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Chelates of Schiff Bases Derived From Thiocarbohydrazide: Synthesis and Applications

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ABSTRACT

Complexes of Fe(III), Co(II) and Cu(II) with a bidentate Schiff base ligand derived from 2-hydroxy-1-naphthaldehyde and thiocarbohydrazide have been synthesized. The complexes have been characterized by IR,UV, magnetic susceptibility, molar conductance and thermal studies. From the data, an octahedral geometry has been suggested for Fe(III) and Co(II) complexes and square planar geometry for Cu(II) complex. 3D molecular modeling and energies of all complexes are furnished and the analysis for bond length has been carried out for one of the complex. The complexes have been tested for their antimicrobial and nuclease activity.

Keywords: Schiff base, molecular modeling, nuclease activity.

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INTRODUCTION

Transition metal Schiff base complexes have been found to play a vital role in medicine, biological systems and industries. A large number of complexes with therapeutic values have been reported; for example, Co(III) Schiff base complexes are potential antiviral agents. Cisdichlorodiammineplatinum(II) is an anticancer agent and Cu(II) Schiff base complex is an ant tubercular agent [1]. Many biologically active nitrogen heterocycles have been synthesized by Cu (II) Schiff base complexes mediated atom transfer radical cyclisation [2].

Thiocarbohydrazide derivatives have attracted much attention in recent years due to their applications in the synthesis of heterocyclic compounds [3], synthesis of transition metal complexes [4] and in pharmacological studies [5]. Macro cycles synthesized in the reactions of thiocarbohydrazide with polycarbonyl compounds and their complexes with the salts of divalent metals are effective fungistatic agents. The cytotoxicity of carbohydrazones and thiocarbohydrazones of some ketones is comparable with or even exceeds the cytotoxicity of the well-known commercial compound melphalan [6].

In the present work, we have synthesized transition metal complexes with Schiff base derived from thiocarbohydrazide and 2-hydroxy-1- naphthaldehyde. The complexes were characterized by analytical and spectral techniques and were evaluated for their DNA cleavage, antibacterial and antifungal activity.

MATERIALS AND METHODS

Chemicals Used

2-hydroxy-1-naphthaldehyde, carbon disulfide, hydrazine hydrate and ethanol were purchased from commercial sources and used as such without further purification.

Synthesis of Ligand (L)

The Schiff base was synthesized by the condensation of thiocarbohydrazide (10 mmol) and 2-hydroxy-1-napthaldehyde in 1:1 molar ratio in hot ethanol. The reaction mixture was stirred well, refluxed for 1 hour and left overnight. The yellowish orange colored solid precipitate of Schiff base obtained was filtered, washed with cold ethanol and dried.

Synthesis of Metal Complexes

A hot ethanolic (20 mmol / 10 ml) solution of Schiff base, 2-hydroxy napthylidene-thiocarbohydrazide was added drop wise to an ethanolic (10 mmol / 10 ml) solution of corresponding metal salts [metal acetate / chlorides (Co,Fe, Cu)] in 2:1 (Schiff base : Metal) ratio. The reaction mixture was stirred well, refluxed for 1 h and left overnight. The resulting solutions were reduced to 1/3 of their original volume and kept aside. The solid complexes obtained were collected by vacuum filtration, washed several times with cold 50% ethanol, ethanol and anhydrous ether and then dried *in vacuo* over anhydrous $CaCl_2$.

Characterization Techniques

Melting points of all the complexes were determined on Cintex apparatus in open glass capillaries. The carbon, hydrogen, sulphur and nitrogen contents of the synthesized Schiff base ligands and the complexes were determined using ElementarVario EL III at STIC, CUSAT, Cochin. Conductance values of the complexes were obtained on EQ-660A Digital conductivity meter using DMF as solvent. All measurements were corrected for the conductance of pure solvent by subtracting the conductance of pure solvent from that of the solution. Magnetic susceptibility measurements were carried out for solid complexes using Gouy balance at room temperature. The Gouy tube was standardized by using copper Sulphate as calibrant. IR spectra of Schiff base and the complexes were recorded in the range of 4000 to 400 cm^{-1} on a Shimadzu FTIR–8400S spectrophotometer as KBr discs. The electronic absorption spectra of the complexes were recorded on a UV 3000+ spectrophotometer (cell length, 1 cm) in the 200 – 800 nm range. The thermograms were recorded in dynamic nitrogen atmosphere (flow rate 20 mL/min) with a heating rate of 10 K/min using a Perkin Elmer (TGS-2 model) thermal analyzer.

RESULTS AND DISCUSSION

The analytical and physical data of the ligand and the complexes are given in table-1.

Table:1 Analytical and physical data of the ligand L and its complexes

Compound	Molecular formula	Mol. weight	Elemental analysis (%) Found (calc.)				Melting point	Λm ($\Omega^{-1}cm^2mol^{-1}$)
			C	H	N	S		
L	$C_{12}H_{12}N_4OS$	260					206	
$[Fe(L)_2 OAc] (OAc)_2$	$C_{28}H_{39}N_6O_8S_2$ Fe	752.85	57.61 (57.13)	4.46 (4.24)	9.60 (10.01)	7.32 (6.70)	228	190
$[Co(L)_2 OAc] (OAc).2H_2O$	$C_{28}H_{34}N_8O_8S_2$ Co	737.93	45.53 (45.32)	4.61 (4.61)	15.18 (15.77)	4.84 (4.99)	225	110
$[Cu(L)_2] Cl_2$	$C_{24}H_{26}N_80_3S_2$ Cu	678.45	42.45 (42.99)	3.83 (3.90)	16.51 (16.80)	9.43 (9.66)	>360	250

Molar Conductance

The Λ_m values determined for the complexes in DMF are reported in table 1. Analysis of the results show that Fe(III) and Cu(II) chelates of the Schiff base ligand have Λ_m values190-250 $\Omega^{-1}cm^2 mol^{-1}$ showing that they are 1:2 electrolytes whereas Co(II) complex shows Λ_m value of 90-110 $\Omega^{-1}cm^2 mol^{-1}$ which is characteristic of 1:1 electrolyte [7].

IR Spectral Data and Mode of Bonding

The IR spectral data of the important functional groups of the ligand and the complexes are presented in table-2. The IR spectrum of the ligand and that of the Fe complex is given in Fig. 1 and 2

Table-2: Characteristic IR stretching bands of Schiff base ligand and the metal complexesin cm^{-1}

Compound	ν(C=S)	ν (C=N)	ν (OH)	ν (M-N)	ν (M-O)	ν (COO$^-$)$_{as}$	ν (COO$^-$)$_s$	ν (NH2)	ν (C-O)
TCH	756.12							3444.98	
L	749.37	1620.26 (s)	3227.98					3591.57	1253.77(s)
[Fe(L)$_2$ OAc] (OAc)$_2$	749.37	1597.11	3233.71-3298.38	421.46	576.74	1539.25	1457.27	3581.57	1254.74(w)
[Co(L)$_2$ OAc] (OAc).2H$_2$O	748.41	1615.44 (b)	3223.16-2884.64	439.78	539.12	1536.35	1454.38	3519.24-3417.01	1248.95
[Cu(L)$_2$] Cl$_2$	750.33	1612.54	3270.42	416.64	591.20			3498.02	1240.27

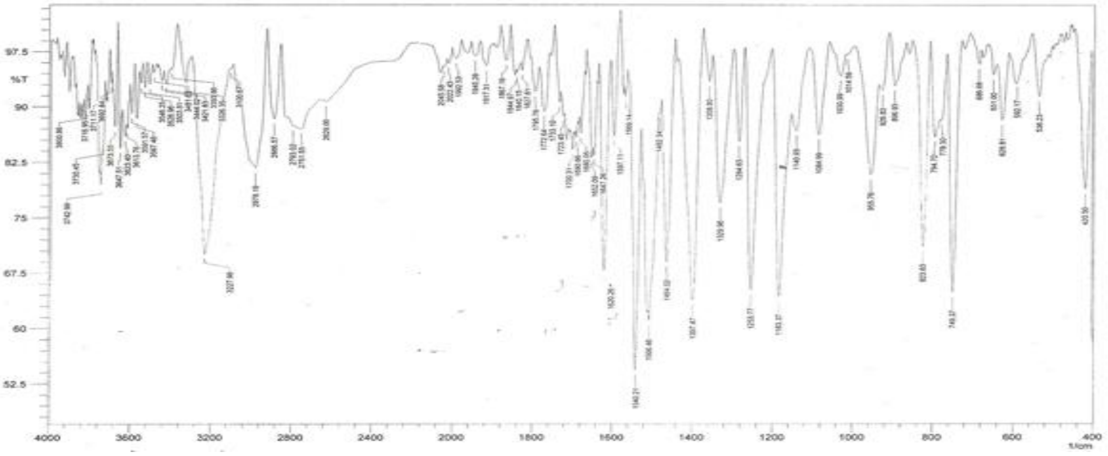


Fig:1 IR spectrum of ligand L

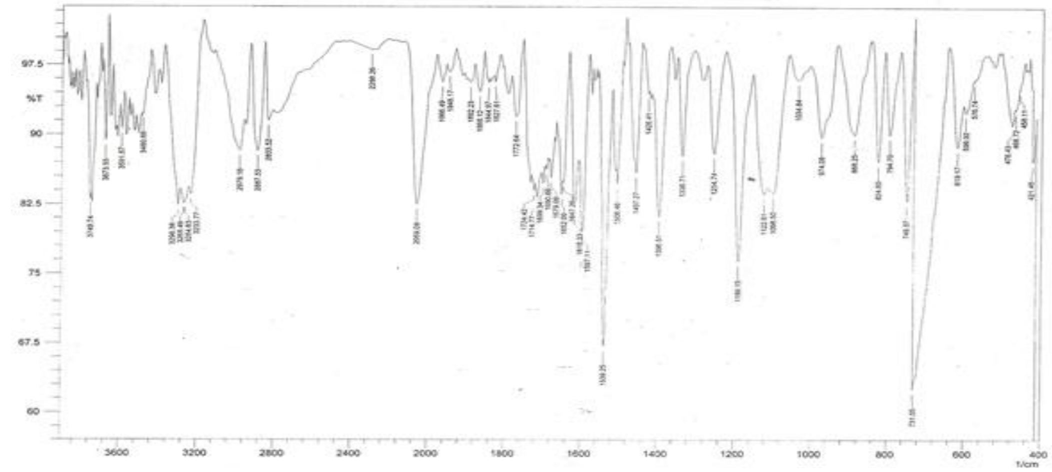


Fig:2 IR spectrum of [Fe(L)$_2$ OAc] (OAc)$_2$

The Schiff base ligand L is formed by condensation of 2-hydroxy naphthaldehyde with thiocarbohydrazide. Formation of Schiff base is proved by the absence of a band around 1700 cm^{-1} due to $_{C=O}$ and the appearance of a band at 1620 cm^{-1} due to azomethine C=N stretching.

Band at 3227 cm^{-1} observed in the spectra of ligand is due to stretching vibrations of phenolic –OH group. A strong band at 749 cm^{-1} shows free ঌ$_{C=S}$ in the ligand.

In the case of the metal complexes, the band due to azomethine group of Schiff base (1620 cm^{-1}) underwent a shift to lower frequency (1590-1612 cm^{-1}) after complexation indicating the co-ordination of azomethine N to metal atom and this can be explained by the donation of electron from nitrogen to the empty d-orbitals of the metal atom. The band at 3227.90 cm^{-1} due to phenolic –OH in the ligands is shifted to higher frequency in all the complexes indicating the co-ordination of phenolic oxygen to the metal ion without deprotonation [8].

The band near 780 cm^{-1} in the Schiff bases which is assigned to free C=S is also present in the IR spectra of all the complexes which indicates that sulphur is not co-ordinating to the metal atom [9].

The far IR spectra of the complexes show bands in the region 420-490 cm^{-1} and 520-590cm^{-1} corresponding to ঌ$_{M-N}$ and ঌ$_{M-O}$ vibrations respectively which identify the co-ordination of azomethine nitrogen and phenolic –OH. IR spectra of all the complexes display the bands corresponding to ঌ$_{NH2}$ at 3440-3591 cm^{-1}.

The acetate ion in aqueous solution is characterized by bands at 1530 and 1440 cm^{-1}, which are commonly assigned to the antisymmetric (ঌ$_{as}$) and the symmetric (ঌ$_{sym}$) stretching vibrations of the carboxylate group. These frequencies and in particular, their difference, Δঌ = ঌ$_{as}$ – ঌ$_{sym}$, have been used as empirical indicators of coordination modes of the acetate group. According to Deacon and Phillips [10], a difference larger than 200 cm^{-1} indicates a monodentate coordination, whereas a difference smaller than 150 cm^{-1} indicates a bridging coordination mode. Moreover, it is accepted that a value of Δঌ smaller than 200 cm^{-1} can indicate a bidentate coordination mode. The ঌ$_{as(COO)}$ vibration band appeared in the domain 1500–1540 cm^{-1} in the spectra of the complexes , while those characteristic of the ঌ$_{sym(COO)}$ appeared in the 1420–1460 cm^{-1} range, which leads to the conclusion that the acetate group in these complexes was in a bidentate mode. From the IR spectral analysis, it has been concluded that the ligand L acts as neutral bidentate in all the synthesized complexes.

Electronic Spectra and Magnetic Moment of the Complexes

The UV spectrum of the ligand and that of the copper complex is given in Fig. 3. The electronic spectrum of the free Schiff base (L) display two bands at 342 nm (29240 cm^{-1}) and 447 nm(22371cm^{-1}) corresponding to π→π* (phenyl ring) and n→π* (CH=N) transitions[11].In the metal complexes this band is shifted to a longer wavelength with increasing intensity. This shift may be attributed to the donation of lone pair of electron of N- atom of the Schiff base to the metal ion (M←N).

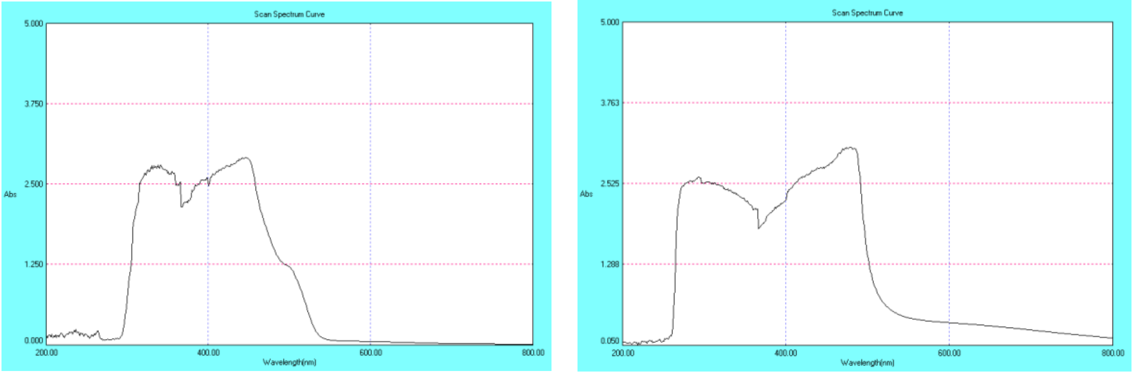


Fig:3 UV spectrum of ligand L and $[Cu(L)_2]\,Cl_2$

Fe (III) is moderately oxidizing and many of its complexes exhibit L→M charge transfer transitions. In most cases d-d absorption in octahedral Fe(III) complexes is rarely observed because the LMCT absorptions obscure it [12]. The electronic spectra of the Fe complex displays a band around ~29800 cm^{-1} which are due to L→M charge transfer transitions while the broad bands in the range 21400-21900 cm^{-1} may be assignable to the spin allowed $^5T_{2g}(F) \rightarrow {}^5E_g$ transition characteristic of octahedral structure [13]. The μ_{eff} for the Fe(III) complex is 5.53BM which is in accordance with high spin octahedral geometry.

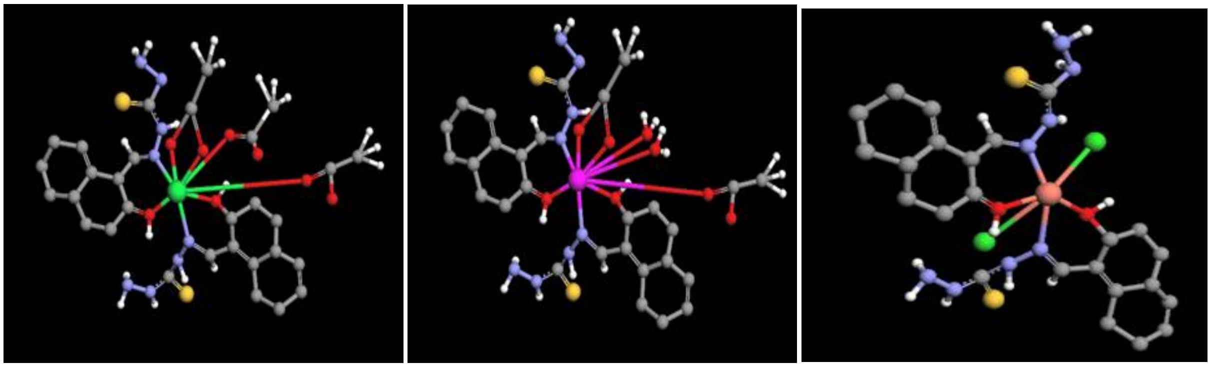
Co(II) complex exhibits two bands at 20800-18200 cm^{-1} and 30300-34500 cm^{-1}in the spectra. The bands can be assigned to $^4T_{1g}(F) \rightarrow {}^4A_{2g}$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions respectively which is in accordance with Co(II) high spin octahedral geometry[14]. Further the magnetic moment of Co(II) complex 3.52 BM suggest a high spin octahedral geometry[15]. The bands around ~33300 cm^{-1}may be assigned for intra ligand charge transfer transition.

According to Lever, the planar Cu (II) complexes for example, the well known Schiff base complexes such as the salicylaldimines exhibit a broad structural band as high as 20,000 cm^{-1}. A second intense band may be seen near 23,000- 30,000cm^{-1} which is often charge transfer in origin.

In the present study, Cu (II) complex exhibits a band around 30000-33000cm^{-1} attributed to intra ligand charge transfer transition. The band around 20000-21500 cm^{-1}may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition characteristic of square planar geometry. The planar geometry may be confirmed by magnetic study which shows a magnetic moment 1.81 BM corresponding to one unpaired electron [13].

3D Molecular Modeling and Analysis

The possible geometries of metal complexes were evaluated using the molecular calculation with Argus lab 4.0.1version software. The metal complexes were built and geometry optimization was done using this software. The molecular modeling pictures are shown in Fig.4.



$[Fe(L)_2\,OAc]\,(OAc)_2$$[Co(L)_2\,OAc]\,(OAc).2H_2O$$[Cu(L)_2]\,Cl_2$

224.38563882 kcal/mol224.34017633 kcal/mol 124.8470 kcal/mol

Fig:4 Molecular Modeling structures for ligands and their complexes

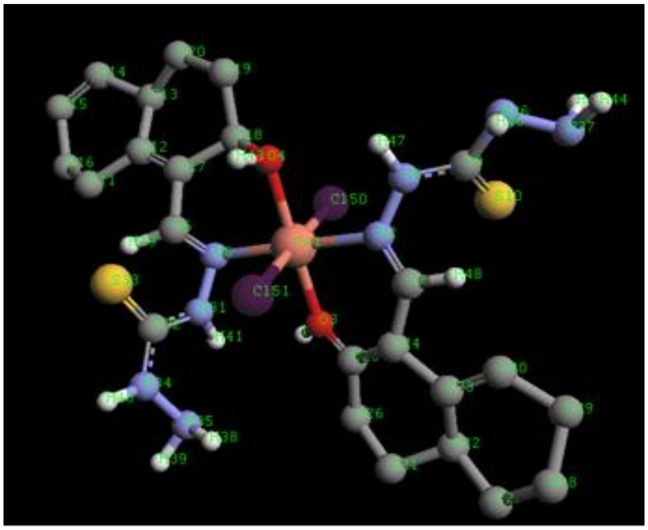


Fig:5 Optimized 3D geometry of $[Cu(L)_2]$ Cl_2complex

The details of important bond lengths as per the 3D structure of Cu (II) (Fig. 5) complex are given in the table 4. These values are obtained as a result of energy minimization of Cu (II) complex in Argus lab 4.0.1version software

The obtained bond lengths of the ligand (L_1) using the software are between C(2)-N(3) and [C(6)-N(7)] 1.279 A°,[C(22)-O(1)] is 1.208 A°.Based on the values in the table 9 and 10, it is observed that when the ligand (L) is coordinated with the copper(II) ion there is an increase in the bond length in between the above mentioned atoms, which confirms the coordination of azomethine group through nitrogen [N(5) and N(2)].When the atoms are coordinated with the metal ion by donating the lone pair of electrons there is decrease of electron density on the coordinating atoms,hence bond length increases in metal complexes. This supports the proposed structure of the complex [16].

Table-3: electronic spectra and magnetic moment of the complexes

Compound	λ_{max} (cm^{-1})	Band assignments	Geometry	Mag. moment BM
L	29240 22371	n→π* π→π*	-	-
[Fe(L)$_2$ OAc] (OAc)$_2$	22272 29851	$^5T_{2g} \rightarrow {}^5E_g$	Octahedral	5.53
[Co(L)$_2$ OAc] (OAc).2H$_2$O	18484 19960 29762	$^3T_{1g}(F) \rightarrow {}^3T_{2g}(F)$ $^3A_{1g} \rightarrow {}^3A_{2g}$&$^3E_g$	Octahedral Intra ligand charge transfer transition	3.52
[Cu(L)$_2$] Cl$_2$	20661 20921 21053 32258 32895	$^2B_{1g} \rightarrow {}^2A_{1g}$	Square planar Intra ligand charge transfer transition	1.81

Table-4: Bond length for selected bonds of [Cu(L)$_2$] Cl$_2$complex

S.No	Bonded atoms	Bond length (A^o)
1	(Cu1)-(N5)	2.0314
2	(Cu1)-(O4)	2.0228
3	(Cu1)-(N2)	2.0161
4	(Cu1)-(O3)	1.9976
5	(O4)-(C18)	1.4361
6	(O3)-(C25)	1.2603
7	(N2)-(C7)	1.2919
8	(N5)-(C6)	1.2919
9	(N8)-(C9)	1.3462
10	(C32)-(S33)	1.4461
11	(O4)-(H43)	1.0337

Antimicrobial Studies

The antimicrobial activity of the metal complexes was studied against two pathogenic bacterial strains, one gram positive (*Staphylococcus aureus*) and one gram negative (*Escherichia coli*) bacteria and one fungal strain (*Candida albicans)*. Ciprofloxacin and clotrimazole were used as standard bactericide and fungicide respectively.

Antibacterial and antifungal potential of metal complexes were assessed in terms of zone of inhibition of bacterial and fungal growth. The results of the antifungal and antibacterial activities are presented in table 5. The minimum inhibitory concentrations (MIC) were calculated as the highest dilution showing complete inhibition of the tested strains and are reported in tables 6 and 7.

Table:5 Antimicrobial activity of Schiff base metal complexes

Micro organisms		**Zone of inhibition Samples (50µg/disc) (mm)**			
		$[Fe(L)_2 OAc] (OAc)_2$	**$[Co(L)_2 OAc] (OAc).2H_2O$**	**$[Cu(L)_2] Cl_2$**	**STD (10µg/disc)(mm)**
Bacteria	*E.coli*	13	11	09	20
	Staphylococcusaureus	12	10	10	25
Fungi	*C.albicans*	19	23	21	18

Table:6 Determination of MIC for antimicrobial activity

SAMPLE NAME	**ORGANISMS**		**500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**62.5 µg/ml**	**31.25 µg/ml**	**15.62 µg/ml**	**7.81 µg/ml**
$[Fe(L)_2 OAc] (OAc)_2$	*Bacteria*	*Staphylococcus aureus*	-	-	-	+	+	+	+
		E.coli	-	-	-	+	+	+	+
	Fungi	*C.albicans*	-	-	-	-	-	+	+
$[Co(L)_2 OAc] (OAc).2H_2O$	*Bacteria*	*Staphylococcus aureus*	-	-	+	+	+	+	+
		E.coli	-	-	-	-	+	+	+
	Fungi	*C.albicans*	-	-	-	-	+	+	+
$[Cu(L)_2] Cl_2$	*Bacteria*	*Staphylococcus aureus*	-	-	-	+	+	+	+
		E.coli	-	-	-	-	+	+	+
	Fungi	*C.albicans*	-	-	-	-	+	+	+

Minus (-) indicates the absence of growth
Plus (+) indicates presence of growth

Table:7 Antimicrobial MIC value

SAMPLE NAME	**ORGANISMS**		**MIC VALUE**
$[Fe(L)_2 OAc] (OAc)_2$	*Bacteria*	*Staphylococcus aureus*	125µg/ml
		E.coli	125µg/ml
	Fungi	*C.albicans*	31.25 µg/ml
$[Co(L)_2 OAc] (OAc).2H_2O$	*Bacteria*	*Staphylococcus aureus*	250µg/ml
		E.coli	62.5µg/ml
	Fungi	*C.albicans*	62.5 µg/ml
$[Cu(L)_2] Cl_2$	*Bacteria*	*Staphylococcus aureus*	125µg/ml
		E.coli	62.5µg/ml
	Fungi	*C.albicans*	62.5 µg/ml

The metal complexes were effective against both bacteria and the fungus. The iron complex has better activity than the other two against both gram positive and gram negative bacteria but in the case of fungal pathogen, cobalt complex shows better activity, its antifungal activity is even superior to that of clotrimazole. Compared with ciprofloxacin the complexes show lesser activity against both the bacteria. The results are also presented as bar graphs (Fig.6).

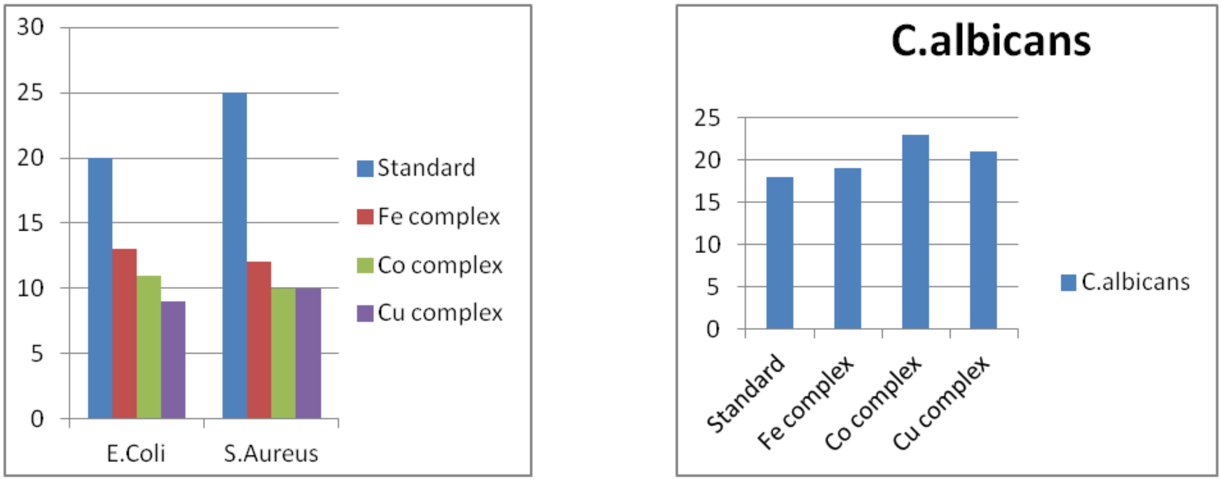


Fig:6 Antimicrobial activity of the Schiff base complexes against bacterial and fungal pathogens

In conclusion, the antimicrobial activity of the metal complexes would help for development of a new alternative medicine system which has no side effects. The Schiff base metal complexes possess a broad spectrum of activity and open the possibilities of finding new clinically effective antimicrobial compounds.

DNA Cleavage Studies

Nucleic acids are often the targets for many chemotherapeutic drugs especially antitumor drugs. Transition metal complexes interact with DNA through covalent bonding, electrostatic interactions, groove binding or intercalation. An example is cisplatin, which functions by cross linking DNA strands through co-ordination of nucleic acid bases. Cisplatin is believed to kill cancer cells by binding to the DNA and interfering with its repair mechanism eventually leading to cell death. However the greatest disadvantage of cispaltin and other heavy metal based drugs is their toxicity. Therefore it will be more appropriate to use soft metal ions and their complexes thus reduce toxic effect and enabling faster and efficient removal of the drug from the body. Some complexes when interact with DNA could induce the breakage of DNA shown by gel electrophoresis technique. After cleavage of a DNA strand, the double strand breaks. The replication ability of the cancer gene is thereby destroyed [17].

In the present study, gel electrophoresis experiments using CT DNA were performed with the complexes in the presence of H_2O_2as an oxidant at 50 micromolar concentration.

The cleavage activities of the complexes are shown in Fig.7, lane C is for the control which does not show any significant cleavage. Lanes 1-3 contain the complexes in presence of oxidant H_2O_2. Cu(II) complex showed significant cleavage activity in the presence of H_2O_2. This may be attributed to the formation of $OH^{\bullet}$ which oxidized Cu(II) to Cu(III) probably through Fenton-type reactions resulting in the formation of reactive oxygen species which could cause oxidative damage to DNA [18].

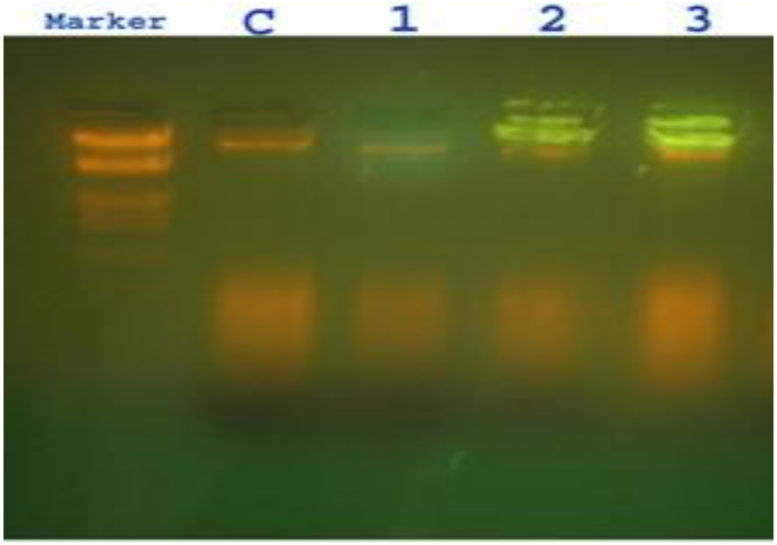


Fig.7 Gel diagram of Calf-thymus DNA induced by Schiff base metal complexes

Lane from Left to Right
(C) CT DNA alone
(1) Fe(II)-Schiff Base (L_1) +DNA + H_2O_2
(2) Co(II)-Schiff Base (L_1) +DNA + H_2O_2
(3) Cu(II)-Schiff Base (L_1) +DNA + H_2O_2

CONCLUSIONS

- The ligand derived from thiocarbohydrazide and 2-hydroxy naphthaldehyde acts as neutral bidentate.
- The geometry of the Fe and Co complexes have been proposed as octahedral and that of Cu complex as square planar.
- All the complexes showed good activity against S.aureus , E.coli and C.albicans.
- Copper complex was found possess DNA cleavage activity

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