

Original Article

SYNTHESIS, CHARACTERIZATION, DNA CLEAVAGE AND ANTIMICROBIAL ACTIVITIES OF SCHIFF BASE LIGAND DERIVED FROM 5-CHLORO-3-PHENYL-1H-INDOLE-2-CARBOXYHYDRAZIDE AND O-VANILLIN AND ITS METAL (II) COMPLEXES

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ABSTRACT

Objective: The main objective of the present work was to synthesize a new Schiff base ligand 5-chloro-N'-(2-hydroxy-3-methoxybenzylidene)-3-phenyl-1H-indole-2-carbohydrazide and its Cu (II), Co (II), Ni (II) and Zn (II) complexes and study their biological activity.

Methods: The compounds are characterized by elemental analysis and various physicochemical techniques like IR, ¹H NMR, ESI-mass, UV-Visible, TGA analyzes and molar conductance data. All the compounds were screened for their antibacterial and antifungal activity by MIC method. Further, antioxidant activity was performed by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and DNA cleavage activity by Agarose Gel Electrophoresis (AGE) method.

Results: Spectral investigations suggested octahedral coordination geometrical arrangement for all the metal (II) complexes, having 1:2 stoichiometric ratio of the type [M(L)₂]. The antimicrobial activity results revealed that the metal complexes were found to be more active than the free ligand. Furthermore, the DNA cleavage activity of the compounds on plasmid DNA pBR322 molecule showed moderate activity.

Conclusion: The newly synthesized ligand acts as ONO donor tridentate chelate and coordinated through an oxygen atom of carbonyl function, azomethine nitrogen and phenolic oxygen atom to the Cu, Co, Ni and Zn metal ions and form octahedral geometrical arrangement. All the complexes are found to be non-electrolytic in nature. Further, all the newly prepared compounds showed moderate biological activity.

Keywords: Indole, *o*-Vanillin, Transition metal complexes, Powder XRD, ESR, DNA cleavage Antimicrobial, Antioxidant

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INTRODUCTION

Schiff base ligands and their transition metal complexes have gained a great deal of attention to the researchers worldwide in view of their potential biological importance *viz.*, antibacterial, antifungal, antioxidant and anticancer activities [1]. Metal complexes of Schiff base ligand have revealed preferable enhancement in their activities after complexation as compared to uncoordinated ligand [2, 3]. The biological relevance of the metal complexes has been rationalized and found to be generally dependent on the nature of the metal ions, its oxidation state, nature and the type of the coordinated ligand and isomers [4-6]. Transition metal complexes surrounded by oxygen and nitrogen donor Schiff base ligands, which can be modified by choosing the suitable amine precursors and the ring may serve in coordination field [7]. Also, Schiff bases and their metal complexes have gained interest owing to their structural diversity and a wide range of potential biological activities such as anticancer, antitumor, antibacterial, antifungal, antitubercular, antioxidant, anti-inflammatory and corrosion inhibition [8-12].

The *o*-vanillin is the most prominent principle flavor and aroma compound in vanilla which is used as a food flavoring agent in foods, beverages, and pharmaceuticals [13]. Also, it possesses numerous biological activities such as anti-inflammatory, analgesic and antiviral activities. It is extensively studied in the medicinal field [14, 15]. In addition to this, it can also be used as an efficient herbicide, pesticide, bactericide [16] and the Schiff bases derived from *o*-vanillin moiety form stable complexes with metal ions [17, 18]. Hence, *o*-vanillin is an optimal candidate for the synthesis of various Schiff base ligands and their metal complexes with significant biological activities. Accordingly, we intend to report here the synthesis, spectral characterization, DNA cleavage and antimicrobial activities of novel Schiff base ligand derived from the reaction between 5-chloro-3-phenyl-1H-indole-2-carboxyhydrazide and *o*-

vanillin and its metal complexes. The newly prepared compounds showed moderate biological activities.

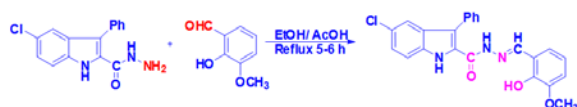
MATERIALS AND METHODS

All the chemicals used were of high purity grade; solvents were dried and distilled before use. Melting points were determined by electrothermal apparatus using open capillary tubes. Metal and chloride contents were determined as per standard procedures [19]. The precursor 5-chloro-3-phenyl-1H-indole-2-carboxyhydrazide was prepared by as per literature method [20] and *o*-vanillin procured from Sigma-Aldrich Chemical Company India.

The IR Spectra were recorded as KBr pellets on a Perkin Elmer-Spectrum RX-I FTIR instrument (4000-400 cm⁻¹). Elemental analysis was obtained from Perkin Elmer 2400 CHN Elemental Analyser. ¹H NMR spectra of the ligand and its Zn complex were recorded on FT NMR Spectrometer model Avance-II (Bruker), 400 MHz instrument using *d*₆-DMSO as a solvent. ESI-mass spectra were recorded on mass spectrometer equipped with electrospray ionization (ESI) source having a mass range of 4000 amu in quadruple and 20000 amu in ToF. UV-Visible spectra of Cu, Co and Ni complexes were recorded on Elico-SL 164 double beam UV-Visible spectrophotometer in the range 200-1000 nm in DMF solution at 1×10⁻³M concentration. The ESR spectra of the Cu complex in the polycrystalline state were recorded on BRUKER Bio-Spin GmbH spectrometer at a microwave frequency of 9.1 GHz. The experiment was carried out by using DPPH as a reference with the field set at 3000 Gauss using tetracyanoethylene as the "g" marker (g = 2.0023). Powder XRD of the complexes was recorded on Bruker AXS D8 Advance (Cu, Wavelength 1.5406 Å sources). Molar conductivity measurements were recorded on an ELICO CM-180 conductivity bridge in dry 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.

Synthesis of Schiff base ligand (L)

An equimolar mixture of 5-chloro-3-phenyl-1*H*-indole-2-carboxyhydrazide (0.001 mol) and *o*-vanillin (0.001 mol) in methanol (25 ml) with 1-2 drops of glacial acetic acid as a catalyst was refluxed on a water bath for about 4-5 h. Yellow colored solid separated in hot, was filtered, washed with hot ethanol, dried and recrystallized from 1, 4-dioxane (Scheme 1). The reaction was monitored by TLC.



Scheme 1: Synthesis of Schiff base ligand (L)

Preparation of metal (II) complexes

To the hot solution of 5-chloro-*N'*-(2-hydroxy-3-methoxybenzylidene)-3-phenyl-1*H*-indole-2-carboxyhydrazide (0.002 mol) in ethanol (30 ml) was added a hot ethanolic solution (15 ml) of respective metal chlorides (0.002 mol). The reaction mixture was refluxed on a water bath for about 5 h. Sodium acetate (0.5 g) was added to the reaction mixture to maintain a neutral pH and to reflux was continued for 1 h more. The reaction mixture was poured into distilled water. The solid colored complexes separated were collected by filtration, washed with sufficient quantity of distilled water, then with hot ethanol to apparent dryness and dried in a vacuum over anhydrous calcium chloride in a desiccator.

Antibacterial and antifungal assay

The newly synthesized ligand and its Cu (II), Co (II), Ni (II) and Zn (II) complexes were screened for their antibacterial and antifungal activities by using Muller-Hinton agar and potato dextrose agar (PDA) diffusion methods respectively [21]. These activities were carried out in four different concentrations (100, 50, 25 and 12.5 µg/ml in DMSO solvent) against, *Escherichia coli* (MTCC 46), *Salmonella typhi* (MTCC 98) and *Bacillus subtilis* (MTCC 736). The antifungal activities were carried out against *Candida albicans* (MTCC 227), *Cladosporium oxysporum* (MTCC 1777) and *Aspergillus niger* (MTCC 1881) by a minimum inhibitory concentration (MIC) method. The above-mentioned organisms were obtained from the Department of Microbiology, Gulbarga University, Kalaburagi, Karnataka, India, which were previously procured from Institute of Microbial Technology Chandigarh (IMTC), India. The lowest concentration of each tested compound where the growth of bacteria/fungi was clearly inhibited is reported as MIC. The results were compared with the Gentamycin and Fluconazole, a broad-spectrum antibiotic for bacterial and fungal strains respectively. The experiment was done in triplicate, and the average values were presented.

DNA cleavage experiment

The extent to which the newly synthesized ligand and its metal complexes could function as DNA cleavage agents was examined using Calf-thymus DNA (Cat. No. 105850) as a target molecule. The electrophoresis method was employed to study the efficiency of cleavage by the synthesized compounds. Each test compound (100 µg) was added separately to the 225ng DNA sample, and these sample mixtures were incubated at 37 °C for 2 h. The electrophoresis of the test compounds was done according to the literature method [22]. Agarose (600 mg) was dissolved in hot tris-acetate-EDTA (TAE) buffer (60 ml) (4.84 g Tris base, pH-8.0, 0.5 M EDTA L-1) and heated to boil for a few minutes. When the gel attains approximately 55 °C, it was then poured into the gas cassette fitted with a comb. Slowly the gel was allowed to solidify by cooling to room temperature and then carefully the comb was removed. The solidified gel was placed in the electrophoresis chamber containing TAE buffer. The DNA sample (20 µL) initially treated with the test compounds were mixed with bromophenol blue dye in equimolar ratios along with standard DNA marker containing TAE buffer was loaded carefully into the wells and the constant 50 V of electricity was supplied for about 30 min. Later, the gel was removed, stained with ethidium bromide solution (10 µg ml⁻¹) for 15-20 min and then the bands were observed and photographed under UV-illuminator.

Antioxidant assay (Free radical scavenging activity)

The Free radical scavenging activity of the test samples was determined by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method [23]. Different concentrations of test compounds (12.5 µg, 25 µg, 50 µg and 100 µg) and standard butylated hydroxyanisole (BHA) were taken in different test tubes, and the volume of each test tube was adjusted to 100 µL by adding distilled DMF. To the tubes containing sample solutions in DMF, 5 ml methanolic solution of DPPH (0.1 mM) was added to these tubes. The tubes were allowed to stand for 30 min. The control experiment was carried out as above without the test samples. The absorbance of test solutions was measured at 517 nm. The reduction of DPPH was calculated relative to the measured absorbance of the control. Radical scavenging activity was calculated using the following formula:

$$\% \text{ of Radical Scavenging activity} = \left[\frac{\text{Control Optical Density} - \text{Sample Optical Density}}{\text{Control Optical Density}} \right] \times 100$$

RESULTS AND DISCUSSION

All the newly synthesized metal complexes are colored solids, amorphous and non-hygroscopic in nature and possess high melting points (>300 °C). The complexes are insoluble in water and common organic solvents but are soluble in DMF and DMSO. Elemental analysis and analytical data (table 1) of the complexes suggest that the metal to ligand ratio of all the complexes were, 1:2 stoichiometry of the type [M (L)₂]. The molar conductance values are too low to account for any dissociation of the complexes in DMF (17-32 ohm⁻¹ cm² mole⁻¹), indicating their non-electrolytic nature.

Table 1: Physical, analytical and molar conductance data

Compounds	M. W.	M. P. (°C)	Color (Yield %)	Elemental Analysis, Calc. (Found) [%]			λ _m	μ _{eff} (BM)
				C	H	N		
C ₂₃ H ₁₈ N ₃ O ₃ Cl (L)	419	269	Yellow (71)	65.87 (65.82)	4.29 (4.22)	10.02 (10.01)	--	--
[Cu(C ₂₃ H ₁₇ N ₃ O ₃ Cl) ₂]	899.5	>300	Green (58)	61.36 (61.33)	3.77 (3.74)	9.33 (9.31)	7.06 (7.00)	32
[Co(C ₂₃ H ₁₇ N ₃ O ₃ Cl) ₂]	894.9	>300	Brown (55)	61.68 (61.63)	3.79 (3.72)	9.38 (9.40)	6.58 (6.40)	21
[Ni(C ₂₃ H ₁₇ N ₃ O ₃ Cl) ₂]	894.6	>300	Brown (53)	61.70 (61.75)	3.80 (3.79)	9.38 (9.36)	6.55 (6.49)	17
[Zn(C ₂₃ H ₁₇ N ₃ O ₃ Cl) ₂]	901.3	>300	Orange (55)	61.23 (61.22)	3.77 (3.75)	9.31 (9.37)	7.25 (7.30)	31

M. W.: Molecular weight; M. P. Melting point

IR spectral studies

The important IR bands of the ligand and its metal complexes are represented in table 2. In the IR spectrum of ligand, absorption due to NH of indole displayed band at 3276 cm⁻¹ and absorption due to NH of

CONH function at 3061 cm⁻¹. Two sharp peaks observed at 1657 and 1604 cm⁻¹ are due to carbonyl and azomethine functions respectively.

In the IR spectra of all the metal complexes, it was observed that the absence of absorption band due to phenolic OH at 3309 cm⁻¹ of

ligand indicates the formation of a coordination bond between the metal ion and phenolic oxygen atom *via* deprotonation. This is further confirmed by the increase in absorption frequency about 72-83 cm^{-1} of phenolic $\nu(\text{C-O})$ which appeared in the region 1303-1314 cm^{-1} in all the complexes indicating the participation of oxygen atom of phenolic oxygen in coordination. The absorption bands due to NH of indole and NH of CONH function displayed the bands in the region 3201-3220 cm^{-1} and 3051-3054 cm^{-1} , which have appeared at about the same region as in the case of ligand, thus confirming their non-involvement in coordination. The absorption frequency of carbonyl

and azomethine functions which have appeared at 1657 and 1604 cm^{-1} in case of the ligand, have been shifted to lower frequency by 38-55 and 20-60 cm^{-1} respectively in all the metal complexes and appeared in the region 1619-1602 cm^{-1} and 1544-1584 cm^{-1} indicating the involvement of oxygen atom of carbonyl function as such without undergoing enolization [24] and nitrogen atom of azomethine [25] function in complexation with the metal ions. This is further confirmed by the appearance of new bands in the region 522-508 cm^{-1} and 462-429 cm^{-1} are due to M-O and M-N stretching vibrations [26] in all the complexes.

Table 2: IR spectral data (cm^{-1}) of Schiff base ligand (L) and its metal complexes

Ligand/Complexes	ν_{OH} (phenolic)	ν_{NH} (amide)	ν_{NH} (indole)	$\nu_{\text{C=O}}$ (carbonyl)	$\nu_{\text{C=N}}$ (azomethine)	$\nu_{\text{C-O}}$ (phenolic)	$\nu_{\text{M-O}}$	$\nu_{\text{M-N}}$
L	3309	3276	3061	1657	1604	1231	--	--
[Cu(L) ₂]	--	3201	3054	1610	1564	1314	522	462
[Co(L) ₂]	--	3220	3053	1608	1544	1303	509	432
[Ni(L) ₂]	--	3209	3052	1619	1584	1305	510	430
[Zn(L) ₂]	--	3218	3051	1602	1546	1304	508	429

¹H NMR spectral data

The ¹H NMR spectrum of ligand (L) displayed three singlets each at 12.160, 11.627 and 11.486 ppm are due to the proton of phenolic OH, amide NH and NH attached to indole moiety respectively. Azomethine proton (CH=N) has resonated as a singlet at 8.399 ppm. The signals due to eleven aromatic protons (ArH) have appeared as multiplets in the region 6.854-7.534 ppm and single appeared at 3.695 ppm is due to three protons of the methoxy group of *o*-vanillin moiety respectively. The Schiff base ligand (L) upon complexation with Zn (II) ion showed the disappearance of signal due to the

proton of phenolic OH confirms the involvement of bonding of phenolic oxygen to metal ion *via* deprotonation. The signals due to amide NH and indole NH have appeared at 11.814 ppm and 11.624 ppm respectively. The signal due to azomethine proton has (CH=N) resonated at 8.699 ppm. The signals due to eleven aromatic protons (ArH) have resonated as multiplets in the region 6.834-7.390 ppm and signal at 3.813 ppm are due to three protons of the methoxy group of *o*-vanillin moiety respectively. When compared to the NMR spectral data of the ligand and its [Zn(L)₂] complex, all the signals due to protons have been shifted towards down field strength confirming the complexation.

Table 3: ¹H NMR spectral data (ppm) of Schiff base ligand (L) and its [Zn(L)₂] complex

Ligand/ complex	¹ H NMR data (ppm)
L	12.160 (s, 1H, Phenolic OH), 11.627 (s, 1H, CONH), 11.486 (s, 1H, indole NH), 8.399 (s, 1H, HC=N), 6.854-7.374 (m, 11H, ArH), 3.695 (s, 3H, OCH ₃).
[Zn(L) ₂]	11.814 (s, 1H, CONH), 11.624 (s, 1H, indole NH), 8.699 (s, 1H, HC=N), 6.834-7.390 (m, 11H, ArH), 3.813 (s, 3H, OCH ₃).

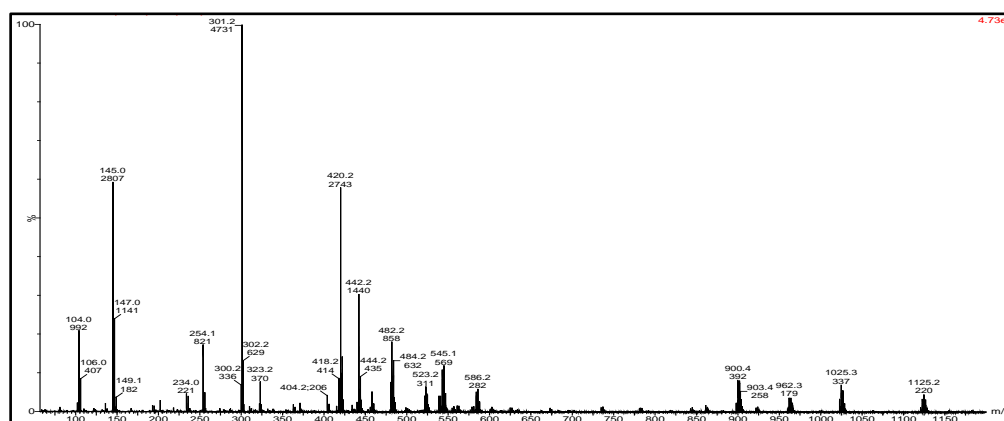


Fig. 1: ESI mass spectrum of [Cu(L)₂] complex

ESI-mass spectral data

The mass spectra of Ligand (L), [Cu(L)₂], [Co(L)₂] and [Ni(L)₂] complexes showed a molecular ion peak recorded at m/z 420, 422 (100%, 33%), M^{+1} 900, 902 (9%, 3%) (fig. 1), 894, 896 (60%, 20%) and M^{+1} 895, 897 (6%, 2%) respectively which are equivalent to their molecular weights.

Electronic spectral studies

The green colored [Cu(L)₂] complex displayed a low-intensity single broad band in the region 15462-17924 cm^{-1} . The broadness of the

band designates 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 give rise to only one broadband and the broadness of the band may be due to dynamic Jahn-Teller distortion. The obtained data suggest the distorted octahedral geometry around the Cu (II) ion [27].

The [Co(L)₂] complex under present study displayed two absorption bands at 16889 cm^{-1} and 20103 cm^{-1} . These bands are assigned to be ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)(\nu_2)$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)(\nu_3)$ transitions, respectively, which are in good agreement with the literature values for octahedral geometry [28, 29]. The lowest band, ν_1 could not be

observed due to the limited range of the instrument used, but it could be calculated using the band fitting procedure suggested by Underhill and Billing [30]. The calculated ν_1 value is presented in table 4. These transition values suggest the octahedral geometry of the Co (II) complex.

The [Ni(L)₂] complex under present investigation exhibited two absorption bands in the region 15098 cm⁻¹ and 25092 cm⁻¹, which are assigned to ³A_{2g} → ³T_{1g} (F) (ν_2) and ³A_{2g} (F) → ³T_{1g} (P) (ν_3) transitions respectively in an octahedral environment [31]. The transition value of band ν_1 was calculated by using a band fitting procedure [31]. The proposed octahedral geometry for the complexes was further supported by the calculated values of ligand field

parameters, such as Racah interelectronic repulsion splitting parameter (B'), the nephelauxetic parameter (β), ligand field splitting energy (10 Dq) and ligand field stabilization energy (LFSE) [31].

The calculated B' values for the [Co (L)₂] and [Ni(L)₂] complexes are lower than the free ion values, which is due to the orbital overlap and delocalization of d-orbitals. The β values are important in determining the covalency for the metal-ligand bond, and they were found to be less than unity, suggesting a considerable amount of covalency for the metal-ligand bonds. The β value for the [Ni (L)₂] complexes was less than that of the [Co (L)₂] complexes, indicating the greater covalency of the Metal-Ligand (M-L) bond.

Table 4: Electronic spectral data

Complexes	Transitions in cm ⁻¹			Dq (cm ⁻¹)	B' (cm ⁻¹)	β	$\beta\%$	ν_2/ν_1	LFSE (k cal.)
	ν_1^*	ν_2	ν_3						
[Cu(L) ₂]	15462-17924			--	--	--	--	--	28.61
[Co(L) ₂]	7879	16889	20103	901	890	0.916	8.34	2.14	15.44
[Ni(L) ₂]	9310	15098	25092	931	817	0.785	21.44	1.62	31.92

*Calculated values

Magnetic susceptibility studies

The room temperature magnetic measurements were obtained for paramagnetic Co (II), Ni (II) and Cu (II) complexes. The observed magnetic moment for Cu (II) complex is 1.86 BM which attributes to one unpaired electron with a slight orbital contribution to the spin only a value of 1.73 BM and the absence of spin-spin interactions in the complex accounting for the possibility of a distorted octahedral geometry [32].

In octahedral Co (II) complex the ground state is ⁴T_{1g} and the orbital contribution to the singlet state lowers the magnetic moment values for the various Co (II) complexes which are in the range 4.12-4.70 and 4.70-5.20 BM for tetrahedral and octahedral complexes respectively [33]. In the present study, the observed magnetic moment values for Co (II) complexes are 5.01 BM indicates octahedral geometry for Co (II) complex. For Ni (II) complex, the observed magnetic moment value is 2.93 BM which is well within the expected range of Ni (II) complex with octahedral geometry, i.e. 2.83-3.50 BM [34].

Thermal studies

The thermal stabilities for Cu (II), Co (II), Ni (II) and Zn (II) complexes been studied as a function of temperature. The proposed stepwise thermal degradation of the complexes with respect to temperature and the formation of respective metal oxides are depicted in table 5.

TG-DTA curve of Cu (II) complex showed that the first stage of decomposition represents a weight loss of two OCH₃ groups of vanillin moieties at 249 °C with a practical weight loss of 7.26% (Cald. 6.89%). The resultant complex underwent the second stage of degradation and gave a break at 345 °C with a practical weight loss of 36.26% (Cald. 36.17%), which corresponds to the loss of C₁₄H₉NCl species of indole moiety and a C₆H₅ group. Thereafter, the compound showed decomposition in a gradual manner rather than with the sharp decomposition up to 627 °C and onwards due to the loss of the remaining organic moiety. The weight of the residue corresponds to cupric oxide.

The thermogram of Co (II) complex showed the first stage of decomposition at 388 °C with a practical weight loss of 7.26% (Cald. 6.92%), which corresponds to weight loss due to two OCH₃ groups of vanillin moieties. Further, the complex underwent decomposition and gave a break at 400 °C with a practical weight loss of 73.70% (Cald. 73.59%), corresponds to weight loss of a deprotonated ligand and C₁₄H₉NCl species of indole moiety. Thereafter the complex showed gradual decomposition up to 715 °C with a weight loss of the remaining organic moiety, the weight of the residue corresponds to cobalt oxide.

In the thermogram of the Ni (II) complex, the first stage of decomposition represents the weight loss due to OCH₃ groups of vanillin moieties at 378 °C with a practical weight loss of 5.70% (Cald. 6.93%). The resultant complex underwent further degradation and gave a break at 405 °C with a practical weight loss of 31.13% (Cald. 31.46%), which corresponds to the loss due to a C₁₄H₁₀NCl species of indole moiety and a chlorine atom. Thereafter, the compound showed a gradual decomposition up to 715 °C with a weight loss of remaining organic moiety. The weight of the residue corresponds to nickel oxide.

In the case of Zn (II) complex, the first stage of decomposition occurs at 364 °C with a practical weight loss of 5.91% (Cald. 6.87%), which represents the loss due to two OCH₃ groups of vanillin moieties. Further, the complex underwent the second stage of decomposition and gave a break at 395 °C with a practical weight loss of 44.56% (Cald. 45.27%), the loss due to a C₁₄H₁₀NCl molecule of indole moiety and two phenyl groups. Thereafter, the compound showed a gradual decomposition up to 716 °C with the weight loss of the remaining organic moiety. The weight of the residue corresponds to zinc oxide.

ESR spectral studies of the Cu (II) complex

The ESR spectrum of the Cu (II) complex in a polycrystalline state was recorded at room temperature to elucidate the geometry and the degree of covalency of the metal-ligand bond or environment around the metal ion. The spin Hamiltonian parameters for the Cu(II) complex is used to derive the ground state. In octahedral geometry the g-tensor parameter with $g_{\parallel} > g_{\perp} > 2.0023$, the unpaired electron lies in the d_{z²} orbital and $g_{\parallel} > g_{\perp} > 2.0023$, the unpaired electron lies in the d_{x²-y²} orbital in the ground state [35]. In the present case, the observed measurements of Cu(II) complex is $g_{\parallel} (2.161) > g_{\perp} (2.0352) > 2.0023$ indicating that the complex are axially symmetric, and the copper site has a d_{x²-y²} ground state characteristic of octahedral geometry [36].

The g_{\parallel} value is an important function for indicating the metal-ligand bond character, for covalent character $g_{\parallel} < 2.3$ and for ionic $g_{\parallel} > 2.3$ respectively [37]. In the present case Cu (II) complex has the g_{\parallel} values were less than 2.3, indicating an appreciable covalent character of the metal-ligand bond. The geometric parameter (G), which is the measure of the extent of exchange interaction and is calculated by using g-tensor values by the expression $G = g_{\parallel} - 2.0023/g_{\perp} - 2.0023$. According to Hathaway [38, 39], if the G value is less than 4, the exchange interaction between the copper centers is noticed, whereas if its value is greater than 4, the exchange interaction is negligible. The calculated G-value for the present Cu (II) complex is 4.879 indicating that the exchange coupling effects are not operating in the present complexes.

Table 5: Thermal degradation pattern of metal complexes

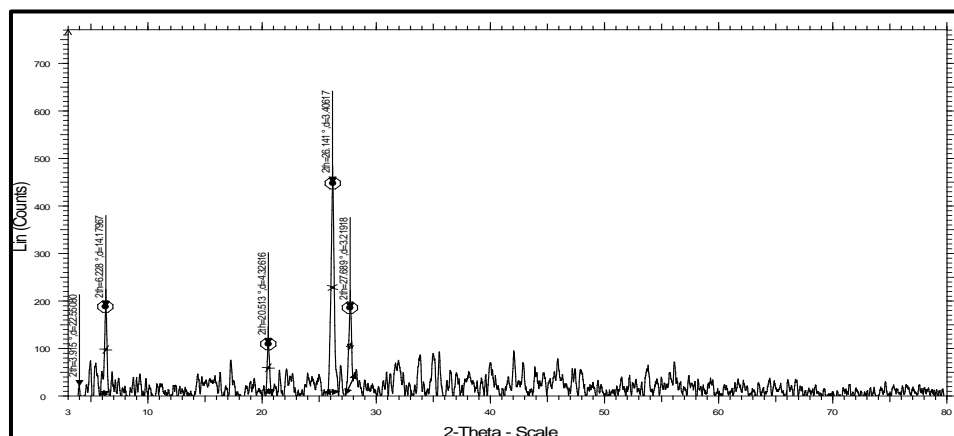
Metal Complexes	Temp. (°C)	Weight loss (%)		Metal oxide (%)		Inference
		Obs.	Calc.	Obs.	Calc.	
[Cu(L) ₂]	249	7.26	6.89	--	--	Loss due to two OCH ₃ groups of vanillin moieties.
	345	36.26	36.17	--	--	Loss due to C ₁₄ H ₉ NCl species of indole moiety and a phenyl group.
	Up to 627	--	--	8.84	8.91	Loss due to remaining organic moiety
[Co(L) ₂]	388	7.26	6.92	--	--	Loss due to two OCH ₃ groups of vanillin moieties.
	400	73.70	73.59	--	--	Loss due to a deprotonated ligand and C ₁₄ H ₉ NCl species of indole moiety.
	Up to 715	--	--	8.37	8.45	Loss due to remaining organic moieties.
[Ni(L) ₂]	378	5.70	6.93	--	--	Loss due to two OCH ₃ groups of vanillin moieties.
	405	31.13	31.46	--	--	Loss due to a C ₁₄ H ₁₀ NCl molecule of indole moiety and a chlorine atom.
	Up to 715	--	--	8.34	8.99	Loss due to remaining organic moiety.
[Zn(L) ₂]	364	5.91	6.87	--	--	Loss due to two OCH ₃ groups of vanillin moieties.
	395	44.56	45.27	--	--	Loss due to a C ₁₄ H ₁₀ NCl molecule of indole moiety and two phenyl groups.
	Up to 716	--	--	9.02	10.21	Loss due to remaining organic moiety.

Powder X-ray diffraction studies (Powder-XRD)

Crystals that are suitable for single-crystal studies were not obtained since all the metal complexes are not soluble in common solvents, but soluble in some polar solvents like DMF and DMSO. Hence powder-XRD pattern of all the metal complexes has been studied in order to test the degree of crystallinity of the complexes. Powder X-ray diffraction pattern for Cu(II) complex (fig. 2) showed 5 reflections in the range of 3-80 ° (2θ), which arise from diffraction of X-ray by the planes of the complex. The interplanar spacing (d) has been calculated by using Bragg's equation, ($n\lambda = 2d \sin\theta$). The calculated inter-planar d-spacing together with relative intensities with respect to the most intense peak have been recorded and depicted in table 6. The unit cell calculations have been calculated for cubic symmetry from the entire important peaks, and $h^2+k^2+l^2$ values were determined. The observed inter-planar d-spacing values have been compared with the calculated ones, and it was found to be

in good agreement. The $h^2+k^2+l^2$ values are 1, 2, 27, 44 and 49 for Cu (II) complex. It was observed that the absence of forbidden numbers (7, 15, 23, 71 etc.) indicates that complexes have cubic symmetry.

Similar calculations were performed for Co (II), Ni (II) and Zn(II) complexes, they showed 5, 6 and 5 reflections each in the range 3-80 ° respectively, which are raised from the diffraction of X-ray by the planes of these complexes. All the important peaks of the complexes have been indexed, and observed values of interplanar distances (d) have been compared with the calculated ones, and it was found to be in good agreement. The unit cell calculations were performed for the cubic system, and the $h^2+k^2+l^2$ values were determined for the above complexes. The $h^2+k^2+l^2$ values were 1, 19, 26, 34 and 54 for Co(II) complex, and 1, 24, 29, 34, 38 and 56 for Ni(II) complex, 1, 1, 1, 2 and 4 for Zn(II) complex respectively. It was observed that the absence of forbidden numbers (7, 15, 23, 71 etc.) indicates that complexes have cubic symmetry.

Fig. 2: Powder X-ray spectrum of [Cu (L)₂] complexTable 6: Powder X-ray data of [Cu (L)₂] complex

S. No.	2θ	θ	Sinθ	Sin ² θ	1000 Sin ² θ	1000 Sin ² θ/CF ($h^2+k^2+l^2$)	h k l	d		a in Å
								Obs.	Calc.	
1	3.915	1.9575	0.0341	0.001	1.162	1.00 (1)	1 0 0	22.55	22.58	22.58
2	6.0228	3.114	0.0543	0.002	2.950	2.50 (2)	1 1 0	14.17	14.18	22.41
3	20.513	10.2565	0.1780	0.031	31.684	27.2 (27)	3 3 3	4.32	4.32	22.58
4	26.141	13.0705	0.2261	0.051	51.12	43.9 (44)	6 2 2	3.40	3.40	22.56
5	27.689	13.8445	0.2392	0.057	57.21	49.2 (49)	7 0 0	3.21	3.22	22.61

Antimicrobial activity results

In most of the cases, the metal complexes exhibited promising antibacterial and antifungal activity greater than the free ligand. This activity was found to be enhanced on coordination with metal ions.

This enhancement in the antimicrobial activity of the complexes over the free ligand can be explained on the basis of chelation theory [40, 41]. The enhancement in the activity may be rationalized on the basis that ligands possess azomethine (C=N) bond. Moreover, in the metal complex, the positive charge of the metal ion is partially shared with

the hetero donor atoms (N and O) present in the ligand, and there may be π -electron delocalization over the whole chelating system [42-44]. Hence, there will be an increase in the lipophilic character of the metal complexes which favors its permeation through the lipid layer of the

bacterial membranes and blocking the metal binding sites in the enzymes of microorganisms. The minimum inhibitory concentration (MIC) values of the compounds against the respective bacterial and fungal strains are summarized in table 7.

Table 7: Size of inhibition zone (mm) formed at different concentrations (12.5, 25, 50 and 100 $\mu\text{g/ml}$ in DMSO solvent) against various Bacteria and fungi

Compounds	Bacteria			Fungi		
	<i>B. Subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>C. oxysporum</i>	<i>A. niger</i>
L	10.29 \pm 0.19	10.12 \pm 0.32	10.31 \pm 0.8	10.71 \pm 0.62	10.91 \pm 0.10	11.48 \pm 0.52
[Cu(L) ₂]	13.24 \pm 0.57	13.10 \pm 0.20	11.15 \pm 0.14	11.49 \pm 0.67	12.63 \pm 0.22	12.41 \pm 0.35
[Co(L) ₂]	13.62 \pm 0.33	12.19 \pm 0.34	13.62 \pm 0.91	12.17 \pm 0.34	12.66 \pm 0.47	14.28 \pm 0.10
[Ni(L) ₂]	12.41 \pm 0.28	11.42 \pm 0.75	13.28 \pm 0.16	13.52 \pm 0.36	13.77 \pm 0.53	13.16 \pm 0.11
[Zn(L) ₂]	13.68 \pm 0.11	11.45 \pm 0.61	13.37 \pm 0.23	13.14 \pm 0.35	13.99 \pm 0.12	12.33 \pm 0.38
Gentamicin	18.34 \pm 0.23	18.13 \pm 0.12	18.91 \pm 0.27	--	--	--
Fluconazole	--	--	--	20.10 \pm 0.30	21.16 \pm 0.13	19.31 \pm 0.29

Note: The stock solutions of the test compounds were prepared by dissolving 10 mg of the test compound in 10 ml of freshly distilled DMSO (1 mg/ml). The experiment was done in triplicate (n = 3), and the average values were calculated. The values presented in the above table are in \pm SEM

Antioxidant assay (DPPH free radical scavenging activity)

The free radical scavenging activity of the ligand and its metal complexes was done by DPPH method. The antioxidant activity of the test compounds was examined by measuring the radical scavenging effect of DPPH radicals. The results of the free radical scavenging activity of the compounds at different concentrations are shown in fig. 3. It was observed that the free radical scavenging activity of these compounds was concentration dependent. Among the examined compounds, ligand (L), Cu and Co complexes have exhibited good scavenging activity, whereas Ni and Zn complex showed moderate activity. The marked antioxidant activity of metal complexes is due to the coordination of metal with azomethine nitrogen and carbonyl oxygen of amide function attached to the 2-position of indole. In the case of metal complexes, the hydrogen of azomethine is more acidic. Hence, hydrogen of azomethine could be easily donated to the DPPH free radical and convert itself into the stable free radical. Moreover, the acidic nature of hydrogen atom attached to azomethine nitrogen increases on complexation with metal ions thereby making that hydrogen atom more liable.

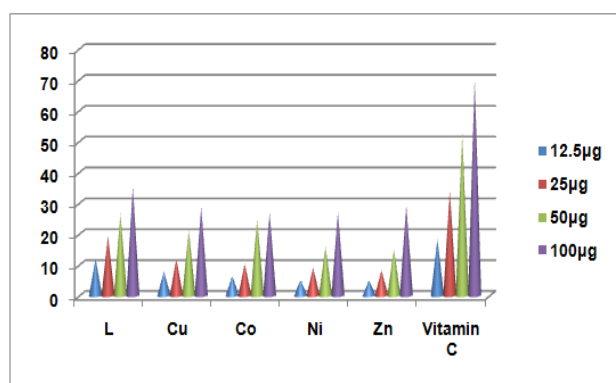


Fig. 3: Antioxidant activity results

The experiment was done in triplicate (n = 3), and the average values were calculated. The values presented in the above fig. are in \pm SEM, Error bars were omitted for simple presentation. On X-axis: Newly prepared compounds and Y-axis: Percentage of scavenging activity

DNA cleavage activity

The ligand and its Cu, Co, Ni and Zn complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method against

Calf-thymus DNA (Cat. No-105850) as a target molecule and the gel picture showing cleavage is depicted in fig. 4. Treatment of DNA on the ligand and complexes revealed that all the complexes have acted on DNA as there was a molecular weight difference between the treated DNA samples and the control. The difference was observed in bands of lanes compared to the control Calf-thymus DNA. The results indicate the important role of nitrogen and oxygen atoms to the metal ions in these isolated DNA cleavage reactions [45]. On the basis of the cleavage of DNA observed in case of ligand and its Cu, Co, Ni and Zn complexes, it can be concluded that all the compounds under present study inhibited the growth of pathogenic organism by DNA cleavage as has been observed on the DNA cleavage of Calf-thymus DNA.

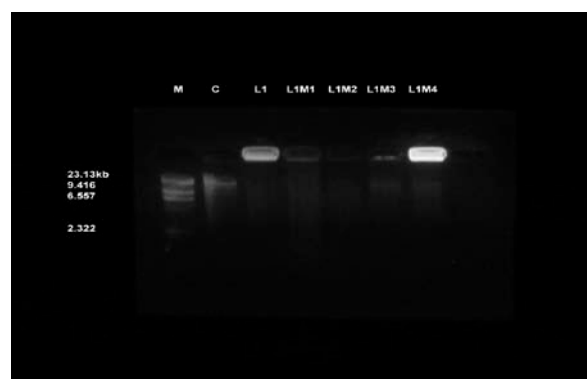


Fig. 4: DNA cleavage: M: Standard DNA, C: Control DNA (untreated pBR 322), L1: Ligand (L), L1M1: Cu (II) complex, L1M2: Co (II) complex, L1M3: Ni (II) complex and L1M4: Zn (II) complex

CONCLUSION

The spectral data revealed that the coordinating ability of the ligand has been proved in complexation reaction with Cu, Co, Ni and Zn ions. The newly synthesized ligand acts as ONO donor tridentate chelate. Metal ions are coordinated through an oxygen atom of carbonyl function, azo methine nitrogen and phenolic oxygen atom to the Cu, Co, Ni and Zn metal ions and form octahedral geometrical arrangement. The antimicrobial activity results showed that all the complexes have exhibited moderate activity when compared to its ligand. The electrophoretic studies indicated that Cu (II) and Co(II) complexes have good efficiency towards DNA cleavage. Based on the analytical data and spectral studies, proposed structure of all the complexes is depicted in fig. 5.

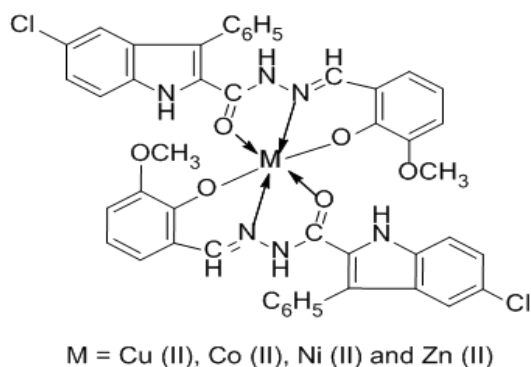


Fig. 5: Proposed structures of the metal (II) complexes

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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