



SYNTHESIS, SPECTROSCOPIC INVESTIGATION AND BIOLOGICAL EVALUATION OF SOME TRANSITION METAL COMPLEXES OF A 3-(((2-HYDROXYQUINOLIN-3-YL) METHYLENE) HYDRAZONO) INDOLIN-2-ONE

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ABSTRACT

A bishydrazone was obtained by the condensation of isatin monohydrazone with 2-hydroxyquinoline-3-carbaldehyde, which formed a series of complexes with Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II). The ligand and its metal complexes were characterized on the basis of elemental analysis, conductance data, magnetic susceptibility measurements, IR, UV-Vis, mass, ¹H NMR, ESR and XRD studies. By these spectral studies it is found that Co(II), Ni(II) and Cu(II) complexes have been assigned octahedral geometry where as the Zn(II), Cd(II) and Hg(II) complexes have been assigned tetrahedral geometry. The Schiff base and its metal complexes have been screened for their *in vitro* antibacterial and antifungal activities by MIC method. The DNA cleavage activities of all of the complexes were studied by the agarose gel electrophoresis method. In addition, the free ligand along with its complexes has been studied for their antioxidant activity.

KEYWORDS: Schiff base, Metal complexes, Spectral studies, Antioxidant, Antimicrobial activity, DNA cleavage.



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INTRODUCTION

Metal complexes play an essential role in agricultural, pharmaceutical and industrial chemistry. Schiff base have been widely investigated for their antibacterial and antitumor activities. The metal complexes of Schiff bases have also received much attention. These complexes play an important role not only in the development of coordination chemistry, but also in catalysis, enzymatic reactions, magnetism and molecular architectures and they exhibit interesting biological activities¹. Hydrazones, possessing an azomethine $-NHN=CH-$ proton, constitute an important class of compounds for drug development. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities. Hydrazones are synthesized by heating the appropriate substituted hydrazine/hydrazides with aldehydes and ketones in solvents like ethanol, methanol, butanol, glacial acetic acid, ethanol-glacial acetic acid². Hydrazones form an interesting class of chelating ligands, which find extensive applications in various fields. Literature survey of transition metal hydrazone complexes revealed that the major contribution to the studies was based on the monohydrazone, but those formed from bishydrazone, especially those containing the isatin moiety, have been studied considerably less so far. We describe here, therefore, the synthesis and characterization of a series of new transition metal complexes of a bishydrazone derived from isatin monohydrazone and 2-hydroxyquinoline-3-carbaldehyde. Isatin is a resourceful endogenous heterocyclic molecule and its derivatives exhibit a wide range of biological activities³. Heterocycles bearing nitrogen, sulphur and oxygen atoms in their structure constitute the core structure of a number of biologically interesting compounds⁴. Because of such significant observations, we thought it worthwhile to incorporate the chemistry of the monohydrazone of isatin with 2-hydroxyquinoline-carbaldehyde to prepare Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes. Thus, the aim of the present work was to synthesize

and characterize Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) metal complexes with newly synthesized Schiff base (HQMHI) derived from isatin monohydrazone and 2-hydroxyquinoline-3-carbaldehyde. The newly synthesized compounds were screened for their antioxidant, DNA cleavage and antimicrobial potency.

MATERIALS AND METHODS

All chemicals used were of analytical grade. Isatin, hydrazine hydrate (99%) was obtained from Fluka and Sisco chemicals.

Synthesis of Schiff base ligand: 3-(((2-hydroxyquinolin-3-yl)methylene)hydrazono)indolin-2-one (HQMHI)

The Schiff base has been synthesized by refluxing the equimolar mixture of methanolic solution of 2-hydroxyquinoline-3-carbaldehyde (0.01 mol, 30 ml) and methanolic solution of isatin monohydrazone (0.01 mol, 30 ml) for 6-7 h in presence of catalytical amount of Conc. HCl. The product obtained after the evaporation of the solvent was filtered, washed with cold methanol and finally recrystallized from methanol to afford Schiff base (HQMHI) as shown in Figure 1. The purity of the compound has been checked by TLC.

Synthesis of metal complexes

The metal complexes were prepared using metal chlorides and the Schiff base (HQMHI) by the general method. An ethanolic solution (40 mL) of Schiff base and Co(II), Ni(II) and Cu(II) chlorides in 1 : 2 molar ratio and 1 : 1 molar ratio for Zn(II), Cd(II) and Hg(II) complexes were refluxed on water bath for about 4 h. An aqueous solution of sodium acetate was added to the reaction mixture to adjust the pH to 6.0-7.0 and reflux was further continued for about an hour. The separated solid complexes were filtered off, washed thoroughly with water and then with little warm ethanol. The complexes obtained were finally dried under vacuum desiccator over fused $CaCl_2$. Elemental analysis data shown in Table 1. The complexes were

analyzed for their metal and chloride contents by standard methods⁵.

Physical measurements

Elemental analysis (C, H and N) was carried out using a Heracus Carlo Erba 1108 CHN analyzer at STIC, Cochin. The IR spectra of the Schiff base and its Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes were recorded in KBr pellets in the region of 4000-450 cm^{-1} on a Perkin Elmer 783 FT-IR spectrophotometer. The electronic spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded on an ELICO SL-164 double beam UV-visible spectrophotometer in the range of 200-1200 nm in DMF (10^{-3} M) solution. Magnetic susceptibility measurements were made at room temperature on a Gouy balance using $\text{Hg}[\text{Co}(\text{NCS})_4]$ as the calibrant.

Molar conductivity measurements were recorded on an ELICO CM-180 conductivity bridge in DMF solution (10^{-3} M) using a dip-type conductivity cell fitted with a platinum electrode, the $^1\text{H-NMR}$ spectra were recorded in DMSO-d_6 on a Bruker 500 MHz spectrophotometer using TMS as an internal standard. The mass spectra were recorded on a JEOL GC mate mass spectrophotometer. The ESR spectrum of the Cu(II) complex in the polycrystalline state was recorded on a Varian-E-4X band EPR spectrophotometer using TCNE as the 'g' marker ($g = 2.00277$) at room temperature. The XRD patterns of the Cu(II) complex was recorded on a Bruker AXS D8 Advance X-ray diffractometer using $\text{Cu K } \alpha = 1.5404$ radiation ($\lambda \text{ \AA}$).

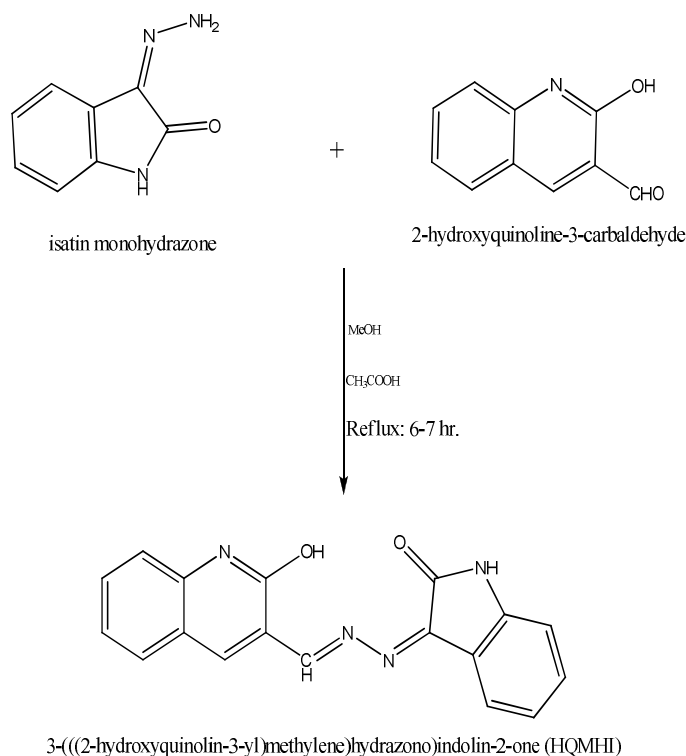


Figure 1
Schematic representation of Schiff base (HQMHI).

Antibacterial and antifungal activities

The biological activities of the synthesized Schiff base and its Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes were studied for *Salmonella typhi* bacteria and *Aspergillus niger*, *Aspergillus flavus*, and *Cladosporium* fungal strains^{6,7}. The stock solution of the test compound was prepared by dissolving 10

antibacterial and antifungal properties by the agar diffusion method respectively in DMSO solvent against *Escherichia coli*, *Staphylococcus aureus* and mg of the test compound in 10 ml of DMSO solvent. The stock solution was suitably diluted with sterilized distilled water to concentrations of 100, 75, 50, 25 and 12.5

µg/ml. The control for each dilution was prepared by diluting 10 ml of solvent instead of stock solution with sterilized distilled water. The bacteria were sub cultured in agar medium. The petri dishes were incubated for 24 h at 37°C. A standard antibacterial drug, gentamycine, was also screened under similar conditions for comparison. The fungi were sub cultured in potato dextrose agar medium. A standard antifungal drug, fluconazole, was used for comparison. The petri dishes were incubated for 48 h at 37°C.

Antioxidant activity: Radical scavenging activity

The radical scavenging activity ligand and its complexes were determined by using 1, 1-diphenyl-2, 2-picryl hyrazyl free radical (DPPH) assay method⁸. DPPH is a stable free-radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule and also contained an odd electron in its structure that frequently used for detection of the radical scavenging activity in chemical analysis. The reduction capability of DPPH radicals was determined by a decrease in its observance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm⁹. Resulting from a color change the absorbance decreased when the DPPH is scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule shows an absorbance at 517 nm, which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic spin-paired molecule. The stock solutions (1mg/ml) of the ligand (HQMHI) and its complexes were diluted to a final concentration of 25, 50, 75 and 100 µg/ml in methanol DPPH in methanol solution (1ml, 0.1 mmol) was added to 2.5 ml of test solution of different concentrations and allowed to react at room temperature. After 30 min the absorbance was measure at 517 nm. A graph was plotted with percentage scavenging effects on the y-axis and concentration (µg/ml) on the x-axis. Radical scavenging activity was expressed as a percentage and was calculated using the following formula.

$$\text{Scavenging effect (\%)} = \frac{[(\text{Control OD} - \text{Sample OD}) / (\text{Control OD})] \times 100}{}$$

The scavenging capability of the ligand (HQMHI) and its complexes were compared with standard drugs namely butylated hydroxyl anisole (BHA) and tertiary butylated hydroxyl quinoline (TBHQ) and ascorbic acid.

DNA cleavage experiment¹⁰

Preparation of culture media

DNA cleavage experiment was done according to the literature¹¹. Nutrient broth (10 g/L of peptone, 5 g/L of yeast extract, and 10 g/L of NaCl) was used for the culturing of Calf-thymus. After 50 ml of medium was prepared, it was autoclaved for 15 min at 121°C under 15 lb of pressure. The autoclaved medium was inoculated with the seed culture and incubated at 37°C for 24 h.

Isolation of DNA

The fresh bacterial culture (1.5 ml) was centrifuged to obtain the pellet, which was then dissolved in 0.5 ml of lysis buffer (100 mM Tris, pH 8.0; 50 mM EDTA; 50 mM lysozyme). To this, 0.5 ml of saturated phenol was added and incubated at 55°C for 10 min. It was then centrifuged at 10,000 rpm for 10 min and to the supernatant, an equal volume of chloroform and isoamyl alcohol (24:1) and a one-twentieth volume of 3 M sodium acetate (pH 4.8) was added. This was then centrifuged at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in Tris buffer (10 mM Tris, pH 8.0) and stored in cold conditions.

Agarose gel electrophoresis

Cleavage products were analyzed by the agarose gel electrophoresis method¹¹. Test samples Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) (1 mg/ml) was prepared in DMSO. The samples Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) (25 µg) were added to the isolated DNA of Calf-thymus. The samples were incubated for 2 h at 37°C and then 20 µl of DNA sample (mixed with bromophenol blue dye at a 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with

a standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0; 0.5 M EDTA/1 L) and finally loaded on to the agarose gel, passing the constant 50 V of electricity for about 30 min. The gel was removed and stained with 10 µg/ml of ethidium bromide for 10-15 min and the bands observed and photographed under the UV transilluminator were used to determine the extent of DNA cleavage. The results were then compared with a standard DNA marker.



Where M = Zn(II), Cd(II), Hg(II) and X = Cl⁻.



Where M = Co(II), Ni(II), Cu(II) and X = Cl⁻

L = 3-(((2-hydroxyquinolin-3-yl) methylene) hydrazono) indolin-2-one (HQMHI).

All complexes are non-hygroscopic solids, insoluble in common organic solvents such as benzene, chloroform, carbon tetrachloride and toluene, but soluble in DMSO and DMF. Analytical data of the complexes presented in Table 1 are in good agreement with the theoretical values. Lower molar conductance values of the metal complexes in DMF indicate their non-electrolytic nature¹².

Table 1
Physical and analytical data of the ligand (HQMHI) and its metal (II) complexes

Compound	Molecular formula	Yield (%)	Found (Calculated) %					Ωm (Ω ⁻¹ cm ² mol ⁻¹)	μ _{eff} (BM)
			C	H	N	M	Cl		
L	C ₁₈ H ₁₂ N ₄ O ₂	80	68.35 (68.29)	3.82 (3.72)	17.71 (17.66)	-	-	-	-
[CoL ₂]	[CoC ₃₆ H ₂₂ N ₈ O ₄]	90	62.71 (62.68)	3.22 (3.18)	16.25 (16.14)	8.54 (8.49)	-	18.23	4.58
[NiL ₂]	[NiC ₃₆ H ₂₂ N ₈ O ₄]	92	62.73 (62.66)	3.22 (3.16)	16.26 (16.11)	8.51 (8.39)	-	20.77	3.17
[CuL ₂]	[CuC ₃₆ H ₂₂ N ₈ O ₄]	80	62.29 (62.12)	3.19 (3.08)	16.14 (16.08)	9.14 (9.08)	-	22.38	1.79
[ZnLCl]	[ZnC ₁₈ H ₁₁ N ₄ O ₂ Cl]	85	51.95 (51.83)	2.66 (2.58)	13.46 (13.31)	15.70 (15.63)	7.65 (7.58)	15.57	-
[CdLCl]	[CdC ₁₈ H ₁₁ N ₄ O ₂ Cl]	70	46.68 (46.55)	2.39 (2.21)	12.10 (12.04)	24.26 (24.12)	8.51 (8.41)	19.39	-
[HgLCl]	[HgC ₁₈ H ₁₁ N ₄ O ₂ Cl]	78	39.21 (39.817)	2.01 (1.99)	10.16 (10.00)	-	-	17.44	-

Electronic spectral and magnetic studies

The electronic absorption spectra of the Co(II), Ni(II) and Cu(II) complexes were recorded in freshly prepared DMF solution (10⁻³ M) at room temperature and the spectral data are presented in Table 2. The electronic spectrum of the Co(II) complex displayed bands at 15923 and 20920 cm⁻¹. These two

RESULTS AND DISCUSSION

The Co(II), Ni(II) and Cu(II) complexes were prepared by treating 1:2 molar ratio of metal salt and ligand (HQMHI), while the other complexes were prepared in 1:1 molar ratio. Formation of the metal complexes can be represented by the following general equations.

bands are assignable to ⁴T_{1g}(F) → ⁴A_{2g}(F) (ν₂) and ⁴T_{1g}(F) → ⁴T_{2g}(P) (ν₃) transitions, respectively, in an octahedral environment¹³. The lowest band ν₇ could not be observed due to the limited range of the instrument used, but could be calculated using the band fitting procedure suggested by Underhill and Billing¹⁴. Co(II) complex display a magnetic

moment value of 4.58 BM, which is within the range of 4.46-5.53 BM which correspond to high spin octahedral environment around the metal ion¹⁵. The Ni(II) complex exhibited two absorption bands, at 15432 and 25125 cm^{-1} assignable to ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2), ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3) transitions, respectively, in an octahedral environment. The band ν_1 was calculated by using a band fitting procedure¹⁶. Ni(II) complex showed the magnetic moment value of 3.17 BM, which is within the range of 2.7-3.3 BM suggesting consistency with their octahedral environment¹⁷. The Cu(II) complex exhibited low intensity single broad asymmetric band in the region 14492-16977 cm^{-1} . The broadness of the band indicates the three transitions ${}^2B_{1g} \rightarrow {}^2A_{2g}$, (ν_1), ${}^2B_{1g} \rightarrow {}^2E_g$ (ν_2) and ${}^2B_{1g} \rightarrow {}^2B_{2g}$ (ν_3), which are of similar in energy and gives rise to only one broad absorption band. The broadness of the band may be due to dynamic Jahn-Teller distortion. All of these

data suggested a distorted octahedral geometry around the Cu(II) ion¹⁸. The Cu(II) complex showed the magnetic moment value of 1.79 BM, which is within the range of 1.75–2.20 BM is consistent with octahedral geometry¹⁹. The octahedral geometry was further supported by the values of ligand field parameters, such as the Racah inter electronic repulsion parameter (B'), ligand field splitting energy (10 Dq), nephelauxetic parameter (β) and ligand field stabilization energy (LFSE)²⁰. The B' values for the complexes were lower than the free ion values, which is an indication of the orbital overlap and delocalization of d-orbitals. The β values obtained were less than unity, suggesting a considerable amount of covalency for the metal-ligand bonds. The β value for the Ni(II) complex was less than that of the Co(II) complex, indicating the greater covalency of the M-L bond²¹.

Table 2
Electronic spectral bands and ligand field parameters of the Co(II), Ni(II) and Cu(II) complexes in DMF (10^{-3} M) solution

Complexes	Transitions in cm^{-1}			Dq (cm^{-1})	B' (cm^{-1})	β	$\beta\%$	ν_2/ν_1	LFSE (kcal)
	ν_1	ν_2	ν_3						
[CoL ₂]	7296	15923	20920	862	996	0.95	4.15	2.18	14.78
[NiL ₂]	9632	15432	25125	963	777	0.74	25.26	1.60	33.02
[CuL ₂]	14492-16977			1573	-	-	-	-	26.97

IR spectral studies

The prominent infrared spectral data with the tentative assignments of the Schiff base (HQMHI) and its Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) complexes are presented in Table 3. The broad peak observed at 3347 cm^{-1} in the IR spectra of the ligand assigned to $\nu(\text{OH})$, which was found to have disappeared in all their respective complexes, there by indicating deprotonation and formation of metal-oxygen bond²². This is further supported by the shifting of phenolic $\nu(\text{C}-\text{O})$ towards higher frequency, indicating the coordination of the phenolate oxygen to metal ion²³. The $\nu(\text{HC}=\text{N})$ vibration of the ligand occurs at 1590 cm^{-1} , which is shifted to a lower frequency in the complexes, indicating the involvement of the azomethine

nitrogen in chelation with the metal ion^{24,25}. The band corresponding to $\nu(\text{C}=\text{O})$ at 1676 cm^{-1} is shifted to a lower frequency, supporting the coordination of the carbonyl oxygen²⁶. However vibrational characteristics of the ring $\nu(\text{N}-\text{H})$ and $\nu(\text{C}=\text{N})$ of the ketimine moiety remain almost unaffected, indicating the non participation of these groups in coordination²⁷. The bands in the regions 500–595 and 449–498 cm^{-1} are ascribed to $\nu(\text{M}-\text{O})$ and $\nu(\text{M}-\text{N})$, vibrations, respectively^{28,29}. From the above observations, it can be concluded that the ligand (HQMHI) binds to the metal ion in a tridentate fashion through the deprotonated phenolate oxygen, Schiff base nitrogen and the carbonyl oxygen of the isatin moiety.

Table 3
IR spectral bands of the ligand and its metal (II) complexes (cm⁻¹)

Tentative assignments	L	[CoL ₂]	[NiL ₂]	[CuL ₂]	[ZnLCI]	[CdLCI]	[HgLCI]
-OH group	3347	-	-	-	-	-	-
Indole ring NH	3210	3210	3210	3210	3210	3210	3210
v(C=O) ring	1676	1650	1645	1652	1655	1636	1660
v(C=N) ring	1600	1600	1600	1600	1600	1600	1600
v(C=N) aldemine	1590	1563	1539	1505	1510	1500	1500
v(C-O)	1249	1309	1260	1311	1320	1347	1254
v(N-N)	965	1035	1015	1039	1090	1057	1030
v(M-N)	-	485	498	496	488	449	449
v(M-O)	-	500	550	595	542	531	589

Mass spectral studies

Mass spectroscopy has been increasingly used as a powerful structural characterization technique in coordination chemistry. The mass spectrum of the Schiff base (HQMHI) showed a molecular ion peak at m/Z 316, which is equivalent to its molecular weight. The spectrum of Zn(II) complex showed a molecular ion peak at m/Z 416.17 which is equivalent to its molecular weight of the complex. Hence supporting the suggested structure for the complex.

¹H-NMR spectral studies

¹H-NMR spectra of the ligand (HQMHI) and its Zn(II) complex was recorded in DMSO-d⁶. The signal at δ (10.6) (s, 1H) was assigned to the OH group on has resonated but in the case of Zn(II) which has been disappeared indicating the involvement of phenolate oxygen in the coordination via deprotonation³⁰. The signal at δ (8.7) (s, 1H) is due to the azomethine group in the ligand (HQMHI), but in case of Zn(II) complex the peak was observed at δ (9.2) (s, 1H)^{31,32}. The peak appeared at δ (9.9) (s, 1H) is due to the hydrogen of -NH in the ligand (HQMHI), but in case of Zn(II) complex the peak was observed at δ (9.9) (s, 1H)³³. In ligand (HQMHI) nine aromatic protons have been observe in the region δ (6.4-7.2) (m, 9H) as a multiplet, in Zn(II) complex nine aromatic protons have been observe in region δ (6.8-7.6) (m, 9H) as multiplet. On comparing the ¹H-NMR spectra of ligand (HQMHI) and the Zn(II) complex, it was observed that the signals of protons of

different functionalities of the ligand have been shifted to the downfield region indicating the coordination of ligand (HQMHI) to the metal Zn(II) ion.

ESR spectra of Cu(II) complex

The ESR spectra of the Cu(II) complex in a polycrystalline state was recorded at room temperature. The g_{\parallel} and g_{\perp} values were found to be 2.198 and 2.042, respectively. The g_{av} was calculated to be 2.094. The spectra showed asymmetric bands with $g_{\parallel} > g_{\perp} > 2.00277$, indicating that the unpaired electrons lay predominantly in the $d_{x^2-y^2}$ orbital with possible mixing of d_{z^2} because of low symmetry³⁴. The axial symmetry parameter 'G' was determined as $G = (g_{\parallel} - 2.00277) / (g_{\perp} - 2.00277) = 4.960$, suggesting that there is no exchange interaction in the Cu(II) complex.

Powder X-ray diffraction

The Cu(II) complex has been characterized by powder XRD studies with a view to find the type of crystal system. The XRD data of Cu(II) complex is given in the Table 4. There are 5 reflections (2θ) between 26.878 to 73.885 with maxima at $2\theta = 50.168$ corresponding to the value of $d = 1.816$. The interplanar spacing (d) has been calculated from the positions of intense peaks using Bragg's equation $n\lambda = 2d \sin \theta$ (Where $\lambda = 1.54056 \text{ \AA}$). The observed and calculated values of d and are quite consistent. The experimental values of $\sin^2\theta/\text{common factor}$

are recorded for each peak in the Figure 2. The $h^2+k^2+l^2$ values of the complex were found to be 1, 2, 3, 4, 7. The presence of

forbidden number 7 indicates the Cu(II) complex belongs to hexagonal or tetragonal system³⁵.

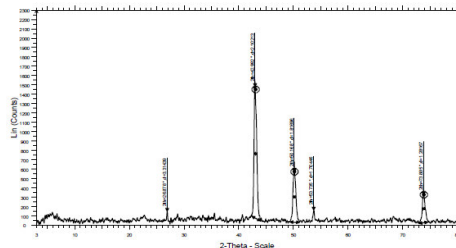


Figure 2
Powder XRD pattern of [CuL₂] Complex.

Table 4
X-ray diffraction data of [CuL₂] Complex

Peak no.	2θ	θ	Sin θ	Sin ² θ	h ² +k ² +l ²	hkl	d Value		a in Å
							Cal	abs	
1	26.878	13.439	0.23240	0.05401	1 (1)	1 0 0	3.31311	3.31439	3.313
2	42.992	21.496	0.36643	0.13427	2.48592 (2)	1 1 0	2.10132	2.10213	3.313
3	50.168	25.084	0.42394	0.17973	3.32745 (3)	1 1 1	1.81626	1.81696	3.313
4	53.735	26.867	0.45192	0.20423	3.78120 (4)	2 2 0	1.70380	1.70446	3.313
5	73.885	36.942	0.60101	0.36121	6.68741 (7)	-	1.28116	1.28167	3.313

Antimicrobial evaluation of ligand and its metal complexes

The ligand and metal complexes were screened for antibacterial activity and the results are presented in Table 5. The MIC was determined by assaying using the broth dilution technique. The synthesized Schiff base (HQMHI) has an inhibitory effect (MIC values of 50–100 mg ml⁻¹) on growth of the tested bacterial strains. All complexes showed greater bactericidal activities against *E. coli* (MIC 12.50–50 µg ml⁻¹), *S. aureus* (MIC 12.50–75 µg ml⁻¹) and *S. typhi* (MIC 12.50–25 µg ml⁻¹) than the ligand (HQMHI). In the fungal studies, the ligand (HQMHI) had an inhibitory effect (MIC values in range 50–100 µg ml⁻¹) on the growth of the tested strains and complexes again showed greater fungicidal activities against *A. flavus* (MIC 12.50–50 µg ml⁻¹), *A. niger* (MIC 12.50–25 µg ml⁻¹) and *Cladosporium* (MIC 12.50–75 µg ml⁻¹). Co(II) and Zn(II) complexes had greater bacterial and fungal activities than the ligand (HQMHI). Chelation reduces the polarity of the metal ion considerably, mainly because of the partial sharing of its positive charge with donor groups and possible π-

electron delocalization on the whole chelate ring. Lipids and polysaccharides are important constituents of cell walls and membranes, which are preferred for metal ion interaction. The cell wall also contains several amino phosphates, carbonyl and cysteinyl ligands, which maintain the integrity of the membrane by acting as a diffusion barrier and also provides suitable sites for binding. Chelation reduces not only the polarity of the metal ion, but increases the lipophilic character of the chelate and the interaction between the metal ion and the lipid is favored. This may lead to the breakdown of the permeability barrier of the cell, resulting in interference with the normal cell processes. If the geometry and charge distribution around the molecule are incompatible with the geometry and charge distribution around the pores of the bacterial cell wall, penetration through the wall by the toxic agent cannot take place and this will prevent the toxic reaction within the pores. Chelation is not the only criterion for antibacterial activity. Some important factors such as the nature of the metal ion, the nature of the ligand, coordinating sites and

geometry of the complex, concentration, hydrophilicity, lipophilicity and the presence of co-ligands have considerable influence on antibacterial activity. Certainly, steric and pharmacokinetic factors also play a decisive role in deciding the potency of an antimicrobial agent. The presence of lipophilic and polar substituents is expected to enhance antibacterial activity. Heterocyclic ligands with multifunctionality have a greater chance of interaction either with nucleoside

bases (even after complexation with metal ion) or with biologically essential metal ions present in the biosystem can be promising candidates as bactericides since they always tend to interact with enzymatic functional groups, in order to achieve higher coordination numbers. Thus antibacterial property of metal complexes cannot be ascribed to chelation alone, but is an intricate of several contributions.

Table 5
The antimicrobial activity of ligand (HQMHI) and its metal (II) complexes evaluated by MIC ($\mu\text{g ml}^{-1}$)

Compounds	<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>Cladosporium</i>
L	75	100	50	75	50	100
[CuL ₂]	25	50	25	25.50	12.50	50
[CoL ₂]	12.50	12.50	12.50	12.50	12.50	12.50
[NiL ₂]	25	75.50	25	12.50	25	25
[HgLCI]	50	25	12.50	25	25	50
[CdLCI]	25	50	25	50	25	75
[ZnLCI]	12.50	12.50	12.50	12.50	12.50	12.50
Gentamycine	12.50	12.50	12.50	-	-	-
Fluconazole	-	-	-	12.50	12.50	12.50

Antioxidant activity: Radical scavenging activity

The antioxidant activity of ligand (HQMHI) and its metal complexes were investigated by comparing with the standard drugs such as BHA, TBHQ and ascorbic acid using spectrophotometrically at 517 nm. From the investigation it was clearly observed that metal complexes scavenge DPPH effectively than ligand (HQMHI), suggesting their stronger free radical scavenge and excellent antioxidant properties. In the present study

the Co(II), Ni(II) and Cu(II) complexes have exhibited good scavenging activity. Whereas, Cd(II), Zn(II) and Hg(II) complexes have shown moderate activity as a radical scavenger compared with standards as shown in Figure 3. Consequently these results are in agreement with earlier studies of metallic complexes in which the ligand (HQMHI) has antioxidant activity and it is expected that the metal ion will increase its activity extensively.

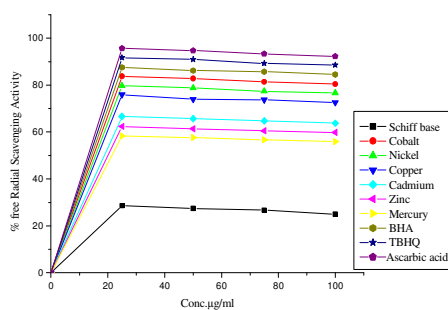


Figure 3
Antioxidant results of Schiff base/complexes.

DNA cleavage efficiency

The Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method against Calf-thymus DNA and the gel picture showing cleavage is presented in Figure 4.

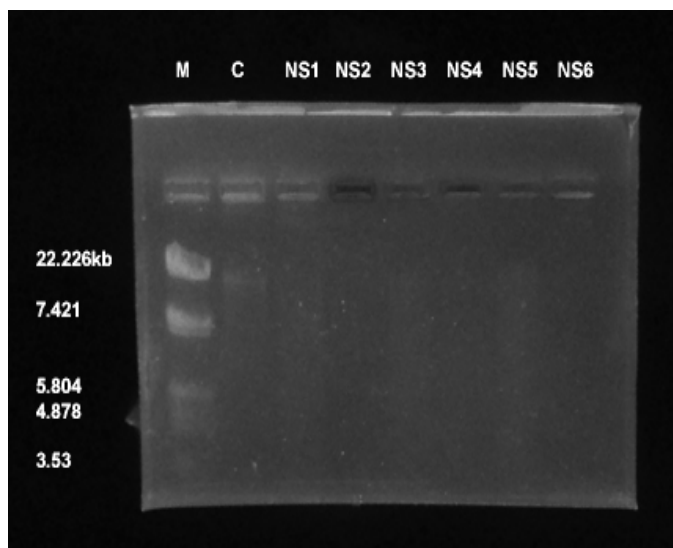


Figure 4
Gel picture showing the cleavage analysis of samples.

Deoxyribonucleic acid (DNA) is the principle target molecule for most of the anticancer and antiviral therapies³⁶. Gel electrophoresis works on the migration of DNA under the influence of electric potential. The cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA-binding ability. DNA-binding studies helps in the rational designing and construction of new and more efficient drugs targeted to DNA³⁷. The gel electrophoresis clearly revealed that, all the complexes (lanes NS1–NS6) have acted on DNA as there was molecular weight difference between the control and the treated DNA samples. The difference was observed in bands of lanes NS1–NS6 compared to the control. However, the nature of reactive intermediates and the mechanism involved in the DNA cleavage by the complexes is not clear. The results indicated the important role of metal ions in isolated DNA cleavage reactions. As the compound was observed to cleave the DNA, it can be concluded that, the compound inhibits the growth of pathogenic organism by cleaving the genome³⁸.

CONCLUSION

The synthesized new Schiff base (HQMHI) and its metal complexes have been confirmed by the analytical data, IR, electronic, mass, ¹H NMR, ESR spectral data, magnetic susceptibility, molar conductance and XRD studies. The antimicrobial activity results shows that all complexes have been found to be more effective than its ligand (HQMHI) as the process of chelation dominantly affects the overall biological behavior of the compounds. All compounds showed varying antioxidant (free radical scavenging) activities when compared to the standards. The results suggest that the antioxidant activity of these compounds may contribute to their claimed antioxidant property and may lead to chemical entities with potential for clinical use. The DNA cleavage studies revealed that the metal complexes showed good efficiency towards DNA cleavage. Based on the analytical and spectral studies, we propose octahedral geometry for the Co(II), Ni(II) and Cu(II) complexes and tetrahedral geometry for the Zn(II), Cd(II) and Hg(II) complexes (Figures 5 and 6).

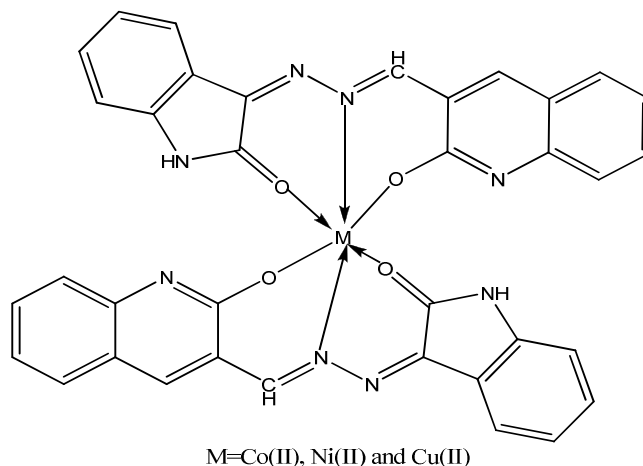


Figure 5
Proposed structure of Co(II), Ni(II) and Cu(II) metal complexes.

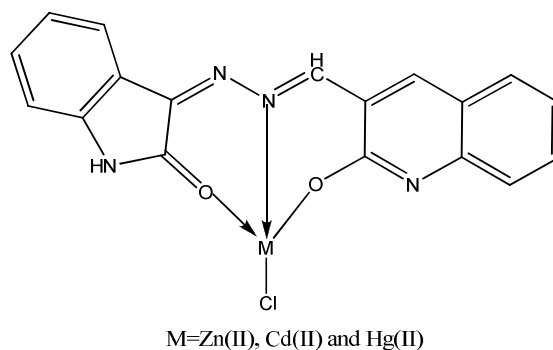


Figure 6
Proposed structure of Zn(II), Cd(II) and Hg(II) complexes.

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