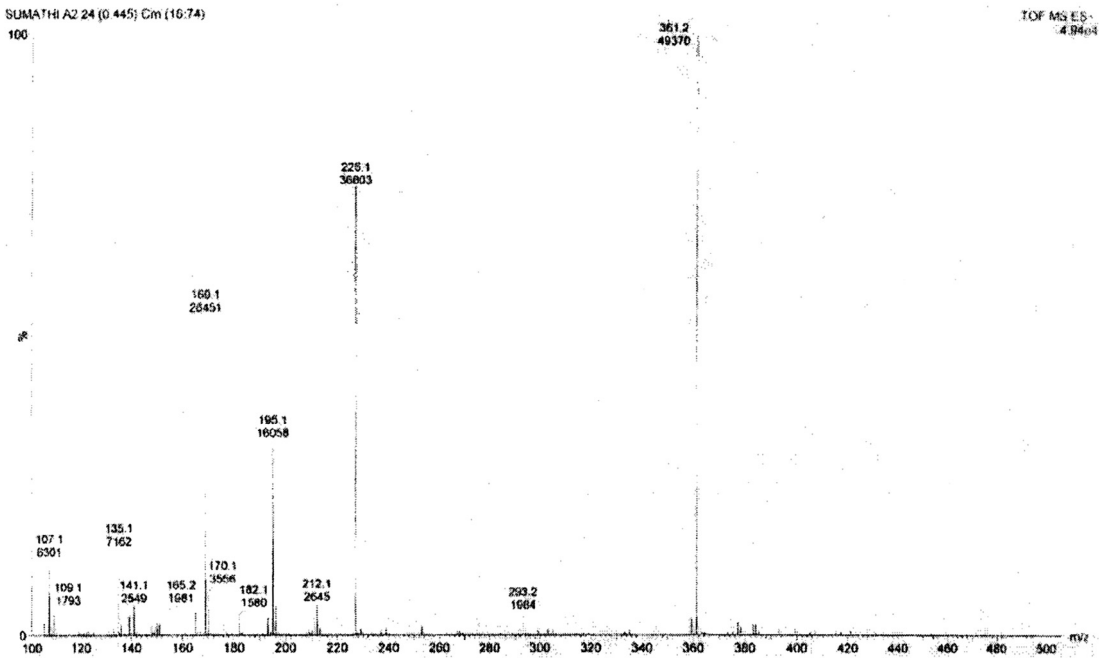


Figure 2 LC-Mass spectrum of Schiff's base ligand.

complex (Fig. 3) showed a molecular ion peak at m/z 851.41 corresponding to the molecular weight of the complex.

4.4. Electronic spectral studies

The electronic absorption spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded in freshly prepared DMF solution (10^{-3} M) at room temperature and the spectral data are presented in Table 3.

The electronic spectrum of the Co(II) complex displayed bands at 14,947 and 18,281 cm^{-1}. These two bands are assigned to $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)(\nu_2)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)(\nu_3)$ transitions, respectively in an octahedral environment (Lever, 1984). The lowest band, ν_1 could not be observed due to the limited range of the instrument used, but could be calculated using the band fitting procedure suggested by Underhill and Billing (Underhill and Billing, 1966).

The Ni(II) complex exhibited two absorption bands, at 15,652 and 25,252 cm^{-1} assignable to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\nu_3)$ transitions, respectively in an octahedral environment.

The light green colored Cu(II) complex exhibited low intensity single broad asymmetric band in the region 15,455–16,207 cm^{-1}. The broadness of the band indicates the three transitions $^2B_{1g} \rightarrow {}^2A_{1g}(\nu_1), {}^2B_{1g} \rightarrow {}^2B_{2g}(\nu_2)$ and $^2B_{1g} \rightarrow {}^2E_g(\nu_3)$, which are similar in energy and gives rise to only one broad absorption band. The broadness of the band may be due to dynamic Jahn–Teller distortion. All of these data suggested a distorted octahedral geometry around the Cu(II) ion (Liu et al., 2007).

The octahedral geometry was further supported by the values of ligand field parameters, such as the Racah inter electronic repulsion parameter (B'), ligand field splitting energy (10 Dq), nephelauxetic parameter (β) and ligand field stabilization energy (LFSE) (Satyanarayana, 2001).

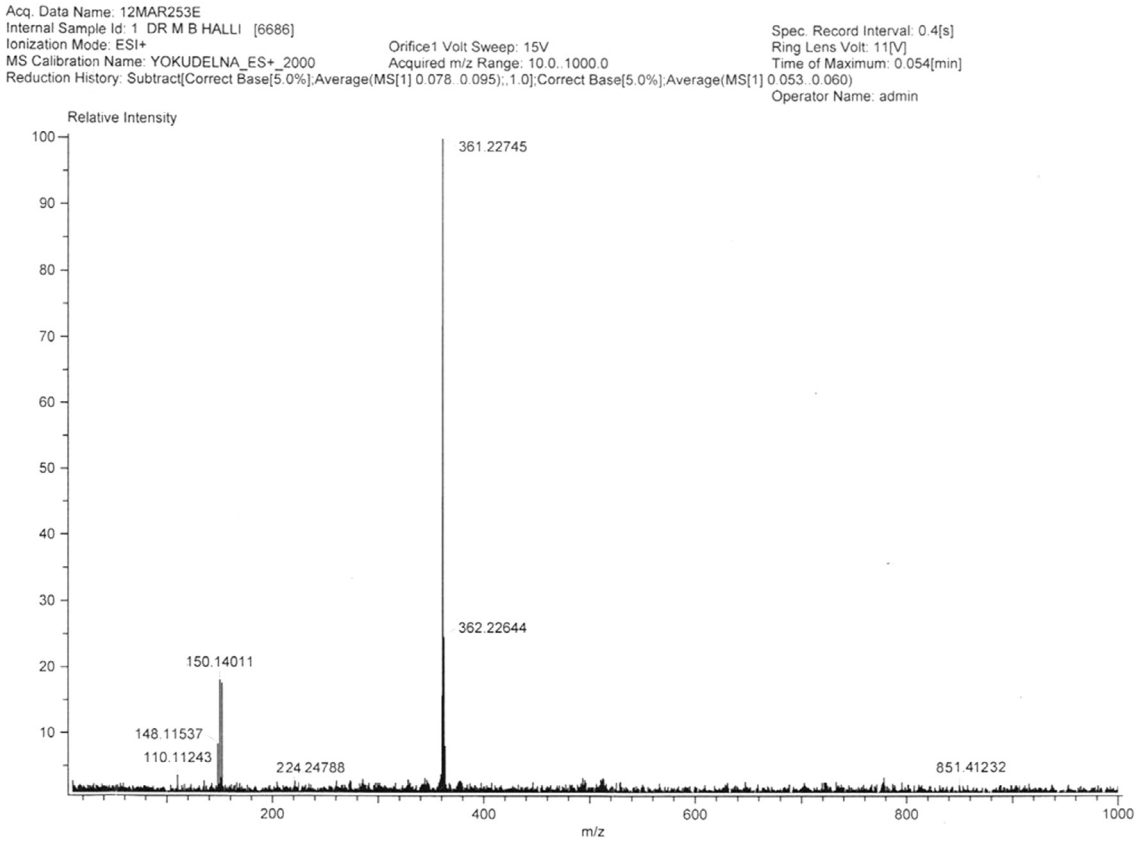


Figure 3 DART-Mass spectrum of Ni(II) complex.

Table 3 Electronic spectral bands and ligand field parameters of the Co(II), Ni(II) and Cu(II) complexes in DMF (10^{-3} M) solution.

Complexes	Transitions in cm^{-1}			Dq (cm^{-1})	B' (cm^{-1})	β	β (%)	ν_2/ν_1	LFSE (k cal)
	ν_1*	ν_2	ν_3						
$[Co(C_{23}H_{25}O_2N_2)_2Cl_2]$	6967	14947	18281	798	822	0.790	15.34	2.145	13.68
$[Ni(C_{23}H_{25}O_2N_2)_2Cl_2]$	9830	15652	25252	983	760	0.730	26.92	1.592	33.70
$[Cu(C_{23}H_{25}O_2N_2)Cl_2{\cdot}2H_2O]$	15455–16207	1560	–	–	–	–	26.74		23.64

* Calculated values.

The B' values for the complexes were lower than the free ion values, which is an indication of the orbital overlap and delocalization of d-orbitals. The β values obtained were less than unity, suggesting a considerable amount of covalency for the metal–ligand bonds. The β value for the Ni(II) complex was less than that of the Co(II) complex, indicating the greater covalency of the M–L bond (Halli and Sumathi, 2012).

4.5. Thermal studies

TG and DTA studies were carried out for some of the complexes. The nature of proposed chemical change with temperature and the percent of metal oxide obtained are given in Table 4.

The thermal decomposition of $[Cu(C_{23}H_{25}O_2N_2)Cl_2{\cdot}2H_2O]$ (Fig. 4) takes place in three steps as indicated by DTA peaks. The first decomposition step in the range 150–192 °C corresponds to the loss of coordinated water molecules with a mass loss of 6.45% (Calc.: 6.76%) (Selwin Joseyphus and Sivasankaran Nair, 2010), the second decomposition step in the range 203–245 °C corresponds to the loss of a chloride molecule with a mass loss of 13.10% (Calc.: 13.34%). The next decomposition step in the range 487–499 °C corresponds to the loss of ligand with a mass loss of 67.61% (Calc.: 67.91%). The $[Co(C_{23}H_{25}O_2N_2)_2Cl_2]$ (Fig. 5) and $[Ni(C_{23}H_{25}O_2N_2)_2Cl_2]$ showed only a single decomposition curve around 325–340 °C and 315–336 °C with a mass loss of 92.78% (Calc.: 93.07%) and 92.85% (Calc.: 93.09%) corresponding to the loss of chloride and $C_{23}H_{25}O_2N_2$ species respectively. Above 550 °C, metal (II) complexes were decomposed leading to the formation of their respective metal oxides. The metal content in the complexes as done by elemental analysis agrees well with that of thermal studies.

Table 4 Thermogravimetric data of Co(II), Ni(II) and Cu(II) complexes.

Complexes	Decomposition temperature (°C)	Weight loss (%)		Metal oxide (%)		Inference
		Found	Calc.	Found	Calc.	
$[Co(C_{23}H_{25}O_2N_2)_2Cl_2]$	325–340	92.78	93.07	6.57	6.91	Loss of chloride and $C_{23}H_{25}O_2N_2$ species
$[Ni(C_{23}H_{25}O_2N_2)_2Cl_2]$	315–336	92.85	93.09	6.51	6.89	Loss of chloride and $C_{23}H_{25}O_2N_2$ species
$[Cu(C_{23}H_{25}O_2N_2)Cl_2{\cdot}2H_2O]$	150–192	6.45	6.76	11.63	11.94	Loss of coordinated water molecules
	203–245	13.10	13.34			Loss of chloride molecule Loss of ligand
	487–499	67.61	67.91			

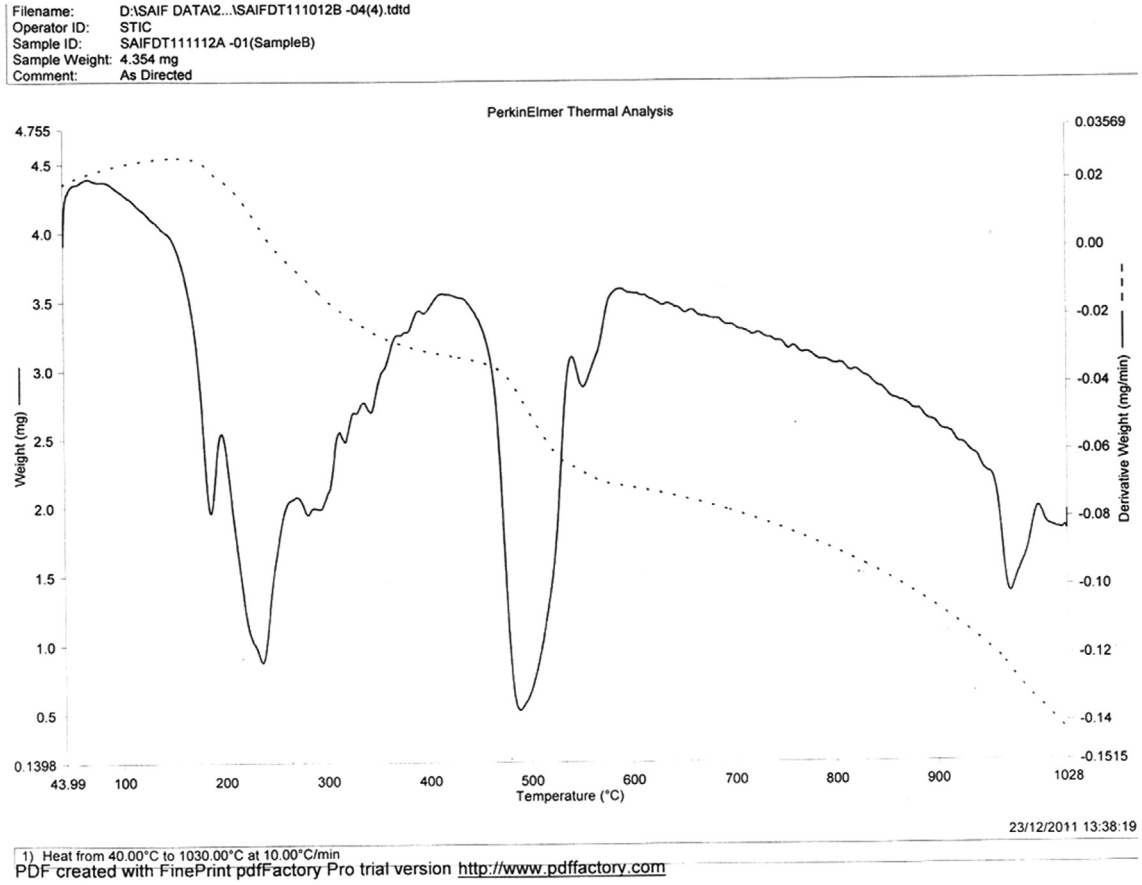


Figure 4 TG–DTA spectrum of the Cu(II) complex.

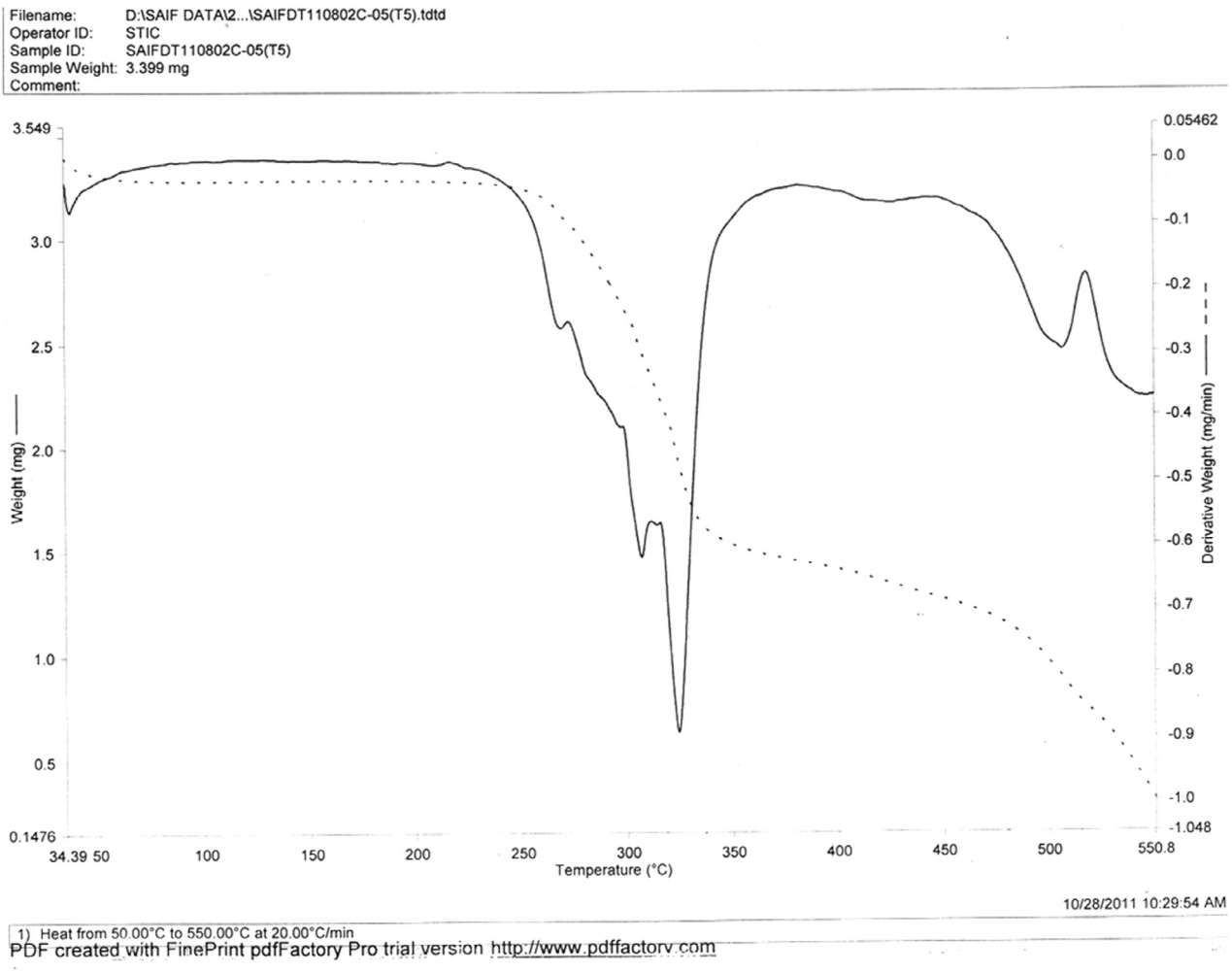


Figure 5 TG–DTA spectrum of the Co(II) complex.

4.6. ESR spectrum of the Cu(II) complex

The ESR spectral studies of Cu(II) complex provides information around the metal ion environment. The ESR spectrum of the Cu(II) complex in a polycrystalline state was recorded at room temperature show $g_{\|} = 2.176$ and $g_{\perp} = 2.033$. The g_{av} value was calculated to be 2.08. The deviation of g_{av} from that of the free electron (2.00277) is due to the covalence property (Masoud et al., 2005). The spectrum showed asymmetric bands with $g_{\|} > g_{\perp} > 2.00277$, indicating that the unpaired electrons lay predominantly in the d_{x2}–d_{y2} orbital with a possible mixing of d_{z2} because of low symmetry (Kivelson and Neiman, 1961). According to Hathway, the value of 'G' was determined as $G = (g_{\|} - 2.00277)/\ (g_{\perp} - 2.00277) = 5.73$, which is more than 4 suggesting that there is less or no interaction between the copper centers (Shakir et al., 2006). Thus, suggesting the Cu(II) complex a distorted octahedral geometry.

The molecular-orbital coefficient parameters, α^2, a measure of the covalency of the in-plane σ-bonding between the 3d and ligand orbitals, β^2, the covalency of the in-plane π-bonding and γ^2, out plane π-bonding were calculated employing the equations,

$$\alpha^2 = -(A_{\|}/0.036) + (g_{\|} - 2.00277) + 3/7(g_{\perp} - 2.00277) + 0.04$$

$$\beta^2(g_{\|} - 2.00277)E/ - 8\lambda\alpha^2$$

$$\gamma^2 = (g_{\perp} - 2.00277)E/ - 2\lambda\alpha^2$$

where $\lambda = -828\ cm^{-1}$ for free Cu(II) and E is the electronic transition energy. The α^2, β^2 and γ^2 values are 0.573, 0.711 and 0.496, respectively. The lower value of α^2 compared to β^2 indicates σ-bonding in plane is more covalent than in-plane π-bonding. These data agree well with the proposed geometry (Hathway, 1973) of the Cu(II) complex

4.7. Magnetic studies

The room temperature magnetic moments indicate paramagnetism for Co(II), Ni(II) and Cu(II) complexes. The values are recorded in Table 1. Co(II) complexes in an octahedral field orbitally degenerate which causes an orbital angular momentum contribution to the magnetic moment and the experimental magnetic moment values lie between spin only, $\mu_{SO} = [4S(S + 1)]^{1/2} = 3.88\ \mu_B$ and $\mu_{S+L} = [4S(S + L) + L(L + 1)]^{1/2} = 5.2\ \mu_B$ values. The present Co(II) exhibited

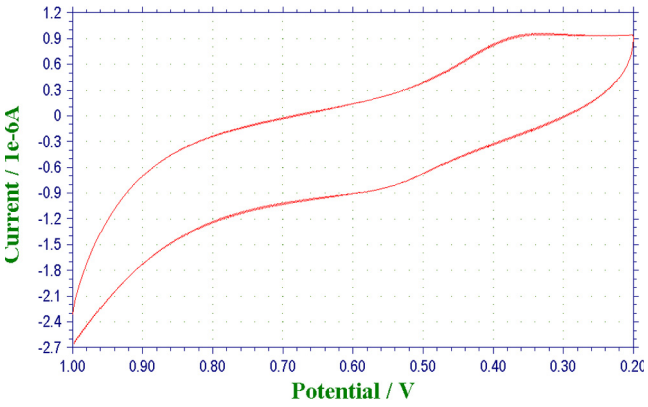


Figure 6 Cyclic voltammogram of Cu(II) complex.

the magnetic moment in the range μ_{eff} = 4.86 BM in agreement with the octahedral geometry (Greenwood and Earnshaw, 1997) and Ni(II) showed the magnetic moment of μ_{eff} = 2.80 BM within the range of 2.8–3.5 BM suggesting (Guptha and Chandra, 2006) consistency with their octahedral environment. The Cu(II) complex showed a magnetic moment of μ_{eff} = 1.91 BM consistent with the expected spin only values of 1.75–2.20 BM (Cotton and Wilkinson, 1988), indicating the absence of spin–spin interactions.

4.8. Electrochemical study

Electron transfer plays a vital role in governing the pathway of chemical reactions. The electrochemical behavior of Cu(II) complex (Fig. 6) was investigated by Cyclic voltammetry. The anodic peak at E_{pa} = 0.54 V versus Ag/AgCl and the associated cathodic peak at E_{pc} = 0.36 V correspond to the Cu(II)/Cu(I) couple. The peak separation (ΔE_p) of this couple is 0.18 V at a scan rate of 0.1 V s^{-1} and increases with the scan rate. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility of one electron transfer reaction. The most significant feature of the Cu(II) complex is a quasi-reversible one electron oxidation. The peak current ratio of cathodic to anodic peak height was less than one. Also, the peak current increases with the increasing square root of the scan rate thus exhibiting electrode process as diffusion controlled (Bard and Izatt, 2001). From the value of peak separation, ΔE_p, and the peak current ratio we can suggest that the electrode process is consistent with the quasi reversibility (Rossiter and Hamilton, 1986) of the Cu(II)/Cu(I) couple (Sangamesh patil et al., 2011).

5. Pharmacology results

5.1. *In vitro* antimicrobial activity

To access their potential as antimicrobial agents the Schiff base and its Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes were assayed against *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa* bacteria and *A. flavus*, *A. niger*, *C. oxysporum* and *C. albicans* fungal strains by the minimum inhibitory concentration (MIC) method as summarized in Table 5.

The biological activity is considered to involve three kinds of mechanisms: (i) inhibition of enzyme ribonucleoside diphosphate reductase (essential for DNA synthesis); (ii) creation of lesions in DNA strand by oxidative rupture; (iii) binding to

Table 5 Antimicrobial results of the Schiff base and its metal complexes (MIC).

Schiff base/complexes	Conc. (μg mL^{-1})	Zone of inhibition against bacteria (mm)				Zone of inhibition against fungi (mm)			
		E. coli	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*A. niger*	*A. flavus*	*C. oxosporium*	*C. albicans*
$C_{23}H_{25}O_2N_2$	100	07	07	06	06	09	09	08	07
	200	14	15	12	13	18	17	16	15
	500	20	21	13	14	22	23	21	20
$[Co(C_{23}H_{25}O_2N_2)_2Cl_2]$	100	08	09	07	07	11	10	09	08
	200	16	15	14	14	20	19	18	17
	500	22	22	15	16	24	25	24	22
$[Ni(C_{23}H_{25}O_2N_2)_2Cl_2]$	100	08	08	07	06	09	10	09	08
	200	14	16	13	14	19	17	16	14
	500	21	22	14	15	24	24	23	21
$[Cu(C_{23}H_{25}O_2N_2)_2Cl_2.\ 2H_2O]$	100	09	09	08	07	10	09	08	07
	200	16	17	14	15	18	19	18	16
	500	21	22	20	19	23	25	24	22
$[Cd(C_{23}H_{25}O_2N_2)_2Cl_2]$	100	09	08	07	06	10	09	08	07
	200	15	17	14	15	19	18	18	15
	500	21	23	15	18	23	24	23	21
$[Zn(C_{23}H_{25}O_2N_2)_2Cl_2]$	100	20	19	18	17	22	21	22	20
	200	22	22	19	18	24	23	23	23
	500	24	23	22	21	27	28	26	24
$[Hg(C_{23}H_{25}O_2N_2)_2Cl_2]$	100	19	19	16	16	22	20	21	20
	200	21	23	17	18	24	26	23	24
	500	23	25	19	19	27	28	26	26
Gentamycine	500	26	27	25	26	–	–	–	–
Flucanazole	500	–	–	–		30	31	30	28

Note: less than 12 mm – inactive; 12–16 mm – moderately active; above 18 mm – more active.

the nitrogen bases of DNA or RNA, hindering or blocking base replication (Sulekh Chandra et al., 2009). The antimicrobial activity results presented in Table 5 reveal that all the complexes exhibited increased antibacterial and antifungal activities than the free ligand. The Co(II), Ni(II), Cu(II) and Cd(II) complexes have shown moderate activity against all the bacterial/fungal strains. The Zn (II) and Hg (II) complexes showed significantly enhanced antibacterial and antifungal activities compared to Schiff's base and Co(II), Ni(II), Cu(II) and Cd(II) complexes, however, less active than the standard drugs. The variation in the activity of different complexes against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells (Poomalai Jayaseelan et al., 2011). Such increased activity of the complexes can be explained on the basis of Overtone's concept and Tweedy's chelation theory (Tweedy, 1964). Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible π-electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane. Furthermore, the mode of action of the compound may involve the formation of a hydrogen bond through the azomethine group with the active center of the cell, resulting in interference with the normal cell processes. In general metal complexes are more active than the ligands because metal complexes may serve as a vehicle for activation of ligands as the principal cyctotoxic species (Petering, 1973). The studies showed that the newly synthesized compounds possess higher antifungal activity than the antibacterial property.

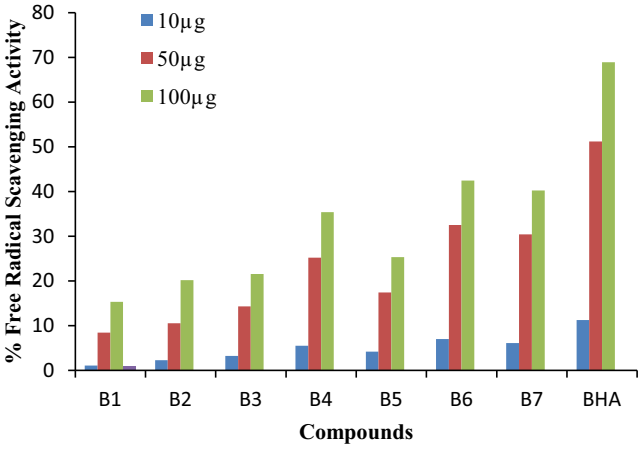


Figure 7 Antioxidant activity results of Schiff base and its complexes. (B1-Schiff base, B2-Co(II), B3-Ni(II), B4-Cu(II), B5-Cd(II), B6-Zn(II), B7-Hg(II) complexes respectively, BHA-standard).

5.2. Antioxidant assay (DPPH free radical scavenging activity)

The Schiff base and its complexes were screened for free radical scavenging activity by the DPPH method (Singh et al., 2002). The results of percentage of free radical scavenging activity are presented in Fig. 7. The coordination of different metal ions into the ligand demonstrated a broad spectrum of results. Cu(II), Zn(II) and Hg(II) complexes have exhibited a good scavenging activity. Whereas, Co(II), Ni(II) and Cd(II) complexes have shown a moderate activity while, all the complexes have exhibited a higher scavenging activity than the Schiff base ligand. Further, the synthesized compounds scavenged the DPPH radical in a concentration dependent manner.

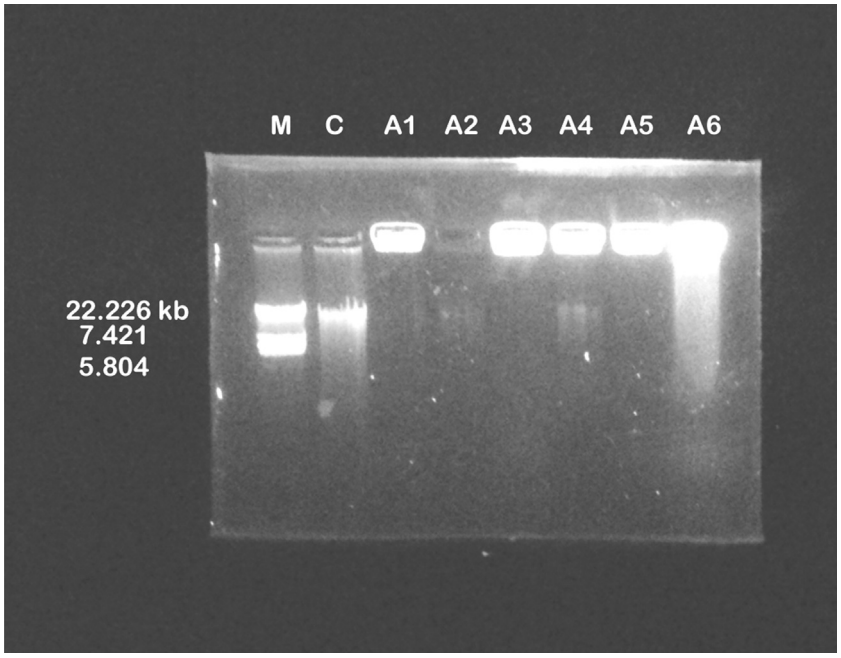


Figure 8 The gel picture of DNA cleavage activity on Calf-thymus DNA. M: Standard Molecular Weight Marker, C: Control DNA, Lanes A1-A6 [Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II)] complexes respectively.

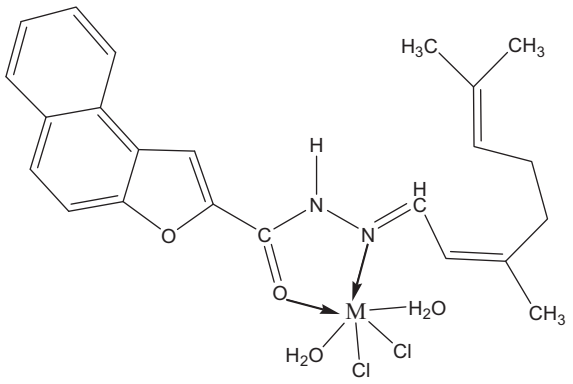


Figure 9 Suggested structure for Cu(II) complex.

5.3. Electrophoretic studies

The interaction of Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes against Calf-thymus DNA at 100 μg m L^{-1} concentration was analyzed with the aid of agarose gel electrophoresis method. The gel picture showing the cleavage is presented in Fig. 8.

Gel electrophoresis works on the migration of DNA under the influence of electric potential. The cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA-binding ability. DNA-binding studies help in the rational designing and construction of new and more efficient drugs targeted to DNA (Waring, 1977). The gel electrophoresis clearly revealed that there was a difference in the migration of the lanes A1–A6 of Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes respectively as compared to the DNA of *E. coli* (Lane – C) at 100 μg concentration. However, the nature of reactive intermediates and the mechanism involved in the DNA cleavage by the complexes is not clear. The results indicated the important role of metal ions in an isolated DNA cleavage reaction. As the compound was observed to cleave the DNA, it can be concluded that, the compound inhibits the growth of pathogenic organism by cleaving the genome (Sangamesh patil et al., 2011).

6. Conclusion

Now we report a neutral bidentate Schiff base with azomethine nitrogen and carbonyl oxygen donor atoms. The bonding of ligand to the metal ions has been confirmed by the analytical data, thermal and various spectral studies. The thermal studies provide useful information about the presence of water molecules and the stability of the complex. Electrochemical studies of Cu(II) complex exhibited one electron transfer quasi-reversible redox couple. The mycological studies revealed that all the complexes are superior to the free ligand and their toxicity has increased upon chelation. The Zn(II) and Hg(II) complexes have exhibited an enhanced antimicrobial activity whereas Co(II), Ni(II), Cu(II) and Cd(II) complexes have shown a moderate activity than its ligand. All the newly synthesized compounds possess higher antifungal properties than the antibacterial property. Electrochemical study indicated that the Cu(II) complex is of a single electron transfer quasi-reversible nature. All compounds showed varying antioxidant activities while Ni(II), Zn(II), and Hg(II) complexes have shown good antioxidant activity compared to the ligand. The DNA cleavage activity studies revealed that all the complexes have the ability to cleave the DNA molecule. All these observations put together led us to propose the octahedral structure to Cu(II) complex (Fig. 9) and dimeric octahedral geometry to Co(II), Ni(II), Cd(II), Zn(II) and Hg(II) complexes (Fig. 10).

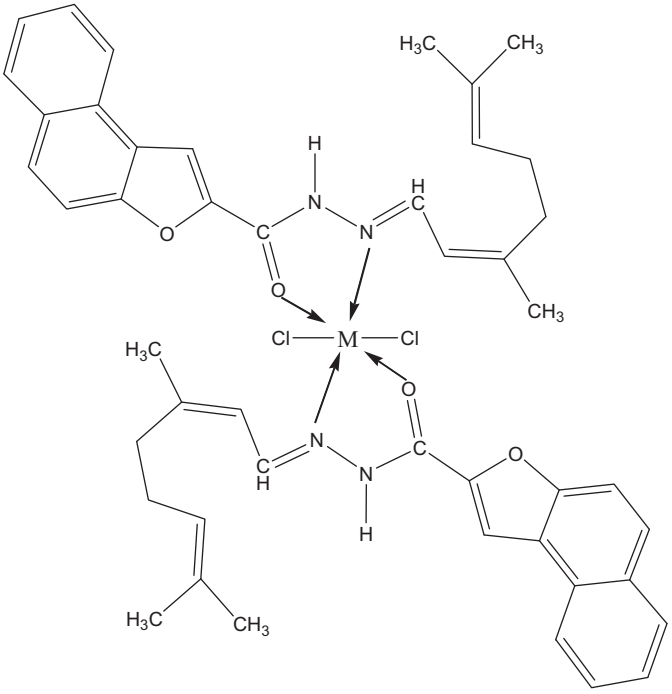


Figure 10 Suggested structure for Co(II), Ni(II), Cd(II), Zn(II) or Hg(II) complexes.

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