JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

SYNTHESIS, CHARACTERIZATION, BIOLOGICAL ACTIVITIES OF NOVEL SCHIFF BASE 5-BROMO-N'-(2-HYDROXY-4-PHENYLDIAZENYL)BENZYLIDENE-3-PHENYL-1*H*-INDOLE-2-CARBOHYDRAZIDE AND ITS METAL (II) COMPLEXES.

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The novel Schiff base ligand 5-bromo-N'-(2-hydroxy-4-phenyldiazenyl)benzylidene)-3-phenyl-1H-indole-2-carbohydrazide and its Cu(II), Co(II), Ni(II) and Zn(II) complexes were prepared, characterized and studied their biological activity. Newly prepared compounds are characterized by elemental analysis and various physico-chemical techniques like FT-IR, 1H NMR, ESI-mass, UV-visible, TGA analysis, molar conductance and ESR spectral techniques. The elemental analysis data and spectral study indicate octahedral geometry for Cu(II), Co(II) and Ni(II) complexes and tetrahedral geometry for Zn(II) complex. 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 Electrophorosis (AGE) method. All the newly prepared compounds were tested for antioxidant activity, The Schiff base ligand **(**L**)**, Cu(II) and Co(II) complexes showed good activity.

Keywords: Schiff base, indole, 2-hydroxy-4-(phenyldiazenyl)benzaldehyde, Transition metal complexes, powder XRD, ESR, DNA cleavage, antimicrobial, antioxidant.

Introduction

Transition metal complexes derived from Schiff base ligands have been among the most widely studied coordination compounds in recent years [1,2], since they are becoming increasingly important as biochemical, analytical and antimicrobial regents. These complexes containing certain metal ions are active in many biological processes. The fact that copper ,magnesium, calcium, zinc, iron, and vanadium are essential metallic elements and exhibit great biological activity when associated with certain metal-protein complexes, participating in oxygen transport, electronic transfer reaction or the storage of ions [3,4] has created enormous interest in the study of systems containing these modification in the structure of the ligands containing hard soft donor atoms(N,S, and/or O), In view of the evolution of resistance to antimicrobial agent that are used to control pathogens in medicine and agriculture, which markedly affected the activity of the compounds [6,7]. There is pressing need to develop new agents and therapeutic strategies for the treatment of pathogenic infectious diseases. Schiff's bases are the compounds containing azomethine group (-HC=N) formed by condensation of primary amine and carbonyl compounds are known as imines. Schiff's base derivatives are the subject of renewed interest because they have been found to be useful intermediates for the synthesis of various heterocyclic compounds and have a wide variety of application in many fields [8]. We intend to report here the synthesis, spectral characterization, DNA cleavage and antimicrobial activities of novel Schiff base ligand 5-bromo-N'-(2-hydroxy-4-phenyldiazenyl)benzylidene)-3-phenyl-1H-indole-2-carbohydrazide derived from the reaction between 5- bromo -3-phenyl-1H-indole-2-carboxyhydrazide and 2-hydroxy-4-(phenyldiazenyl)benzaldehyde and its metal complexes.

MATERIALS AND METHODS

Materials

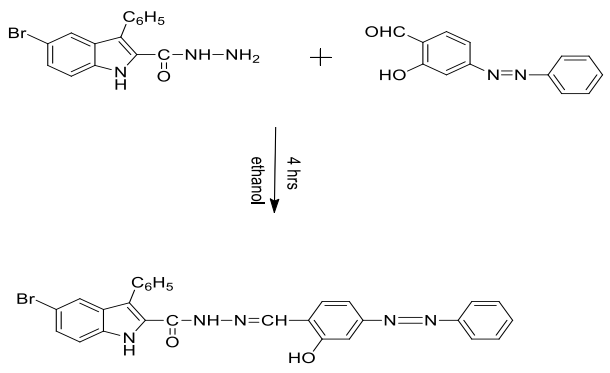
All the chemicals used were of high purity grade; solvents were dried and distilled before use. Melting points were determined by electro-thermal apparatus using open capillary tubes. Metal and chloride contents were determined as per standard procedures [8]. The precursor 5- bromo-3-phenyl-*1H*-indole-2-carboxyhydrazide [9] and 2-hydroxy-4-(phenyldiazenyl)benzaldehyde[10] were prepared as per literature methods.

Analysis and physical measurement

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. ^{1}H NMR spectra of the ligand and its Zn complex were recorded on FT NMR Spectrometer model Avance-II (Bruker), 400 MHz instrument using *d6* -DMSO as solvent. ESI-mass spectra were recorded on mass spectrometer equipped with electrospray ionization (ESI) source having 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^{-3} M concentration. The ESR spectra of the Cu complex in the polycrystalline state was recorded on BRUKER Bio Spin Gmbh spectrometer at microwave frequency of 9.1 GHz. The experiment was carried out by using DPPH as a reference with field set at 3000 Gauss using tetracynoethylene as the "g" marker (g = 2.0023). Powder XRD of the complexes was recorded in 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- bromo -3-phenyl-*1H*-indole-2-carboxyhydrazide (0.001mol) and 2-hydroxy-4-(phenyldiazenyl)benzaldehyde (0.001mol) 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 **(Scheme1).**The reaction was monitored by TLC.



Scheme 1: Synthesis of ligand.

Preparation of metal (II) complexes

To the hot solution of 5- bromo -N'-(2-hydroxy-4-phenyldiazenyl)benzylidene)-3-phenyl-1*H*-indole-2-carbohydrazide (0.002mol) in ethanol (30 ml) was added a hot ethanolic solution (15 ml) of respective metal chlorides (0.002mol). 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 refluxing continued for 1 h more. The reaction mixture was poured into distilled water. The colored solid 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 [11]. 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 target molecule. The electrophoresis method was employed to study the efficiency of cleavage by the synthesized compounds. Each test compound (100µg) was added separately to the 225µg of pBR322 DNA sample and these sample mixtures were incubated at 37 °C for 2 h. The electrophoresis of the test compounds were done according to the literature method [12].

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 (20µL) 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 (10µg ml^{-1}) for 15-20 min and then the bands were observed and photographed under UV-illuminator.

Antioxidant assay (DPPH 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 [13]. Different concentrations of test compounds (12.5µg, 25µg, 50µg and 100µg) and standard butylated hydroxyanisole (BHA) were taken in different test tubes and the volume of each test tube was adjusted to 100µL by adding distilled DMF. To the tubes containing sample solutions in DMF, 5ml 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:

RESULTS AND DISCUSSION

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

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 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 and 1:1 stoichiometry of the type $[M(L)_2]$ for Cu, Co, Ni complexes and [M(L)Cl] for Zn complex respectively. The molar conductance values are too low to account for any dissociation of the complexes in DMF (18-33 $ohm^{-1}cm^2$ $mole^{-1}$), 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	M		
$C_{28}H_{20}N_5O_2Br$ **(L)**	503	290	Yellow	68.39	4.08	14.21	--	--	--
			(74)	(68.12)	(4.06)	(14.24)			
$[Cu(C_{56}H_{38}N_{10}O_4Br_2)]$	1137.5	>303	Green	64.42	3.72	13.26	6.09	34	1.82
$[Cu(L)_2]$			(61)	(64.39)	(3.68)	(13.25)	(6.10)		
$[Co(C_{56}H_{38}N_{10}O_4Br_2)]$	1132.9	>302	Brown	64.43	3.64	13.42	5.65	25	5.09
$[Co(L)_2]$			(59)	(64.46)	(3.62)	(13.45)	(5.70)		
$[Ni(C_{56}H_{38}N_{10}O_4Br_2)]$	1132.6	>305	Brown	64.44	3.64	13.42	5.62	18	2.95
$[Ni(L)_2]$			(58)	(64.46)	(3.69)	(13.46)	(5.68)		
$[Zn(C_{28}H_{19}N_5O_2Br_2)]$	682.3	>302	Orange	56.71	3.20	11.81	11.03	17	Dia.
$[Zn(L)Cl]$			(50)	(56.73)	(3.16)	(11.85)	(11.07)		

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 CONH and indole NH displayed bands at 3395, 3273cm^{-1}respectively. Sharp peaks observed at 1693 and 1611 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 NH of indole and NH of CONH function of the above metal complexes of ligand(L) have displayed bands in the region 3398-3374 cm^{-1} and 3243-3289 cm^{-1} which have appeared at about the same region as in the case of ligand, thus confirming the non-involvement of either indole NH or NH of CONH function in coordination with the metal ions. The absorption frequency of amide carbonyl and azomethine function which have appeared at 1694 and 1610 cm^{-1} in case of ligand, has been shifted to lower frequency by 21-38 and 15-72 cm^{-1} in the complexes and appeared in the region 1673-1656 cm^{-1} and 1595-1538 cm^{-1} indicating the involvement of oxygen atom of carbonyl function as such without undergoing any enolization[53] and nitrogen atom of

azomethine[54] function in complexation with the metal ions. This is further confirmed by the appearance of new bands in the region 575-534 cm^{-1}, 462-410 cm^{-1} and 360-317 cm^{-1} due to M-O, M-N and M-Cl stretching vibrations [55] 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)	$\nu_{C=O}$ (carbonyl)	$\nu_{C=N}$ (azomethine)	ν_{C-O} (phenolic)	ν_{M-O}	ν_{M-N}	ν_{M-Cl}
L	3424	3313	3064	1684	1606	1284	--	--	--
$[Cu(L)_2]$	--	3309	3056	1650	1539	1335	591	496	--
$[Co(L)_2]$	--	3316	3056	1664	1537	1342	544	483	--
$[Ni(L)_2]$	--	3309	3061	1657	1540	1368	615	486	--
[Zn(L)Cl]	--	3382	3059	1614	1545	1329	511	468	377

^{1}H NMR spectral data

The ^{1}H NMR spectrum of ligand **(L)** displayed three singlets each at 12.684, 11.880 and 11.775ppm are due to the proton of phenolic OH, amide NH and NH of indole moiety respectively. Azomethine proton (CH=N) has resonated as a singlet at 9.451 ppm. The signals due to sixten aromatic protons (ArH) have appeared as multiplets in the region 7.023-7.999 ppm. The Schiff base ligand **(L)** upon complexation with Zn (II) ion showed the disappearance of signal due to the proton of phenolic OH confirming the involvement of bonding of phenolic oxygen to metal ion *via* deprotonation. The signals due to amide NH and indole NH are appeared at 12.369 ppm and 11.810 ppm respectively. The signal due to azomethine proton (CH=N) resonated as a singlet at 10.769 ppm. The signals due to sixteen aromatic protons (ArH) have resonated as multiplets in the region 6.931-8.501ppm. When compared to the ^{1}HNMR spectral data of the ligand and its [Zn(L)Cl] complex, all the signals due to protons have been shifted towards down field strength confirming the complexation of ligand with metal ion.

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

Ligand/ Complex	^{1}H NMR data (ppm)
L	12.684 (s, 1H, Phenolic OH), 11.880 (s, 1H, CONH), 11.775 (s, 1H, indole NH), 9.451 (s, 1H, HC=N), 7.023-7.999 (m, 16H, ArH),
$[Zn(L)_2]$	12.369 (s, 1H, CONH), 11.810 (s, 1H, indole NH), 10.769 (s, 1H, HC=N), 6.931-8.501(m, 16H, ArH),

ESI-mass spectral data

The mass spectrum of Ligand **(L)** showed the molecular ion peak $M^{+\cdot}$-1=502,504 (100%, 33%) due to the loss of a hydrogen radical from the molecular ion which is also a base peak. Further, no significant fragment peaks. Were observed, Some fragment ion peaks which observed were of very minute intensity.

In the mass spectrum of $[Cu(L)_2]$ complex, molecular ion peak was observed at $M^{+\cdot}$ 1137 1138 (11.1%, 2.6 %) This fragment ion peak on loss of $C_{14}H_7NBr$ radical gave a fragment ion peak recorded at m/z 823, 825 (4.27%, 2.98 %) which on simultaneous expulsion of 5-bromo-2-phenyl indole radical and seven hydrogen radicals gave a fragment ion peak observed at m/z 590 (27.35%). This fragment ion on simultaneous loss of benzene diazonium radical and a $C_6H_4N_2$ molecule gave a fragment ion peak recorded at m/z 298 (49.57%).

In the mass spectrum of $[Co(L)_2]$ complex, molecular ion peak was observed at $M^{+\cdot}$1132, 1134 (85.29 %, 35.29 %) which is equal to its molecular weight. The molecular ion on loss of a two hydrogen radicals gave a fragment ion peak observed at m/z 1132 (100%) which is also a base peak. No significant fragment peaks were observed further. In the mass spectrum of $[Ni(L)_2]$ complex, the molecular ion peak was observed at m/z 1132, 1134 (8.39%,4.58%). This fragment ion by the simultaneous expulsion of $C_{15}H_8NOCl$ molecule, $C_6H_4N_2$ molecule and phenyl diazonium radical gave a fragment ion peak recorded at m/z 580, 582 (10.68%, 6.87%), which on loss of $C_{15}H_8N_2OCl$ radical gave fragment ion peak observed at m/z 313 (2.86%), this fragment on loss of NH_2 radical gave a fragment ion peak recorded at m/z 297 (12.97%). This fragment ion of m/z 297 was also obtained from the molecular ion peak by the expulsion of two 5-chloro-3-phenyl-2-isocyanate molecules, one $C_6H_4N_2$ molecule and a phenyl diazonium radical simultaneously. The molecular ion

in another route by the simultaneous loss of two benzene molecule, a $C_6H_4N_2$molecule and a phenyl diazonium radical gave a fragment ion peak recorded at m/z 677, 679 (3.81%,2.29%).

In mass spectrum of [Zn(L)Cl] complex molecular ion peak was recorded at $M^{+\cdot}$ 682, 684, (100%, 33%) which is also a base peak. This fragment on simultaneous loss of a chloride, phenyl diazonium and hydrogen radicals gave a fragment ion peak recorded at m/z 451, 453 (5.46%,3.12%) which on simultaneous expulsion of chloride and C_7H_3O radicals gave a fragment ion peak recorded m/z 313, 315 (4.68%,3.12%). The fragmentation patterns of above complexes are in consistency with their structures.

Electronic spectral studies

The green colored $[Cu(L)_2]$ complex displayed a low intensity single broadband in the region 15490-17894 cm^{-1}. The broadness of the band designates the three transitions $^2B_{1g} \rightarrow {}^2A_{1g}$ (v_1), $^2B_{1g} \rightarrow {}^2B_{2g}$ (v_2) and $^2B_{1g} \rightarrow {}^2E_g$($v_3$), which are similar in energy and give rise to only one broad band 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 [17].

The $[Co(L)_2]$ complex under present study displayed two absorption bands at 16880 cm^{-1} and 20111 cm^{-1}. These bands are assigned to be $^4T_{1g}$ (F) $\rightarrow$ $^4A_{2g}$ (F) (v_2) and $^4T_{1g}$ (F) $\rightarrow$ $^4T_{2g}$ (P) (v_3) transitions, respectively, which are in good agreement with the literature values for octahedral geometry [18, 19]. The lowest band, v_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 [20]. The calculated v_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 the present investigation exhibited two absorption bands in the region 15090 cm^{-1} and 25102 cm^{-1}, which are assigned to $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (v_2) and $^3A_{2g}$ (F) $\rightarrow$ $^3T_{1g}$ (P) (v_3) transitions respectively in an octahedral environment [31]. The transition value of band v_1 was calculated by using a band fitting procedure [21] and presented in the **Table 4.**The proposed octahedral geometry for the complexes was further supported by the calculated values of ligand field parameters, such as Reachinter electronic repulsion parameter (B'), nephelauxetic parameter (β), ligand field splitting energy (10 Dq) and ligand field stabilization energy (LFSE) [22]. 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.

Complexes	Transitions in cm^{-1}			Dq	B′	β	β%	ν_2/ν_1	LFSE
	ν_1^*	ν_2	ν_3	(cm^{-1})	(cm^{-1})				(k cal.)
$[Cu(L)_2]$	15490-17894			--	--	--	--	--	28.65
$[Co(L)_2]$	7873	16880	20111	900	891	0.918	8.91	2.14	15.44
$[Ni(L)_2]$	9304	15090	25102	930	818	0.787	21.29	1.62	31.90

Table 4: Electronic spectral data

*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 1.83 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 [22]. 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 [23]. In the present study the observed magnetic moment values for Co (II) complexes are 5.10 BM indicates octahedral geometry for Co (II) complex. For Ni (II) complex the observed magnetic moment value is 2.96 BM which is well within the expected range of Ni (II) complex with octahedral geometry, i.e. 2.83-3.50 BM [24].

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 chlorine atoms of indole moieties at 310 °C with practical weight loss of 6.12% (Cal. 6.68%). The resultant complex underwent second stage of degradation and gave break at 365 °C with a practical weight loss of 20.82% (Cal. 19.64%), which corresponds to the loss of $C_{14}H_9N$ species of indole moiety. Thereafter, the compound showed decomposition in a gradual manner rather than with the sharp decomposition up to 700 °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 308°C with practical weight loss of 4.72% (Cal. 3.35%), which corresponds to weight loss due to one chlorine atom of a indole moiety. Further the complex underwent decomposition and gave a break at 410 °C with a practical weight loss of 32.35% (Cal. 32.83%), corresponds to weight loss of $C_{14}H_9NCl$ species of indole moiety and $C_6H_5N_2$group. Thereafter the complex showed gradual decomposition up to 700 °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 one chlorine atom of a indole moiety at 189°C with a practical weight loss of 3.91% (Cal. 3.35%). The resultant complex underwent further degradation and gave break at 439°C with a practical weight loss of 28.35% (Cal. 30.06%), which corresponds to the loss due to a $C_{14}H_9NCl$ species of indole moiety and a phenyl group. Thereafter, the compound showed a gradual decomposition up to 720 °C with a weight loss of remaining organic moiety. The weight of the residue corresponds to nickel oxide.

Table 5: Thermal degradation pattern of metal complexes

Metal complexes	Temp. °C	Weight loss (%)		Metal Oxide (%)		Inference
		Obs.	Cal.	Obs.	Cal.	
[Cu(L)$_2$]	310	6.12	6.68	--	--	Loss due to two bromine atoms of indole moieties.
	365	20.82	19.64	--	--	Loss due to $C_{14}H_9N$ species of indole moiety.
	Up to 700	--	--	7.54	7.59	Loss due to remaining organic moiety.
[Co(L)$_2$]	308	4.72	3.35	--	--	Loss due to one bromine atom of indole moiety.
	410	32.35	32.83	--	--	Loss due to $C_{14}H_9NBr$ species of indole moiety and $C_6H_5N_2$ species
	Up to 700	--	--	7.21	7.18	Loss due to remaining organic moiety.
[Ni(L)$_2$]	189	3.91	3.35	--	--	Loss due to one bromine atom of indole moiety.
	439	28.35	30.06	--	--	Loss due to $C_{14}H_9N$ bromine species of indole moiety and a phenyl group
	Up to 720	--	--	7.34	7.15	Loss due to remaining organic moiety.
[Zn(L)$_2$]	450	5.51	5.90	--	--	Loss due to one bromine atom of indole moiety.
	465	40.26	40.56	--	--	Loss due to $C_{14}H_9NBr$ species of indole moiety
	Up to 720	--	--	13.52	13.73	Loss due to remaining organic moiety.

In case of Zn (II) complex, the first stage of decomposition occurs at 450 °C with practical weight loss of 5.51% (Cal. 5.90%), which represents the loss due to one chlorine atom of a indole moiety. Further the complex underwent second stage of decomposition and gave a break at 465 °C with practical weight loss of 40.26% (Cald.40.56%), which corresponds to the loss due to a $C_{14}H_9NBr$ species of indole moiety. Thereafter, the

compound showed a gradual decomposition up to 720 °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 $g_{\perp} > g_{\parallel} > 2.0023$, the unpaired electron lies in the d_{z^2} orbital and $g_{\parallel} > g_{\perp} > 2.0023$, the unpaired electron lies in the $d_{x^2-y^2}$orbital in the ground state [25]. In the present case the observed measurements of Cu(II) complex is $g_{\parallel}$ (2.184) > $g_{\perp}$ (2.0352) > 2.0023 indicating that the complex are axially symmetric and copper site has a $d_{x^2-y^2}$ ground state characteristic of octahedral geometry [26]. 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 [27]. 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 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 [28, 29], if the G value is less than 4, the exchange interaction between the copper centres is noticed, where as if its value is greater than 4, the exchange interaction is negligible. The calculated G-value for the present Cu (II) complex is 5.590 indicating that the exchange coupling effects are not operating in the present complex.

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.1**) showed 9 reflections in the range of 3-80° (2θ), which arise from diffraction of X-ray by the planes of the complex. The inter-planar 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 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, 5, 8, 9, 11, 18, 25 and 36 for Cu (II) complex. It was observed that the absence of forbidden numbers (7, 15, 23, 71 etc.) indicates that the Cu (II) complex has cubic symmetry.

Similar calculations were performed for Co(II), Ni(II) and Zn(II) complexes, they showed 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 inter-planar 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 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, 3, for Co(II) complex and 1, 1, 2, 2, 3, 3 and 5 for Zn(II) complex respectively. It was observed that the absence of forbidden numbers (7, 15, 23, 71etc) indicates that Co(II) complex and Zn(II) complex have cubic symmetry. The $h^2+k^2+l^2$ values were 1, 1, 2, 3, 5, 5, 7, 8 and 14 for Ni(II) complex. The presence of forbidden number 7 indicates that the Ni(II) complex may belong to hexagonal or tetragonal system.

Fig. 1: Powder X-ray spectrum of $[Cu(L)_2]$ complex

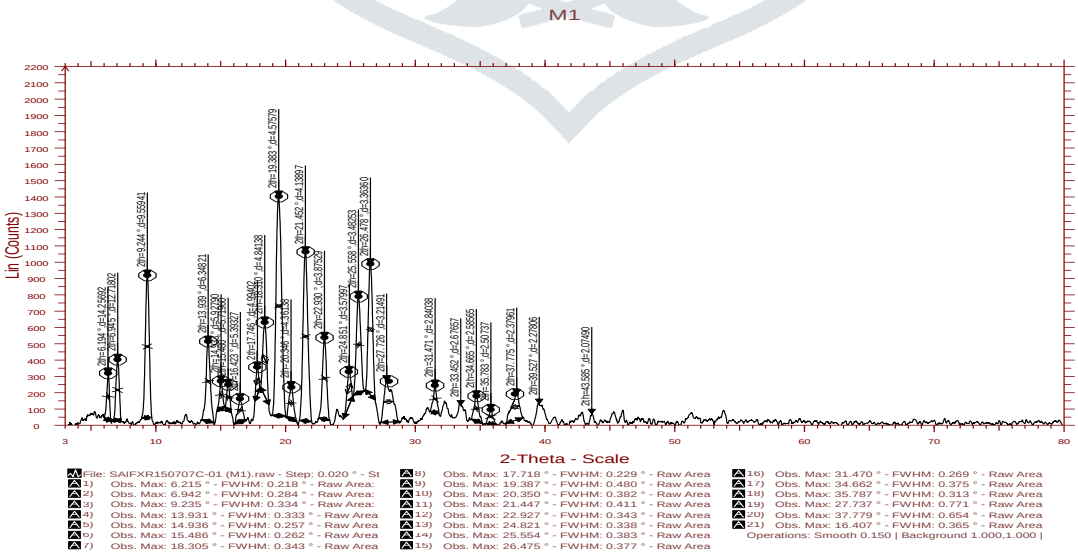


Table 6: Powder X-ray data of $[Cu(L)_2]$ complex

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 [30, 31]. The enhancement in the activity may be rationalized on the basis that ligands possess azomethine (C=N) bond. Moreover, in 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 [32-34]. Hence there will be an increase in the lipophilic character of the metal complexes which favours its permeation through the lipoid layer of the bacterial membranes and blocking of 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: Antimicrobial studies of Schiff base and its metal complexes

Note: The stock solutions of the test compounds were prepared by dissolving 10 mg of the test Compounds in 10 ml of freshly distilled DMSO (1 mg/ml).

The experiment was done in triplicate (n = 3), and the average values were calculated.

S. No	2θ	θ	Sinθ	$Sin^2θ$	1000 $Sin^2θ$	1000 $Sin^2θ/CF$ $(h^2+k^2+l^2)$	h k l	d Obs.	d Calc.	a in A
1	6.194	3.097	0.0540	0.00291	2.91	1.00 (1)	1 0 0	14.25	14.25	14.27
2	9.244	4.622	0.0805	0.00649	6.49	2.23(2)	1 1 0	9.55	9.56	14.27
3	13.939	6.969	0.1213	0.01472	14.72	5.05 (5)	2 1 0	6.34	6.34	14.26
4	17.746	8.873	0.1542	0.02379	23.79	8.17(8)	2 2 0	4.99	4.99	14.26
5	19.383	9.691	0.1683	0.02833	28.33	9.73 (10)	3 1 0	4.57	4.57	14.26
6	21.452	10.726	0.1861	0.03463	34.63	11.90 (12)	2 2 2	4.13	4.13	14.27
7	26.478	13.239	0.2290	0.05244	52.44	18.02 (18)	4 1 1	3.36	3.36	14.27
8	31.471	15.735	0.2711	0.07351	73.54	25.27 (25)	5 1 0	2.84	2.84	14.27
9	37.778	18.887	0.3237	0.10478	104.78	36.00 (36)	6 0 0	2.37	2.37	14.27

Compounds	Bacteria			Fungi		
	B. Subtilis	*E. coli*	*S. typhi*	*C. albicans*	*C. oxysporum*	*A. niger*
L	11.25±0.18	11.12±0.31	11.31±0.9	10.91±0.96	11.09±0.11	11.43±0.53
$[Cu(L)_2]$	13.23±0.58	13.10±0.22	12.91±0.14	13.39±0.67	13.13±0.16	13.41±0.36
$[Co(L)_2]$	13.35±0.29	13.19±0.11	13.10±0.11	13.09±0.07	12.66±0.44	13.49±0.33
$[Ni(L)_2]$	13.10±0.11	13.39±0.31	13.17±0.22	12.91±0.81	12.99±0.46	13.16±0.22
[Zn(L)]	12.89±0.41	12.46±0.41	13.00±0.11	13.96±0.99	13.22±0.16	12.31±0.33
Gentamicin	18.00±0.22	18.22±0.12	18.29±0.27	--	--	--
Fluconazole	--	--	--	20.22±0.33	21.16±0.14	20.31±0.39

The values presented in the above table are in ±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 radical scavenging effect of DPPH radicals. The results of the free radical scavenging activity of the compounds at different concentrations are shown in **Fig.2**. It was observed that the free radical scavenging activity of these compounds was concentration dependent. Among the examined compounds, ligand **(L),** Cu(II) and Co(II) 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 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 there by making that hydrogen atom more liable.

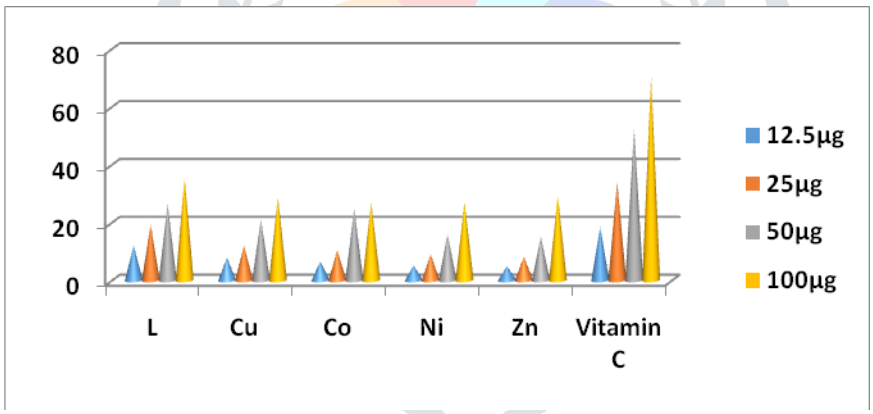


Fig. 2: Antioxidant activity results

The experiment was done in triplicate (n = 3), and the average values were calculated.

The values presented in the above figure are in ±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. 3.** Treatment of DNA on the ligand and complexes revealed that all the

complexes have acted on DNA as there was 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 [35]. 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.

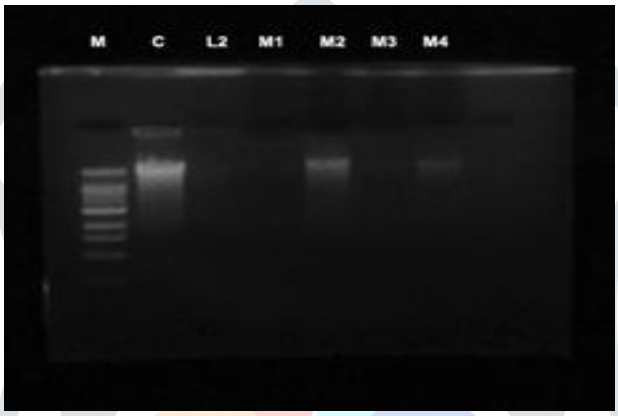
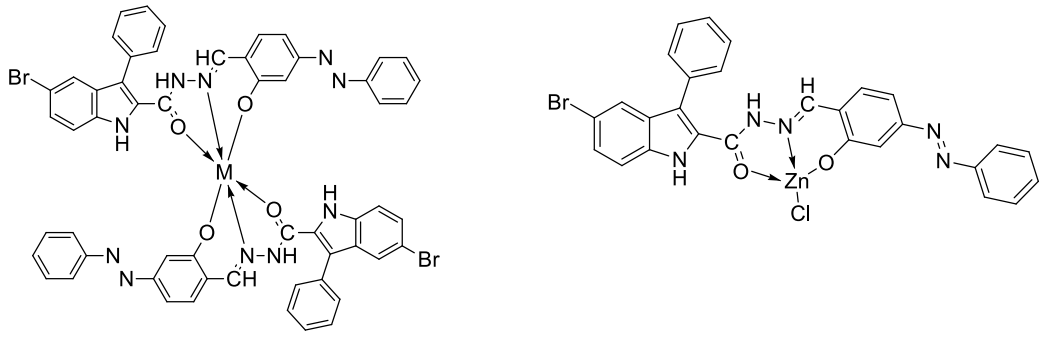


Fig 3 : DNA cleavage: M: Standard DNA, C: Control DNA (untreated pBR 322)L2:Ligand(L),M1:Cu(ll) complex,M2:Co(ll) complex,M3:Ni(ll) complex and M4:Zn(ll) complex.

CONCLUSION

A series of Cu(II), Co(II), Ni(II) and Zn(II) complexes were prepared with tridentate ONS donor novel Schiff base ligand (L) 5-bromo-3-phenyl-*1H*-indole-2-carboxyhydrazideand 2-hydroxy-4-(phenyldiazenyl)benzaldehyde by various physicochemical techniques. The physicochemical results demonstrate that Cu(II), Co(II)and Ni(II) complexes have octahedral geometry and Zn(II) complex has a tetrahedral geometry. Based on physic-chemical evidence, the following structures were proposed for the complexes **(Fig. 4).** The non-electrolytic nature of the complexes was confirmed on the basis of their molar conductance values. Also, the Schiff base ligand **(L)** and its Cu(II) and Co(II) complexes showed good antioxidant activity.



were, M=Cu(II),Co(II),and Ni(II)

Figure 4: Proposed structures of metal complexes

Acknowledgments

Authors are thankful to Professor and Chairman, Department of Chemistry, Gulbarga University, Kalaburagi, and to Professor Department of Chemistry Govt college Kalaburagi. By providing laboratory facilities, we are grateful to the SAIF Punjab University, Chandigarh, STIC Cochin University, for providing spectral data. We are thankful for BioGenics Research and Training Centre in Biotechnology, Hubli for biological activities.

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