

Water Soluble Mixed Ligand Complexes

Spectral, antioxidant, antimicrobial and DNA interaction studies

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A new series of water soluble mixed ligand complexes $[M^{\text{II}}(L)(\text{bpy})\text{AcO}]\cdot n\text{H}_2\text{O}$ $M = \text{Cu}$, ($n=1$) (1); Co (2), Mn (3), Ni (4) ($n=4$) and Zn (5) ($n=2$) have been synthesized from 2-(2-Morpholinoethylimino)methylphenol Schiff base ligand (HL) and 2,2'-bipyridine in a 1:1:1 molar ratio. The resulting complexes were characterized by spectral techniques. The spectral data of these complexes suggest an octahedral geometry. *In vitro* antioxidant activity results of DPPH assay, hydroxyl radical, super oxide and nitric oxide for complexes (1-5) were compared with ligand (HL) and *in vitro* antimicrobial activities of all compounds were examined against selected bacterial and fungal strains which indicate that the complexes exhibit higher antimicrobial activity than free ligand (HL). Gel electrophoresis results indicated that, complexes (1) and (2) have exhibited more DNA cleavage efficiency than others. The intrinsic binding constant (K_b) values for the complexes (1-5) observed by electronic absorption technique were in the order of (1) > (4) > (5) > (2) > (3) and DNA binding affinity values for these complexes obtained by viscosity measurements were in the order of ethidium bromide > (1) > (2) > (3) > (4) > (5).

Keywords: Schiff base, bipyridine, octahedral, DNA interactions

Schiff base complexes of transition metal have played a prominent role in the development of coordination chemistry. Over recent years a huge number of mixed ligand transition metal(II) complexes having N, O and S donor binding sites have been found to play a vital role in biological systems, clinical, medicinal, agricultural, industrial, analytical and therapeutic applications [1-3]. Heterocyclic bases like morpholine, pyrimidine derivative complexes have been extensively studied because of their potential antimicrobial and anticancer activities [4, 5] and they are exhibited unusual electronic properties, diverse chemical reactivity and peculiar structure which result in non-covalent interactions with DNA [6]. The ternary Cu(II) complexes of 2,2'-bipyridine that are strongly bound to DNA and cleave DNA oxidatively and exhibit cytotoxicity [7, 8] and also reported that the binding studies of mixed ligand Cu(II) complexes of diimines with DNA. When it interacts with DNA may induce the breakage of DNA, after cleavage of DNA double strands, the replication ability of the cancer gene is thereby destroyed. They exhibit more efficient anticancer activity than that of cisplatin [9].

Vitamins C and E are found in generous amounts in fruits and vegetables which are natural antioxidants belong to the group of polyphenolic compounds prevents the risk of several aging related diseases and human pathologies, including cancer and cardio cerebral-vascular diseases, diabetes mellitus, etc. The antioxidant compounds can act as reduction agents which reduces the effects of dangerous oxidants by binding together with these harmful molecules, decreasing their destructive power and antioxidants can also help to repair damage already sustained by cells [10]. It is well known that the antioxidant activity is reasonably related to the electrochemical behavior where low oxidation potential meant high antioxidant power [11]. Keeping the above facts in mind, we are enticed to continue our research work on the synthesis of mixed ligand transition metal complexes (1-5) from morpholine substituted Schiff base incorporating 2,2'-bipyridine, structural elucidation, antioxidant, antimicrobial and DNA interaction studies.

Experimental part

Materials and methods

All required materials and solvents were purchased from Sigma Aldrich Chemical Company and solvents were of analytical grade.

Synthesis of mixed ligand complexes

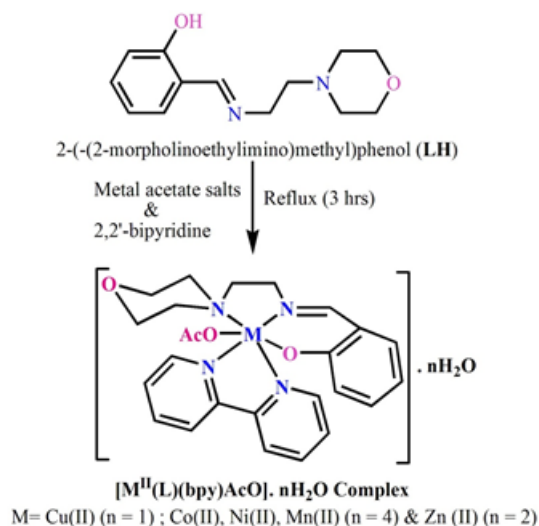
The preparation of 2-(2-morpholinoethylimino)methylphenol Schiff base ligand (HL) has been reported in our previous research paper [12]. A solution of 2-(2-Morpholinoethylimino)methylphenol Schiff base (HL) (Primary ligand) (0.001 M) in methanol (40 mL) was added slowly to a solution of Metal(II) acetate (0.001 M) $\text{Cu}(\text{OAc})_2\cdot\text{H}_2\text{O}$, $\text{Zn}(\text{OAc})_2\cdot 2\text{H}_2\text{O}$, $\text{Mn}(\text{OAc})_2\cdot 4\text{H}_2\text{O}$, $\text{Co}(\text{OAc})_2\cdot 4\text{H}_2\text{O}$ and $\text{Ni}(\text{OAc})_2\cdot 4\text{H}_2\text{O}$ in 30 mL of absolute methanol and the resulting mixture was stirred for 30 min and the hot mixture of solution slowly added into methanolic solution of 2,2'-bipyridine as secondary ligand (0.001 M). The obtaining mixture was refluxed for 3 h. The solid product so formed was separated by filtration and purified by recrystallization using methanol and petroleum ether. Trace of water and solvents were recovered by keeping in vacuum desiccators over anhydrous CaCl_2 . The preparation of all complexes was followed by the similar method and the yield was found to be 71-82 % (scheme 1) and (table 1).

In vitro antioxidant assay

The antioxidant activity of ligand (HL) and complexes (1-5) were analysed by various free radicals such as 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl, hydrogen peroxide, superoxide radical and nitric oxide [12-16].

Pharmacological study - *In vitro* antimicrobial assay

Antimicrobial activities of the ligand (HL) and complexes (1-5) were screened *in vitro* against the selected pathogenic bacterial strains and fungi species by the disc diffusion method [17].



Scheme 1. Schematic representation of synthesis of ligand (**HL**) and complexes (**1-5**)

Table 1
ANALYTICAL AND PHYSICAL DATA OF SCHIFF BASE LIGAND (**HL**) AND ITS MIXED LIGAND COMPLEXES (**1-5**)

Compounds (EF & FW)	Colour	Yield(%)	MP °C	Found (Calcd), %				\wedge_m
				C	H	N	M	
(HL) (C ₁₃ H ₁₈ N ₂ O ₂) (234.31)	Yellow	87.48	--	66.90 (66.64)	07.60 (07.74)	11.39 (11.95)	--	--
(1) (C ₂₅ H ₃₁ N ₄ O ₅) Cu (530.72)	Green	82.00	105	56.18 (56.52)	05.50 (05.84)	10.21 (10.55)	11.74 (11.97)	40.24
(2) (C ₂₅ H ₃₇ N ₄ O ₅) Co (580.11)	Dark Brown	73.66	112	51.34 (51.71)	06.13 (06.42)	09.13 (09.65)	10.01 (10.15)	35.92
(3) (C ₂₅ H ₃₇ N ₄ O ₅) Mn (576.12)	Black	75.34	220	52.78 (52.07)	06.10 (06.47)	09.15 (09.72)	09.05 (09.53)	30.86
(4) (C ₂₅ H ₃₇ N ₄ O ₅) Ni (579.88)	Dark green	75.40	85	51.07 (51.73)	06.18 (06.43)	10.03 (09.65)	10.04 (10.12)	38.21
(5) (C ₂₅ H ₃₃ N ₄ O ₆) Zn (550.55)	Pale brown	78.55	75	54.03 (54.49)	05.88 (06.04)	09.85 (10.17)	11.66 (11.87)	32.40

ML(bpy), (bpy = 2,2'-bipyridine), M = Cu, Co, Mn, Ni, Zn,
EM = Empirical Formula, FW = Formula Weight, \wedge_m = Molar conductance (ohm⁻¹ cm² mol⁻¹)

DNA interaction studies - DNA cleavage study by gel electrophoresis

DNA cleavage activities of ligand (**HL**) and complexes (**1-5**) with CT-DNA were monitored by agarose gel electrophoresis method [18, 19].

DNA binding study by electronic absorption titration

DNA binding studies of ligand (**HL**) and complexes (**1-5**) with CT-DNA were assessed by electronic absorption spectral titrations and viscosity methods [12, 20, 21].

Results and discussions

Synthesis and characterization

The synthesized Schiff base ligand (**HL**) and its mixed ligand complexes (**1-5**) were found to be intensely coloured and they were slightly hygroscopic nature at room temperature. The synthesized mixed ligand complexes were soluble in water, methanol, ethanol, CHCl₃ and DMSO. The analytical data and physical properties of the ligand (**HL**) and complexes

(**1-5**) are listed in the table 1. The low value of conductivity shows that they are non electrolytic nature due to lack of dissociation.

Electro spray ionization Mass spectra

ESI-MS mass spectrometry is used to confirm the stoichiometry composition of compounds. The mass

spectrum of ligand (**HL**) shows the molecular ion peak at m/z 235 corresponding to C₁₃H₁₈N₂O₂ and the [Cu^{II}(L)(bpy)AcO]·H₂O complex (**1**) molecular ion peak at m/z 531.2 corresponding to [(C₂₅H₃₁N₄O₅)Cu] which confirms the formation of [MLL²] stoichiometry (Scheme 1). The molecular ion peaks of other complexes were observed at m/z with relative abundance 581.3 (**2**), 577.2 (**3**), 580.9 (**4**) and 551.1 (**5**) and they are in good agreement with the formula weight.

¹H Nuclear Magnetic Resonance spectra

The ¹H NMR spectra of the Schiff base ligand (**HL**) and complex (**5**) show the following signals (fig. 1); δ values of Schiff base ligand: aromatic protons (m, 4H) at 6.84-7.32 ppm; azomethine proton (-HC=N-) (s, 1H) at 8.35 ppm; morpholino-OCH₂ protons (t, 4H) at 3.72 ppm; morpholino-N-CH₂ (t, 4H) at 2.44 ppm; phenolic-OH proton (s, 1H) at 13.52 ppm [22]. Complex (**5**): aromatic protons (m, 10H) at 6.88-7.51 ppm; azomethine proton(-HC=N-) (s, 1H) at 8.53 ppm; bpy-CH=N proton (s, 2H) at 8.88 ppm; morpholino-OCH₂ (t, 4H) at 3.72 ppm; morpholino-N-CH₂ (t, 4H) at 2.78 ppm; acetate protons (CH₃COO-) (s, 3H) at 2.038 ppm. The above ¹H NMR spectra data assigned that the azomethine proton and morpholino-N-CH₂ proton signals in the spectrum of the complex (**5**) are shifted down field compared to the free ligand suggesting deshielding

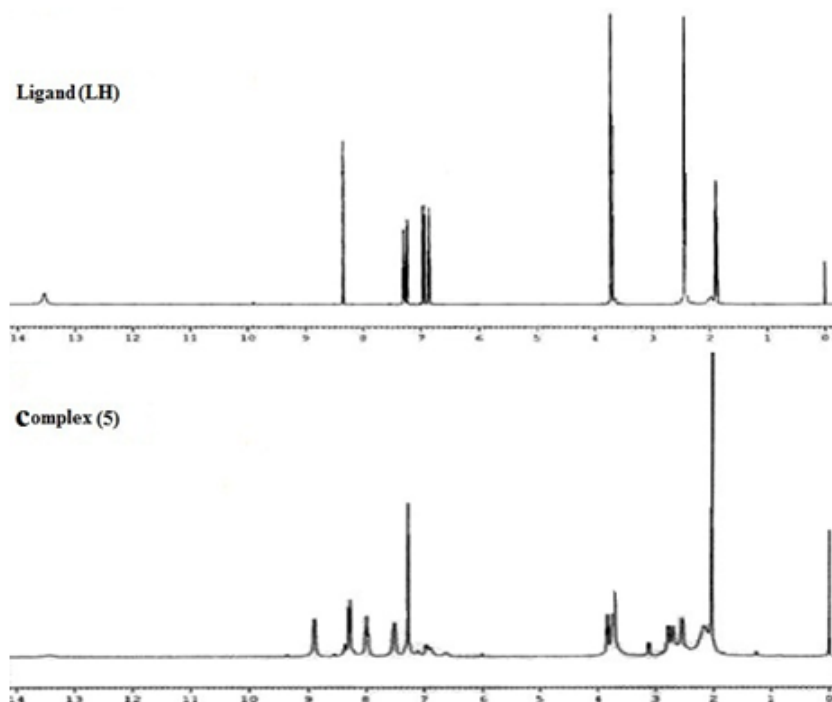


Fig. 1. ^1H -NMR spectra of Schiff base ligand (**HL**) and complex (**5**)

due to nitrogen atom is taking part in complexation [23, 24]. The absence of singlet peak at the range of 13 ppm was noted that the complex (**5**) indicates the loss of the -OH proton due to complexation [25] and there is no appreciable change in other signals in the complex (**5**).

FTIR spectra

The FTIR spectra of the complexes (**1-5**) were compared with the free ligand (**HL**) for the frequency changes during the complexation (table 2). In the FTIR spectrum of Schiff base ligand (**HL**) a strong sharp band was observed at 1635 cm^{-1} which is assigned to the azomethine group ($-\text{HC}=\text{N}-$) and shifted to lower frequencies in the spectra of the complexes (**1-5**) indicating the involvement of imino nitrogen in coordination to the central metal ion [26] and aliphatic morpholino $-\text{C}-\text{N}-\text{C}$ bands at 1342 cm^{-1} found in the ligand (**HL**) is shifted lower frequencies in the spectra of the complexes (**1-5**) indicating the involvement of $\text{C}-\text{N}-\text{C}$ nitrogen in coordination to the metal ion. The peak of ligand (**HL**) belonging to $-\text{OH}$ group is identified at 3676 cm^{-1} , the peak is disappeared in spectra of the complexes (**1-5**) which indicates the deprotonation of $-\text{OH}$ group upon complexation [27] and also the peak at 1278 cm^{-1} for the phenolic $\text{C}-\text{O}$ group in the ligand (**HL**) is shifted to higher frequencies in the spectra of the complexes (**1-5**) indicating confirming deprotonation of

the phenolic $-\text{OH}$ on chelation [28]. In the spectra of metal complexes (**1-5**) a broad diffuse band was identified in the range $3500\text{--}3350\text{ cm}^{-1}$ and another weak band in-plane bending (rocking) in the range $858\text{--}828\text{ cm}^{-1}$. It suggests that the presence of water molecules in the complexes (**1-5**) [29, 30]. In the complexes (**1-5**), two bands appeared in the region $1668\text{--}1664\text{ cm}^{-1}$ ($\gamma_{\text{asymmetry}}$) and $1404\text{--}1398\text{ cm}^{-1}$ (γ_{symmetry}) which attribute the carboxylate part in the acetate group and also suggest that they are responsible for the consisting of unidentate coordination site with the metal ion due to the value of differences between asymmetry and symmetry was greater than 200 cm^{-1} ($\Delta\gamma \geq 200\text{ cm}^{-1}$) [31]. The far IR spectra of the complexes (**1-5**) show medium bands in the region $471\text{--}462\text{ cm}^{-1}$ and $516\text{--}498\text{ cm}^{-1}$ corresponding to $\nu\text{ M}-\text{N}$ and $\nu\text{ M}-\text{O}$ vibrations respectively [32] and other absorption bands were no appreciable change in the ligand (**HL**) and metal complexes (**1-5**).

Electronic spectra and Magnetic susceptibility

The electronic spectra of the free ligand (**HL**) displayed two bands at 258 nm (38760 cm^{-1}) and 319 nm (31348 cm^{-1}) were intraligand charge transfer assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions for phenyl ring and the azomethine chromophore ($-\text{CH}=\text{N}-$) respectively [33]. The bands for the metal complexes (**1-2**) and (**4**) are shifted to a longer

Compounds	$\text{HC}=\text{N}$	bpy $\nu(\text{C}=\text{N})$	Ph- $\text{C}-\text{O}$	Aliphatic $\text{C}-\text{N}-\text{C}$	Acetate (s) / (as)	Ph-OH	(H_2O)	ν M-N	ν M-O
Ligand (LH)	1635	--	1278	1342	--	3676	--	--	--
Complex (1)	1596	1571	1310	1325	1398 1664	--	848(b)	464	508
Complex (2)	1600	1573	1311	1320	1402 1664	--	846(b)	466	498
Complex (3)	1621	1575	1302	1336	1402 1668	--	828(b)	470	508
Complex (4)	1596	1571	1309	1334	1402 1668	--	836(b)	462	516
Complex (5)	1627	1573	1308	1335	1402 1664	--	858(b)	471	510

bpy = 2,2'-bipyridine, Ph-C-O = Phenolic C-O group, Aliphatic C-N-C = Morpholino C-N-C,
s = symmetry, as = asymmetry, Ph-OH = Phenolic OH, b = In plane bending (rocking)

Table 2
FTIR SPECTRAL DATA
OF SCHIFF BASE
LIGAND (**HL**)
AND COMPLEXES
(**1-5**) (cm^{-1})

wavelength which may be attributed to the donation of lone pair electron in a sp^2 -hybridized orbital of the imino nitrogen atom of the ligand to the metal ($N \rightarrow M$). Complex (1) exhibited only one low intensity broad band d-d transition at 647 nm (15455 cm^{-1}) due to dynamic Jahn-Teller distortion [34, 35]. It is assigned to the ${}^2E_g \rightarrow {}^2T_{2g}$ transition and its magnetic moment (μ_{eff}) value is 1.81 BM which is slightly higher than the spin only value (1.73 BM) for one unpaired electron which suggests possibility of a distorted octahedral geometry [36, 37]. The electronic spectrum of complex (2) showed three bands at 886 nm (11286 cm^{-1}), 525 nm (19047 cm^{-1}) and 404 nm (24752 cm^{-1}) corresponding to the transitions ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(F)$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$. The magnetic moment of the complex (2) was observed at 4.82 BM which was within the range 4.7-5.2 BM for octahedral geometry [38, 39]. The electronic spectrum of complex (4) showed three bands at 926 nm (10799 cm^{-1}), 553 nm (18083 cm^{-1}) and 346 nm (28901 cm^{-1}) corresponding to the transitions ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$. The magnetic moment of the complex (4) was observed at 3.13 BM which was within the range 2.5-3.5 BM for octahedral Ni(II) complexes [40]. Complexes (3) and (5) have no absorption in the visible domain which is in agreement with the electronic structure of the central metallic ion with d^5 and d^{10} electronic configurations respectively. The crystal field theory does not predict the d-d transitions of these complexes (3) and (5) due to absence of absorption bands in the visible region. The observed all spectral data results and magnetic moment values suggest an octahedral environment around the metal(II) ion.

EPR spectra

The X-band EPR spectra of the complex (1) in powder state was recorded at room temperature and liquid nitrogen temperature under 9.10 GHz Microwave field modulation using tetracyanoethylene ($g_e = 2.00277$). The complex (1) exhibited one intense absorption band at 300 K in the high field region was isotropic due to the tumbling motion of the molecules and another one exhibited at frozen state (77 K) was anisotropic pattern with well-resolved hyperfine lines (fig. 2 and table 3).

The results are summarized in table 3. The spin Hamiltonian parameters have been calculated by Kivelson's method [12]. The observed g -values are in the order $g_{\parallel} (2.1068) > g_{\perp} (2.01827) > g_e (2.00277)$ indicating that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital of Cu(II) [41] and the observed g_{\parallel} values for complex (1) was less than 2.3 in agreement with the covalent environment character of the M-L bond [42]. The covalent nature of the M-L bond in the complex (1) was further supported by the g_{eff} value which observed value was 1.3750 and less than 2.00277 [43]. The observed hyperfine

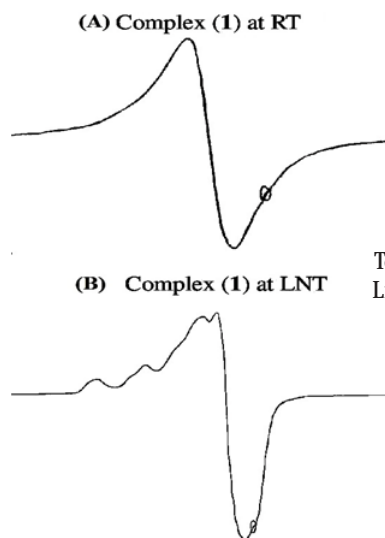


Fig. 2. The EPR spectra of complex (1) at (A) Room Temperature (300 K) and (B) Liquid Nitrogen Temperature (77 K)

constant parameters for the complex (1) were in the order $A_{\parallel} = 130 \text{ G} > A_{\text{av}} (103.33 \text{ G}) > A_{\perp} = 90 \text{ G}$ [12]. In the complex (1) the observed interaction coupling constant (G) value (6.6820) was larger than 4 suggesting that there is no interaction between Cu-Cu centers in the solid state complex and the absence of half field signal at 1600G corresponding to the $\Delta M_s = \pm 2$ transition rules out a Cu-Cu interaction [44]. The values of molecular orbital coefficient parameters (α^2 , β^2 , γ^2) were calculated by Kivelson and Neimann formulae in the previous published report [12]. The observed in-plane π -bonding parameter (α^2) value was 0.5 which indicates that the complex (1) has covalent character. The observed in-plane π -bonding (β^2) and out-plane π -bonding (γ^2) values were less than 1.0 which also indicate that the π -bonding is completely covalent character. The observed orbital reduction factor K_{\parallel} value was larger than K_{\perp} value for the complex (1) which indicates the presence of out-plane π -bonding in metal-ligand π -bonding [45]. The observed Co-factor (f_{\parallel}) value of degree of geometrical distortion for the complex (1) was 175.56 cm^{-1} which indicates an octahedral geometry around the Cu(II) ion. Spectral data and magnetic measurements of the complex (1) have been proposed as a distorted octahedral geometry (scheme 1).

Cyclic voltammetry

The electrochemical techniques are the most effective and versatile methods available for the mechanistic study of redox systems of the complexes. The cyclic voltammogram for complexes (1-5) reveals that one electron reduction peak (E_{red}) corresponding to $M(\text{II})$ to $M(\text{I})$ and oxidation peak (E_{ox}) corresponding to $M(\text{I})$ to $M(\text{II})$. The observed values of peak-to-peak separation (ΔE_p) for complexes (1-5) was in the range of 255- 862 mV which is

Complex	g_{tensor}			Hyperfine constant $\times 10^4 \text{ cm}^{-1}$			
	g_{\parallel}	g_{\perp}	g_{av}	A_{\parallel}	A_{\perp}	A_{av}	G
Complex (1)	2.11	2.02	2.05	130	90	103.33	6.682
	Bonding parameters						
	α^2	β^2	γ^2	K_{\parallel}	K_{\perp}	$f_{\parallel}, \text{ cm}^{-1}$	$\mu_{\text{eff}}, \text{ BM}$
	0.512	0.480	0.280	0.242	0.160	175.56	1.77

$g_e = 2.00277$, Microwave frequency (γ^1) = 9.114×10^9 cycle/sec, Resonance magnetic field (H) = 3250 gauss, $H_a = 3240$, $H_{\parallel} = 3080$, $H_{\perp} = 3215 \text{ G}$, ($1 \text{ G} = 10^{-4} \text{ cm}^{-1}$) $E_{d-d} = 15,455.95 \text{ cm}^{-1}$, one-electron spin orbit coupling constant of free Cu(II) ion $\lambda_0 = -828 \text{ cm}^{-1}$, $\mu_{\text{eff}} = 1.81 \text{ BM}$ (exp), Free ion dipolar term (P) = 0.036 cm^{-1} , $f_{\parallel} = g_{\parallel} / A_{\parallel}$, $\mu_{\text{eff}} = g_{\text{av}}$ $[S(S+1)]^{1/2}$

Table 3
THE EPR SPECTRAL DATA FOR
POLYCRYSTALLINE
SAMPLE OF THE COMPLEX (1)
AT 77K

Complexes	Couple	E_{pa} (V)	E_{pc} (V)	ΔE_p (mV)	E^0 (V)	i_{pa} $10^{-6} \mu A$	i_{pc} $10^{-6} \mu A$	i_{pa}/i_{pc} (μA)
(1)	Cu(II)/ Cu(I)	-0.5424	-0.9827	440	-0.7625	1.7509	-3.7512	-0.4667
(2)	Co(II)/ Co(I)	-0.2520	-1.1145	862	-0.6832	2.3174	-3.6223	-0.6397
(3)	Mn(II)/ Mn(I)	0.0983	-0.3213	419	-0.2230	1.4185	-2.2920	-0.6189
(4)	Ni(II)/ Ni(I)	-0.8646	-1.1212	256	-1.0029	-1.6443	-1.9493	0.8435
(5)	Zn(II)/ Zn(I)	-0.6024	-0.8578	255	-0.7301	-1.0567	-3.1011	0.3400

Formal electrode potential E^0 (or) $E_{1/2} = \frac{1}{2} (E_{pa} + E_{pc})$, Scan rate = 100 mV s⁻¹

Table 4
ELECTROCHEMICAL DATA OF
MIXED LIGAND
COMPLEXES (1-5) IN METHANOL
MEDIUM

larger than the Nernstian value [$(E_{pa} - E_{pc})$ (or) $\Delta E_p = 2.303RT/nF = 59/n$ mV] for the $M(II)/M(I)$ redox couple revealing that this process is the best quasi-reversible and if the peak current ratio is greater or less than unity ($i_{pa}/i_{pc} >$ or < 1) which represents quasi-reversible one electron transfer process [46]. The observed values of peak current ratio of complexes (1-5) were less than unity. In quasi-reversible the peak current (i_p) is proportional to the root of the scan rate (\sqrt{U}) and separation in peak potential (ΔE_p). The observed values of formal electrode potential (E^0) were in the ranges -0.2230 to -1.0029 V which indicate that each couple corresponds to one electron transfer process (table 4).

(a) DPPH radical scavenging assay

Antioxidants are chemical substances that donate an electron to the free radical and convert into a harmless molecule. They may decrease the energy of the free radical or suppress radical formation or break chain propagation or repair damage and reconstitute membranes. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in methanol [47, 48]. DPPH free radical is a stable at room temperature which can accept hydrogen or electron from donors and gets reduced by a color change from purple to yellow. When the concentration of complexes increases the antioxidant potential also increases. It was observed that the complex (1) has exhibited better antioxidant potential than others (fig. 3). The DPPH radical scavenging assay provides a simple and rapid approach to evaluate antioxidants by

electronic spectrophotometer and it can be useful to assess various products at a time.

(b) Hydroxyl radical scavenging assay

In biological systems, uncontrolled accumulations of hydrogen peroxide leads to the formation of oxygen free radicals which cause immense damage to cells membrane. Hydroxyl radical scavenging activity is indicted as the percentage of inhibition of the complexes (1-5) was found to be comparable to that of the ligand at all the concentration used in this study. The 50 % inhibition of all compounds was observed from 14 to 16.0×10^{-5} M concentration [49]. The complex (1), complex (2) and complex (3) showed significant antioxidant activity compared to others (fig. 3).

(c) Superoxide scavenging assay

It is well known for the antioxidant properties and it is clear understand that the antioxidants have the ability to prevent oxidative damage induced by reactive oxygen species as it can interfere with the oxidation process by inhibiting the initiation or propagation of oxidizing chain reactions. In this study the antioxidant potential of ligand (HL) and complexes (1-5) was further assessed by their ability to quench superoxide ions. It was observed that both ligand (HL) and complexes (1-5) showed an increasing trend in their superoxide radical quenching ability with increasing concentrations (fig. 3). In fact at 20×10^{-5} M concentration they exhibited above 50 % activity [50].

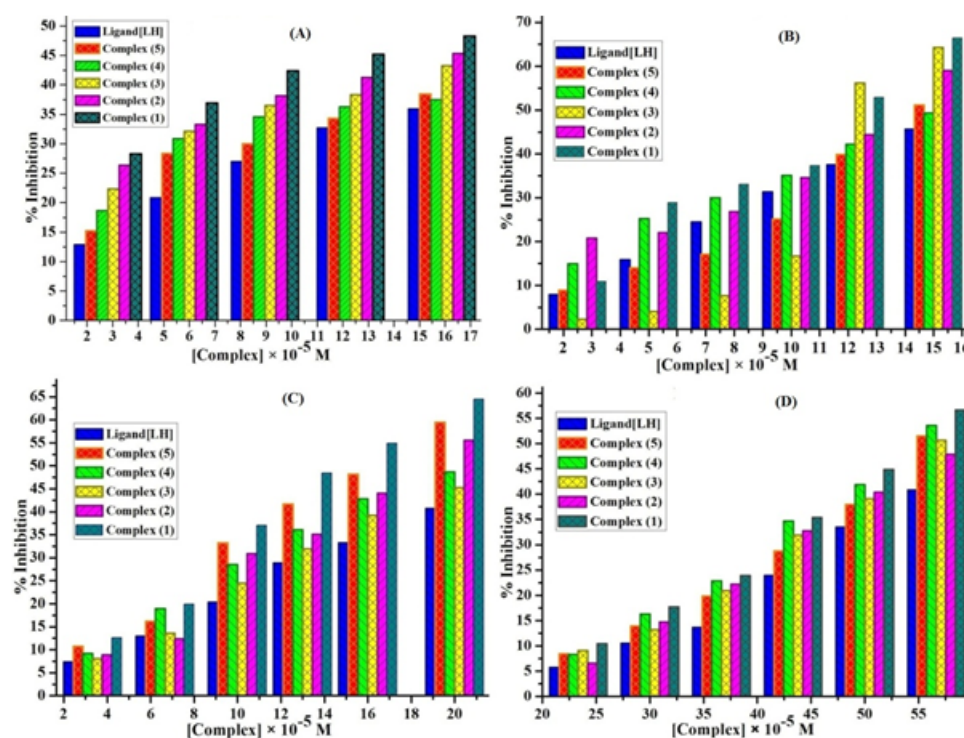


Fig. 3. (A) DPPH assay, (B) Hydroxyl radical scavenging, (C) Super oxide scavenging and (D) Nitric oxide scavenging activity of ligand (HL) and complexes (1-5)

(d) Nitric oxide scavenging assay

Free radical scavenging potential of ligand (**HL**) and complexes (**1-5**) was further confirmed by nitric oxide scavenging assay [51]. The nitric oxide radical inhibition was increased with increasing concentration of all compounds and they are exhibited moderate nitric oxide radical scavenging effect. The observed maximum nitric oxide radical inhibition of all compounds was found at concentration 55×10^{-5} M. The all observed antioxidants results of ligand (**HL**) and complexes (**1-5**) are shown in (fig. 3).

Antimicrobial assay

It was observed the zones inhibition value (mm) that complexes (**1-5**) exhibit good antimicrobial activity than the free ligand (**HL**) [52]. The observed higher activity of the metal complexes could be explained on the basis of Overtone's concept and Tweedy's chelation theory [53]. A possible explanation of higher antimicrobial activity for the complexes was clear attributed that the polarity of the metal ion in a chelated complex is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion. It increases the delocalization of π - and d-electrons over the whole chelated ring and enhances the lipophilicity of the metal complexes. The increased lipophilicity of complexes enhances the cell permeability into lipid membranes which leads to breakdown of the barrier of the cell and thus retards the normal cell processes [54]. The observed values of complexes (**1**), (**2**), (**5**) were significant antimicrobial activity than others and less than the standard drugs (Amikacin and Ketokonazole) [55] (table 5).

DNA interaction studies

(a) DNA nuclease activity by gel electrophoresis

The DNA cleavage efficiency of the complexes (**1-5**) was compared with DNA control (fig. 4). Lane: 1 for the control (DNA + H_2O_2) does not exhibit significant cleavage even on longer exposure time [56] and lane: 2 for the ligand (**HL**) alone was inactive in the presence and absence of external agents. Lane: 3 complex (**1**) and Lane: 4 complex (**2**), were cleaved DNA more efficiently in the presence of hydrogen peroxide than others. This may be attributed to the formation of hydroxyl free radicals ($OH\cdot$) which can be produced by metal ions reacting with H_2O_2 to produce the diffusible hydroxyl radical or molecular oxygen, which may damage DNA through Fenton type chemistry [57]. This hydroxyl radical participates in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of sugar-phosphate backbone [58] and also the smear in the gel diagram indicates the presence of radical cleavage.

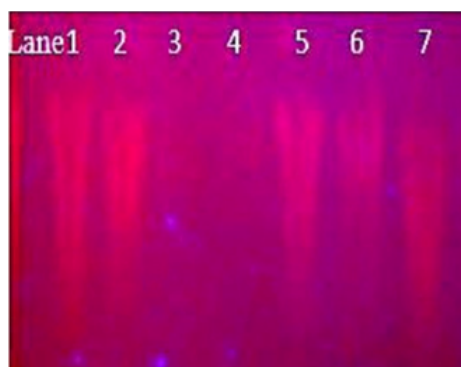
(b) Evaluation of DNA binding constants by Electronic absorption titration

Intrinsic binding constant (K_b) and Gibbs free energy change (ΔG_b°) values for complexes (**1-5**) have been evaluated by electronic absorption titration (fig. 5). The complexes (**1-5**) exhibit two intense absorption bands. One intense band appeared in the region of 292-367 nm which assigned to π - π^* intraligand charge transfer transitions and other one intense band in the region of 270 nm which attributed to ligand to center transfer [59]. The binding of the complexes to DNA helixes were characterized by monitoring the changes in the absorbance of π - π^* bands and shift in wavelength on each addition of DNA solution

Compounds	Antibacterial activity					Antifungal activity		
	(-) E. coli	(-) S. typhi	(-) C. bacterim	(+) S. aureus	(+) B. cereus	A. flavus	A. niger	C. albicans
Ligand (HL)	10	9	7	9	10	8	9	11
Complex (1)	15	14	12	14	14	12	13	15
Complex (2)	12	13	12	12	13	10	12	14
Complex (3)	10	11	8	9	10	8	10	11
Complex (4)	14	13	12	10	13	11	12	14
Complex (5)	13	12	10	13	12	10	11	14
Amikacin	16	19	18	18	17	--	--	--
Ketokonazole	--	--	--	--	--	17	15	16

Escherichia coli, Salmonella enterica typhi, Chromo bacterium, Staph aureus, Bacillus cereus, Aspergillus flavus, Aspergillus niger, Candida albicans, Standard drugs: Amikacin and Ketokonazole

Table 5
EVALUATION OF ANTIMICROBIAL ACTIVITIES (DIAMETER OF ZONE OF INHIBITION IN (mm) OF THE INVESTIGATED COMPOUNDS BY AGAR DISC DIFFUSION METHOD



Lane:1 DNA alone
Lane:2 Ligand [LH] + DNA + H_2O_2
Lane:3 Complex (1) + DNA + H_2O_2
Lane:4 Complex (2) + DNA + H_2O_2
Lane:5 Complex (3) + DNA + H_2O_2
Lane:6 Complex (4) + DNA + H_2O_2
Lane:7 Complex (5) + DNA + H_2O_2

Fig. 4. Gel electrophoresis showing the chemical nuclease activity of DNA by the synthesized ligand (**HL**) and complexes (**1-5**) in the presence of hydrogen peroxide (lane 1-7)

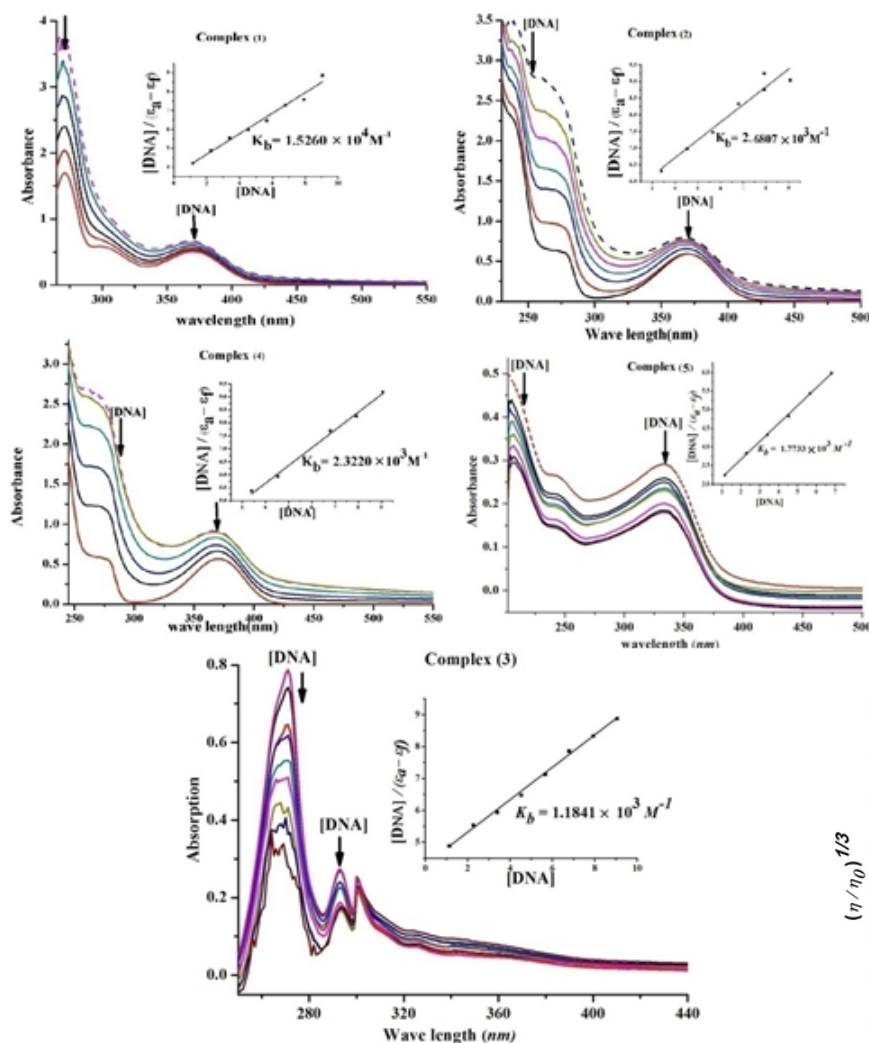


Fig. 5. Electronic absorption spectra of complexes (1-5) in buffer pH = 7.2 at 25°C in the presence of increasing amount of CT DNA. Arrow indicates the changes in absorbance upon increasing the CT DNA concentration. Inset: plot of $[DNA] / (\epsilon_a - \epsilon_p)$ vs $[DNA]$ for the absorption titrations of CT DNA with complexes (1-5)

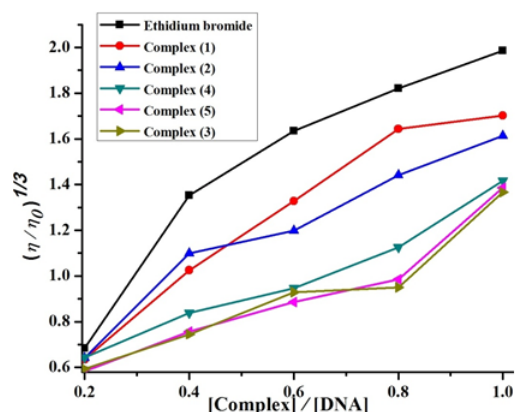


Fig. 6. Plot of relative specific viscosity (η/η_0) versus $R = [Complex] / [DNA]$

Table 6
SPECTRAL PARAMETERS FOR DNA INTERACTION
WITH THE SYNTHESIZED COMPLEXES (1-5)

Complexes	λ_{max} free (nm)	λ_{max} bound (nm)	$\Delta\lambda$ (nm)	Types of Chromism	Chromim %	Binding constant K_b M^{-1}	ΔG_b° $KJ.mol^{-1}$
(1)	367	372	05	Hypo & Red shift	28.47	1.5260×10^4	-23.8707
(2)	366	371	05	Hypo & Red shift	26.98	2.6807×10^3	-19.5516
(3)	292	294	02	Hypo & Red shift	36.10	1.1841×10^3	-17.5362
(4)	366	372	06	Hypo & Red shift	37.60	2.3220×10^3	-19.2050
(5)	333	336	03	Hypo & Red shift	36.97	1.7733×10^3	-18.5281

to the complex. While the concentration of DNA increases from 1.1308×10^{-5} to $9.0464 \times 10^{-5} M$, hypochromic shifts of the intraligand bands were observed accompanied by a moderate bathochromic (red) shifts with range of 2-6 nm for complexes (1-5). The observed results of hypochromism effect with a red shift revealed that the interaction between complexes (1-5) and CT DNA could be non-covalent intercalative binding. The binding constant (K_b) values are calculated from the ratio of the slope to the intercept of the plot of $[DNA] / (\epsilon_a - \epsilon_p) \times 10^8$ Vs $[DNA] \times 10^{-5} M$ by Wolfe-Shimmer equation and ΔG_b° values for these complexes were calculated by Van't Hoff equation which indicate that the mixed ligand complexes can interact with DNA in a spontaneous manner and also the percentage of chromicity (H , %) for these complexes were determined [12]. The results are summarized in table 6. From the observed results, it is clear that complex (1) has higher binding efficacy via intercalation and other complexes may bind to DNA via groove binding.

(c) Evaluation of DNA binding affinity by Viscosity titration measurements

The interaction between the complexes (1-5) and DNA was investigated by viscosity measurements. Ethidium bromide is used as a well known DNA classical intercalator which increases the viscosity strongly by lengthening the DNA double helix through intercalation. While increasing the concentration of complexes (1-5), the relative viscosity of complexes also increases steadily similar to the performance of ethidium bromide. The increased degree of viscosity may depend on the binding affinity to DNA and the observed values were in the following order **EB** > (1) > (2) > (3) > (4) > (5) (fig. 6). The significant increase in viscosity of the complexes is obviously due to the partial insertion of the ligand between the DNA base pairs leading to an increase in the separation of base pairs at intercalation locations, hence an increase in overall DNA contour length [60].

Conclusions

The work described in this report involves the synthesis of novel water soluble mixed ligand complexes. The spectral data of the complexes (**1-5**) suggest an octahedral geometry of the monomeric type and [MLL'] stoichiometry. The lower electrical conductivity values reveal that they are nonelectrolytes. Cyclic voltammogram showed that complexes (**1-5**) were of M(II)/M(I) redox couple correspond to quasi reversible one electron transfer process. Complexes (**1-5**) have exposed higher potent activity than ligand (**HL**) in antimicrobial and antioxidant studies. The Gel electrophoresis results revealed that complexes (**1**) and (**2**) have been revealed a significant DNA cleavage efficiency than others in the presence of hydrogen peroxide. The observed DNA binding results for the complexes (**1-5**) by electronic absorption and DNA binding affinity results by viscosity titration measurements were lesser than Ethidium bromide (control). DNA binding results also indicates that complex (**1**) is bound to DNA via intercalation and other complexes may bind to DNA via groove binding. The negative Gibbs free energy change values (ΔG_b°) indicate that the mixed ligand complexes can interact with DNA in a spontaneous manner.

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