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Synthesis, spectral, thermal, fluorescence, antimicrobial, anthelmintic and DNA cleavage studies of mononuclear metal chelates of bidentate 2H-chromene-2-one Schiff base

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Abstract

The Co(II), Ni(II) and Cu(II) complexes have been synthesized with Schiff base (**HL**), derived from 8formyl-7-hydroxy-4-methylcoumarin with benzylamine. The Schiff base and its metal complexes were structurally characterised based on IR, ¹H NMR, ¹³C NMR, UV-visible, ESR, magnetic, thermal, fluorescence, mass and ESI-MS studies. The complexes are completely soluble in DMF and DMSO. The molar conductance values indicate that, all synthesized metal complexes are non-electrolytic in nature. Elemental analysis reveals [ML2(H2O)2] stoichiometry, here M = Co(II), Ni(II) and Cu(II), L = deprotonated ligand. The coordination between metal ion and Schiff base was supported by IR data, through deprotonation of phenolic oxygen of coumarin and azomethine nitrogen atoms. Solution electronic spectral results unveiled that all the synthesized complexes posses six coordinated geometry around metal ion. Thermal studies suggest the presence of coordinated water molecules. The Schiff base and its metal complexes have been screened for their antibacterial (*Escherichia coli, Pseudomonas aureginosa, Klebsiella pneumoniae* and *Staphylococcus aureus*) and antifungal (*Penicillium chrysogenum* and *Aspergillus niger*), anthelmintic (*Pheretima posthuma*) and DNA cleavage (Calf Thymus DNA) activities.

Keywords: Fluorescence; Coumarin Schiff base; DNA Cleavage; Anthelmintic; Metal comple

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1. Introduction

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Coumarin (5,6-Benzo-alpha-pyrone) is a white crystalline oxygen heterocyclic compound, having fluorescence emission properties. It occurs naturally throughout plant kingdom in the form of its derivatives and can be synthesised chemically. Coumarin was first isolated from Tonka bean (Dipteryx odorata) in 1820 by Vogel [1] and synthesised by Perkin in 1868 [2]. Coumarin, a secondary metabolite is reported in plant families such as Umbelliferae, Rutaceae etc. However, higher concentrations are found in fruits followed by roots, stems and leaves. Few chief coumarin compounds have also been reported in microorganisms e.g. novobiocin and coumermycin from *Streptomyces* and aflatoxins from *Aspergillus* species [3, 4]. Since many years, especially organic and medicinal chemists have been carry out their investigation on coumarin, because of its remarkable uses like antimicrobial [5], antioxidant [6], hypotensive [7], anthelmintic [8], inhibition of platelet aggregation [9], cytotoxic activity [10], optical brighteners [11] and fluorescent probes [12]. Since, last few decades, inorganic chemists have paid their considerable attention towards the synthesis of coumarin derivatives because of their enhanced bioactivity upon complexation. Several metal complexes with various Schiff bases, which are well characterized by spectral techniques and bioactivity assays conducted, have been reported from our laboratory [13-16]. 7-Hydroxycoumarin (Umbelliferone) on its structural and biogenetic sense, is regarded as the parent coumarin among structurally more complex coumarins [17]. Generally, bioactivity of various coumarin derivatives depends on the presence and position of hydroxyl groups [18]. A recent study has shown that 7-hydroxycoumarin inhibits the release of Cyclin D1, which is over expressed in many types of cancer. Literature survey revealed that no work has been done on Schiff base metal complexes of aldehydo hydroxy coumarin with benzylamine. Hence, in view of their significant usage, synthesis of new Schiff base derivative of hydroxy coumarin would be worthwhile. All these developments in the synthesis of Schiff base with metal ions are most fascinating. Here, in this investigation we have synthesized the Co(II), Ni(II) and Cu(II) complexes with newly synthesized ON donor Schiff base derived from 8-formyl-7-hydroxy-4-methylcoumarin and benzylamine, which are characterized by spectral (IR, NMR, UV-Vis, Mass, ESI-MS, ESR), fluorescence, thermal and molar conductivities. The synthesised, Schiff base and its metal complexes were studied for their antibacterial, antifungal, anthelmintic and DNA cleavage activities.

2. Experimental

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2.1 Reagents

All chemicals are of reagent grade, used without further purification.

2.2 Physical measurements

Carbon, hydrogen and nitrogen were estimated using TRUSPEC CHN analyzer. IR spectra of Schiff base and its Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the region 4000 - 400 cm⁻¹ using KBr disc. Electronic spectra of metal complexes were recorded in HPLC grade DMF solvent on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200 - 1100 nm. The ¹H NMR and ¹³C NMR spectra of Schiff base were recorded in D₆-DMSO on a BRUKER 400 MHz spectrometer at room temperature using TMS as an internal reference. The fluorescence studies of Schiff base and its metal complexes were carried out on HITACHI F-7000 Fluorescence Spectrophotometer. Mass spectrum of the Schiff base and electro spray mass spectra of the metal complexes were recorded using QP 2010S and THERMO Finnegan LCQ Advantage max ion trap mass spectrometer respectively. The ESR spectrum of Cu(II) complex was recorded on Varian-E-4X-band EPR spectrometer and the field set was 3000 G at modulation frequency of 100 KHz under liquid nitrogen temperature using TCNE as 'g' marker. The electrochemistry of the synthesized complexes was recorded on CHI1110A-electrochemical analyzer (Made in U.S.A.) using dimethyl sulphoxide (DMSO) containing 0.05M n-Bu₄NClO₄ as the supporting electrolyte. Thermo gravimetric analyses data measured for synthesized metal complexes by using a PERKIN-ELMER DIAMOND TG/DTG instrument. Platinum crucible was used for thermal study under nitrogen atmosphere with flow rate of 100 mL/min from a range of room temperature to 1000 °C at a heating rate of 10 C/min. Molar conductivity measurements were recorded at room temperature on ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51 and magnetic susceptibility was determined using Faraday balance for all the synthesized metal complexes.

2.3 Synthesis

2.3.1 Synthesis of 7-hydroxy-4-methylcoumarin [19]

A mixture of dry resorcinol (0.2 mol) and ethylacetoacetate (0.2 mol) was cooled to 0 - 5 °C and conc. sulphuric acid (25 mL) was added gradually with constant shaking. The reaction mixture was then kept in a refrigerator for 24 h. and poured into crushed ice with stirring. The separated solid was filtered washed with water and recrystallized using ethanol as cream colored needles. Yield: 81%; Melting point: 180-182 °C.

2.3.2 Synthesis of 8-formyl-7-hydroxy-4-methylcoumarin [20]

A mixture of 0.03 mol of 7-hydroxy-4-methylcoumarin and 0.07 mol of hexamine in 50 ml of glacial acetic acid were heated for 4-5 h and then 75 mL of 20% hydrochloric acid was added and heating was continued for 20 min. Resulting mixture was cooled and extracted with ether. Pale yellow solid of 8-formyl-7-hydroxy-4-methylcoumarin was obtained after evaporating the ether. Yield: 21.0 %; Melting point: 176-178 °C.

2.3.3 Synthesis of Schiff base HL

The Schiff base (**HL**) has been synthesized (Scheme 1) by refluxing a reaction mixture of hot ethanolic solution (30 mL) of benzylamine and hot ethanolic solution (30 mL) of 8-formyl-7-hydroxy-4-methylcoumarin (0.01mol) for 5-6 h. with the addition of 2-3 drops of glacial acetic acid. The precipitate formed during reflux was filtered, washed with cold ethanol and recrystallized using ethanol. Melting point 189-193 °C.



Scheme 1. Synthesis of Schiff base HL.

2.3.3.1 NMR spectral studies

The Schiff base has been characterized by ¹H NMR and ¹³C NMR to elucidate differently positioned proton and carbon atoms. The ¹H NMR and ¹³C NMR spectrum of **HL** are reproduced in Figs. 1 and 2 respectively.

In the ¹H NMR spectrum of **HL**, a singlet signal at 14.86 ppm is ascribed to phenolic -OH. A characteristic singlet proton signals at 9.09, 4.95 and 2.49 ppm are assigned to -CH=N, $-CH_2$ and $-CH_3$ respectively. In addition to this, the multiplet signals in the 6.05-7.63 ppm range are due to aromatic protons.

In the ¹³C NMR spectrum of **HL**, signals at 173.22, 160.45, 56.84 and 18.33 ppm are due to C=N, C=O, $-CH_2$ and $-CH_3$ respectively. Signals appeared in the region 104.039 - 159.39 ppm are assigned to aromatic carbons.

2.3.3.2 Mass spectrum of the Schiff base

The formation of ligand was further investigated by its mass spectrum analysis. The mass spectrum of **HL** is depicted in Fig. 3, exhibited a molecular ion peak at m/z 293; which is equivalent to its molecular weight. The fragmentation peak at m/z 276 is due to cleavage of -OH group.



Fig. 1. ¹H NMR Spectrum of **HL**.



Fig. 2. ¹³C NMR Spectrum of HL.



Fig. 3. Mass spectrum of HL.

2.3.4 Synthesis of Co(II), Ni(II) and Cu(II) complexes [1-3]

An ethanolic solution of Schiff base (2mmol) (30 mL) was refluxed with CoCl₂.6H₂O/NiCl₂.6H₂O/CuCl₂.2H₂O (1mmol) in ethanol (30 mL) on water bath for 1h. Then, to the reaction mixture 1 mmol of sodium acetate was added and reflux was continued for 3 h. The separated complex was filtered, washed thoroughly with water, ethanol and ether. Finally dried in vacuum over fused CaCl₂.

2.4 Pharmacology

2.4.1 In vitro antibacterial and antifungal activities

The biological activities of the newly synthesized Schiff base and its metal complexes were studied for their antibacterial and antifungal activities by disc diffusion method [21]. The antibacterial and antifungal activities were carried out at 200 µg/mL concentrations of metal complexes dissolved in DMF solvent, against four bacterial species {*Escherichia coli (MTCC-1687), Pseudomonas aureginosa (MTCC-1688), Klebsiella pneumoniae (MTCC-3384)* and *Staphylococcus aureus (MTCC-3160)*} and two fungal species {*Penicillium chrysogenum (MTCC-5108)* and *Aspergillus niger (MTCC-1344)*}. Bacteria and fungi cultures were sub cultured on nutrient agar and potato dextrose agar medium respectively. Bacteria and fungi culture petridishes treated with synthesised Schiff base and its metal complexes were incubated for 24 h and 48 h at 37 °C respectively. The standard antibacterial drug (*Gentamicin*) and antifungal drug (*Fluconazole*) were also screened as explained above.

2.4.2 Anthelmintic activity

The assay was executed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological similarity with intestinal roundworm parasite of human beings [22]. Anthelmintic assay was studied as per the method given in literature [23] with minor modifications. The earthworms, *Pheretima posthuma* collected from moist soil, washed with normal saline (0.5%) for about 30 s to remove all faecal matter, were used for anthelmintic study. Earthworms of 2-4 cm in length were used in this experiment. All the synthesized compounds were subjected to study anthelmintic activity against earthworms at 2 and 10 μ g/mL concentrations. DMSO in distilled water was used as control and Albendazole as a reference drug. The

paralyzing and death times were noted and their mean was calculated for triplicate sets. Death time was recorded by placing earthworms in warm water (50 °C) and observed for stimulated movement, if the worm was alive.

2.4.3 DNA Cleavage activity

DNA cleavage activity was studied for the synthesised Schiff base and its metal complexes using Calfthymus DNA (CT-DNA, Purchased from Bangalore Genei, Bengaluru, Cat. No. 105850). The synthetic compounds were added separately to the CT-DNA, sample mixtures were then incubated at 37 °C for 2h. The cleaved product were run on agarose gel electrophoresis according to the procedure [24] and observed under UV transilluminator for DNA cleavage.

2.4.3.1 Agarose gel electrophoresis

In brief, 250 mg of agarose was dissolved in 25 mL of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) by boiling. The warmed agarose was poured into gel cassette fitted with comb. Later comb was removed carefully after the gel solidified and gel placed in electrophoresis chamber flooded with TAE buffer. 20 μ L of sample mixture, CT-DNA (mixed with bromophenol blue dye @ 1:1 ratio), were loaded carefully into individual wells, along with standard DNA marker and gel was run in electrophoresis chamber by passing constant 50 V of electricity for 45 min. Gel was then immersed in ETBR solution (10 μ g/mL) for 10-15 min to stain and then observed for bands under UV transilluminator and gel band pictures were recorded. The cleavage ability of samples were compared with the CT- DNA and standard DNA marker (λ DNA/ HindIII digest, purchased from Merck, Bengaluru).

3. Results and Discussion

All synthesized Co(II), Ni(II) and Cu(II) complexes of Schiff base are non-hygroscopic coloured solids, stable at room temperature and remarkably soluble in DMF and DMSO. Elemental analysis was carried out using CHN analyzer. Synthesized metal complexes (1-3) having stoichiometry of the type [ML2(H2O)2], here M = Co(II), Ni(II) and Cu(II). Molar conductivity of the metal complexes at 10⁻³ M concentrations were carried out using DMF solvent at room temperature. Results obtained are given in Table 1, which indicate that all synthesized metal complexes have conductivity values in the range, agreeable for non-electrolytic nature.

3.1 Infrared spectra

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IR spectra of Schiff base and its metal complexes are tabulated (Table 2) in order to confirm the coordination mode of Schiff base. In Schiff base spectrum, broad band in the region 3062-3052 cm⁻¹ assigned to v (O-H). Absence of this band in its respective metal complexes spectrum, confirms the deprotonation of hydroxyl group and formation of the M-O bond [25]. The characteristic high intensity band at 1622 cm⁻¹ is attributed to v (C=N) [26, 27]. The strong bands at 1318 and 1717 cm⁻¹ are assigned to phenolic v (C-O) and v (C=O) to lactonyl respectively.

In all synthesized metal complexes, the band assigned to azomethine group is shifted to lower frequency in the region 1605-1601 cm⁻¹ which indicates that chelation takes place through the nitrogen of the azomethine group [28]. The position of the v (C=O) band remained largely unchanged, suggesting that lactone carbonyl oxygen is not involved in coordination to the metal. The strong band in the region 1337-1332 cm⁻¹ of metal complexes is assigned to phenolic v (C-O), these observations supports the formation of v (M-O) bond. Presence of coordinated water molecules in the metal complexes were confirmed by a broad band around 3450 cm⁻¹ and two bands in the region 745-735 cm⁻¹ and 780-770 cm⁻¹ due to v (-OH), rocking and wagging modes of vibrations respectively. The bands observed for metal complexes between 538-522 and 496-484 cm⁻¹ were metal sensitive and are assigned to v (M-O) and v (M-N) [29] respectively. In Figs. 4 and 5 are presented the IR Spectra of the Schiff base **HL** and its Ni(II) complex respectively.



Fig. 4. IR spectrum of the Schiff base HL.

Compound Name / No.	Empirical Formula	C	%	H	%	N %		Colour / % Yield	Molar Conductance
		Calcd.	Found	Calcd.	Found	Calcd.	Found		value Ohm ⁻¹ cm ² mole ⁻¹
HL	C ₁₈ H ₁₅ NO ₃	73.71	73.69	5.15	5.13	4.78	4.76	Light orange / 88%	
1	C ₃₆ H ₃₂ CoN ₂ O ₈	63.63	63.61	4.75	4.78	4.12	4.10	Brown / 61%	11.4
2	C ₃₆ H ₃₂ NiN ₂ O ₈	63.65	63.60	4.75	4.73	4.12	4.11	Light green / 66%	14.4
3	$C_{36}H_{32}CuN_2O_8$	63.20	63.21	4.71	4.70	4.09	4.11	Pale brown / 62%	17.3

Table 1. Elemental analyses of Schiff base (HL) and its metal complexes (1-3) along with molar conductance data.

Table 2. The prominent infrared frequencies (in cm⁻¹) of Schiff base (**HL**) and its metal complexes (1-3).

Compound	Compounds	Coordinated	Lactonyl		Phenolic		
Name / No.		water	$v(C=O)^{s}$	$v(C=N)^{s}$	<i>v</i> (C-O) ^s	$v(M-O)^m$	$v(M-N)^{s}$
		v(O-H) ^{br}					
HL	C ₁₈ H ₁₅ NO ₃	I	1717	1622	1318		
1	Co(C ₁₈ H ₁₄ NO ₃) ₂ . 2H ₂ O	3448	1715	1602	1332	522	496
2	Ni(C ₁₈ H ₁₄ NO ₃) ₂ . 2H ₂ O	3449	1716	1601	1337	527	485
3	Cu(C ₁₈ H ₁₄ NO ₃) ₂ , 2H ₂ O	3452	1717	1605	1337	538	484

^s = strong, ^m = medium, ^{br} = broad.



Fig. 5. IR spectrum of $Ni(C_{18}H_{14}NO_3)_2 H_2O$.

3.2 Solution electronic absorption spectra and magnetic studies

In order to obtain information concerning the stereochemistry of the metal ions, the UV–Vis spectra of the ligand and complexes were recorded in DMF. The Schiff base (**HL**) exhibited two absorption bands around 255 and 330 nm. The spectral data below 350 nm are similar and are ligand centred transitions (intraligand π - π * and n- π *) of aromatic ring and non-bonding electrons present on the nitrogen of the azomethine group in the Schiff base [30, 31].

Solution electronic absorption spectra of the synthesized metal complexes are recorded in DMF (Table 3). The electronic spectrum of Co(II) complex exhibited absorption band in the region at 568 and 980 nm, corresponding to ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F)(v_{1})$ and ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)(v_{3})$ transitions respectively. These bands are characteristic of high spin octahedral Co(II) complexes [32]. Observed magnetic moment of Co(II) complex was 4.90 BM and it supports octahedral geometry for Co(II) complex [33].

The spectrum of light green Ni(II) complex exhibited three bands at 989, 674 and 365 nm are attributed to the ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{2g}(F)(v_1)$, ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)(v_2)$ and ${}^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(P)(v_3)$ transitions respectively, which indicate octahedral geometry around Ni(II) ion [34]. Magnetic moment value obtained was 3.15 BM, which is agreeable with the octahedral environment around Ni(II) ion [35, 36].

The electronic spectrum of Cu(II) complex (Fig. 6) showed two absorption bands. A low intensity broad band in the region 690 nm is assignable to ${}^{2}E_{g} \rightarrow {}^{2}T_{2g}$ transition in support of distorted octahedral configuration of metal complex [37]. Another high intensity band at 391 nm is due to symmetry forbidden ligand \rightarrow metal charge transfer. Obtained magnetic moment value for Cu(II) complex is 1.77 BM, further it supports the electronic spectral data [38, 39].



Fig. 6. Electronic absorption spectrum of Cu(II) complex.

Table 3. Solution electronic spectral results along with magnetic susceptibility data of metal complexes (1-3).

Complex No.	λ_{\max} in cm ⁻¹ (nm)	Band assignments	Geometry	µ _{eff} (BM)
1	10204 (980) 17605 (568)	${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F) (v_{1})$ ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P) (v_{3})$	octahedral	4.90
2	10111 (989) 14836(674) 27397(365)		octahedral	3.15
3	14492 (690) 25575 (391)	$\begin{array}{c} {}^{2}T_{2g} \leftarrow \overset{2}{E}_{g}\\ L \rightarrow M \end{array}$	distorted octahedral	1.77

3.3 Electron spin resonance

Fig. 7 shows the ESR spectrum of $Cu(C_{18}H_{14}NO_3)_{2.}2H_2O$ was recorded in X band spectrometer (microwave frequency of 9.1GHz and field set was 3000 G at modulation frequency of 100 KHz) under liquid nitrogen temperature in DMSO with g factor quoted relative to standard marker TCNE (g = 2.0027). ESR

spectra of the metal chelates provide important information regarding the environment around the metal ion in the complexes.

The ESR spectrum of Cu(II) complex show typical axial behaviour with slightly different g^{\parallel} and g^{\perp} values. The g^{\parallel} , g^{\perp} , g_{av} and G values of Cu(II) complex calculated from ESR spectrum was 2.0692, 2.0402, 2.0498 and 1.7651 respectively. The trend $g^{\parallel}(2.0692) > g^{\perp}(2.0402) > g_e$ (2.0023) observed for copper complex suggest that, unpaired electron is localized in d_{x2-y2} orbital of Cu(II) ion with 3d⁹ configuration [40].

Here, $g^{\parallel} < 2.3$ is characteristic of a covalent nature of the M-L bonding. The ESR data provide further evidence to magnetic and solution electronic spectral results.



Fig.7. ESR spectrum of Cu(II) complex (3).

3.4 Fluorescence Spectra

As we know that coumarin itself shows good fluorescent material, we studied fluorescent effect of synthesized coumarin Schiff base and its metal (II) complexes at room temperature. The Schiff base showed λ_{max} value at 502 and 507 nm in DMSO and DMF respectively (Fig 8). Upon addition of 2-3 drops of 2% NaOH to the Schiff base solution, we observed λ_{max} at 499 nm in DMSO and 501 nm in DMF (Fig. 9). The shift of λ_{max} to lower wavelength (*i.e.* blue shift) after addition of 2-3 drops of aqueous alkali indicates that, proton transferred (H-bonded ion pair) species exist in equilibrium [41, 42]. The spectra of ligand and its metal complexes have been recorded in 10⁻³ M DMF solution. Fig. 10 shows the overlapping spectra of ligand and its

Co(II), Ni(II) and Cu(II) complexes in DMF. Here, ligand exhibits strong fluorescence intensity compared to other three metal complexes. The weak intensity of band corresponding to complexes is due to decrease in electron density on ligand [43, 44]. The decreased fluorescence intensity of synthesized metal complexes (1-3) compared with free ligand (HL) is in the order Ni(II) > Co(II) > Cu(II).



Fig. 8. Emission spectra of Schiff base (HL) in DMSO and DMF.



Fig. 9. Fluorescence spectra of Schiff base (HL): before and after adding 2% NaOH in DMSO and DMF.



Fig. 10. Fluorescence spectra of Schiff base (HL) and its metal complexes (1-3).

3.5 ESI-Mass studies of metal complexes

The ESI-Mass spectra of Co(II) (1), Ni(II) (2) and Cu(II) (3) complexes showed a M+1 peak at m/z 681, 680 and 685 respectively, which are equivalent of their molecular weight, supports the formation of respective metal complexes of type ML2(H2O)2 and a base peak at m/z 292 is due to the formation of deprotonated ligand. The representative Ni(C₁₈H₁₄NO₃)_{2.}2H₂O is shown in Fig. 11.



Fig. 11. Mass spectrum of $Ni(C_{18}H_{14}NO_3)_{2.}2H_2O$.

3.6 *Thermo gravimetric analysis*

As a function of temperature, thermal features of the synthesized Co(II), Ni(II) and Cu(II) complexes are studied. The obtained results are tabulated in Table 4. Peaks in the regions 170-225 and 330-850 °C are corresponding to mass loss of coordinated water molecules and organic moieties respectively. Finally, the metal complexes decompose gradually with the formation of metal oxide above 850 °C. The representative TG/DTG spectrum of Cu(C₁₈H₁₄NO₃)_{2.}2H₂O is shown in Fig. 12. Here, decomposition of coordinated water molecules occurred in the region 170-225 °C centred at 203 °C and loss of organic moieties occurred in between 330-850 °C. Thus, TG and DTG data provide useful information on the presence coordinated water molecules and stability of metal complexes.

Compound	Decomposition	Weight	loss (%)	Metal oxide (%)		Inference
name	temperature (°C)	Found	Calcd.	Found	Calcd.	
1	173-183	5.31	5.30	11.02	11.04	Loss of coordinated water
						molecules
	345-433	83.67	83.66			Loss of organic moieties
2	187-198	5.34	5.30	10.91	10.92	Loss of coordinated water
						molecules
	340-459	83.79	83.78			Loss of organic moieties
3	170-225	5.28	5.27	11.54	11.57	Loss of coordinated water
						molecules
	330-850	83.18	83.16			Loss of organic moieties

Table 4. Thermo gravimetric data of metal complexes (1-3).



Fig. 12. TG/DTG spectrum of $Cu(C_{18}H_{14}NO_3)_2 H_2O$.

3.7 Electrochemistry

The redox behavior of the synthesized Cu(II) complex was investigated in DMSO by cyclic voltammetric studies using a glassy carbon working electrode. Fig. 13, illustrate a cyclic volammogram of Cu(C₁₈H₁₄NO₃)_{2.}2H₂O, it shows a oxidation peak at E_{pa} = 0.3270V with a corresponding reduction peak at E_{pc} = 0.1450V. The ratio of the anodic and cathodic peak currents (Ipc/Ipa≈1), provide a rough evaluation of the degree of the reversibility of one electron transfer reaction. Copper complex had a large separation between the cathodic and anodic peak of 182 mV and it increase with the scan rates, indicating a quasi-reversible character [45, 46].



Fig. 13. Cyclic voltammogram of Cu(II) complex.

3.8 Antimicrobial activity

Figs. 14 and 15, shows the results of antibacterial and antifungal activities of all synthesized compounds. By observing the results it was found that, metal complexes exhibited higher lethal effect on bacteria and fungi growth than their parent ligand. The Cu(II) complex showed promising antibacterial and antifungal activity against all strains. It is suspected that, factors such as solubility, conductivity, dipole moment and cell permeability mechanism (influenced by the presence of metal ions) may be the possible reasons for increase in activity.



Fig. 14. Bacterial growth inhibition in millimetres (mm) for different compounds at 200 µg/mL concentration,



Gentamicin used as reference compound.

Fig. 15. Fungal growth inhibition in millimetres (mm) for different compounds at 200 μg/mL concentration, Fluconazole used as reference compound.

3.09 Anthelmintic activity

Table 5 shows, anthelmintic activity results of all the synthesized compounds. The obtained results clearly indicate that metal complexes are more active than their parent ligand. In particularly Cu(II) complex i.e. compound (3) showed prominent activity, compared with the standard drug Albendazole at 10 μ g/mL concentration.

Compound	Conc.(µg/mL)	Time of paralysis (min)	Time of death (min)
Albendazole	10	3.48 ± 0.06	7.25±0.14
DMSO ^a			
HL	2	20.18±0.02	24.30±0.12
	10	9.28±0.21	14.29±0.11
1	2	12.14±0.14	19.50±0.02
L	10	7.40±0.04	10.12±0.03
2	2	16.10± 0.25	21.20±0.09
2	10	9.13±0.01	15.51±0.00
2	2	10.27±0.03	16.41±0.06
3	10	5.39±0.22	9.31±0.01

Table 5. Anthelmintic studies of Schiff base (HL) and its metal complexes (1-3).

^aDMSO as control.

3.10 DNA Cleavage Activity

The DNA cleavage activity of Schiff base and its metal complexes were studied by agarose gel electrophoresis method using calf thymus DNA(CT-DNA). The CT- DNA cleavage activity of Schiff base **HL** and its complexes are shown in Fig. 16. M- is standard DNA molecular weight marker, C- is Control CT-DNA i.e. untreated CT-DNA and lanes **1-3** and **HL** are CT-DNA treated with different metal complexes and Schiff base respectively. Gel electrophoresis works on, migration of DNA under the influence of electric potential.

From the gel picture, we found that compound $Cu(C_{18}H_{14}NO_3)_2.2H_2O$ shown complete cleavage, whereas $Co(C_{18}H_{14}NO_3)_2.2H_2O$, $Ni(C_{18}H_{14}NO_3)_2.2H_2O$ and Schiff base HL shown partial cleavage. As Cu(II) complex cleaved CT-DNA completely, this implies that Cu(II) complex is more effective than the other metal complexes.



Fig. 16. DNA Cleavage activity of Schiff base (HL) and its metal complexes (1-3).

4. Conclusion

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The newly synthesized Schiff base act as bidentate ligand through azomethine nitrogen and phenolic oxygen atoms of coumarin moiety. The bonding of ligand to metal ion was confirmed by various spectral studies. Antimicrobial study reveals that, Cu(II) complex was found to be active against all the bacterial and fungal species. Cu(II) complex (**3**) also showed prominent anthelmintic and DNA cleavage activities. It reveals that, the Cu(II) complex is more effective than other synthesized compounds. All these observations put together lead us to propose the following structure (Fig. 17) in which, metal complexes having stoichiometry of type [ML2(H2O)2] (M=Co(II), Ni(II) and Cu(II), L= deprotonated Schiff base).



Fig. 17. Proposed structures of metal complexes (1-3).

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Highlights

- Characterization of metal complexes using various spectroscopic techniques. •
- Fluorescence emission study reveal the quenching of Schiff base fluorescence. •
- Cu(II) complex of coumarin Schiff base has shown promising biological activities. •

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Graphical abstract : Synthesis of Schiff base HL.

High resolution figures (3,4,11 and 11) of the paper [JPB_9997]







Fig. 4. IR spectrum of the Schiff base HL.



Fig.7. ESR spectrum of Cu(II) complex (3).



Fig. 11. Mass spectrum of $Ni(C_{18}H_{14}NO_3)_2 H_2O$.