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Synthesis and Characterization of Bioactive Schiff Base Complexes from Cardanol

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Abstract: *Schiff base complexes derived from DFMPM and n-propylamine with Co(II), Ni(II) and Cu (II) were synthesized and characterized by IR, UV-Vis, ^{1}HNMR, elemental analysis, molar conductance, powder XRD and SEM. The IR results indicated the bidentate binding mode of the ligand involving azomethine nitrogen. The electronic spectral results indicate that Co(II) & Cu(II) complexes have square planar geometry, while Ni (II) complex is octahedral. Powder XRD and SEM indicate the nano crystalline state of the complexes. The antimicrobial activity of the synthesized ligand and its complexes were screened by disc diffusion method. The results show that the metal complexes were found to be more active than the ligand. The nuclease activity of the ligand and its complexes were assayed on lamda- DNA using gel electrophoresis in the presence of H_2O_2. The DNA cleavage activity of complexes is in the order Co(II) > Cu(II) > Ni(II). The in-vitro anticancer studies reveal that the Co (II) complex had the moderate anticancer activity against HT-29 (Colon Carcinoma) cell with IC_{50} value of 100 μM and Ni(II) complex had IC_{50} value of 37.5 μM.*

Keywords: DFMPM, n-propyl amine, XRD, SEM, DNA cleavage, HT-29 (Colon Carcinoma)

Introduction

Schiff base ligands and their metal complexes have a variety of applications in biological, clinical, analytical, industrial fields[1,2] and have pharmacological properties[3,4]. Further more, the interaction of these complexes with DNA has gained much attention due to their possible applications as new therapeutic agents[5]. The present investigation deals with the synthesis, characterization, antibacterial, antifungal, DNA cleavage, and anticancer studies of Schiff base derived from DFMPM and n-propylamine. Di-α-formylmethoxybis(3-pentadecenylphenyl) methane (DFMPM) is prepared from cardanol using formaldehyde, epichlorohydrin and sodiumperiodate in three stages[6,7].

Cardanol was obtained from M/S Satya Cashew, Chennai, India. Formaldehyde (37% solution) hydrochloric acid, epichlorohydrin, propylamine, sodium hydroxide and other chemicals used were of GR/AR grade quality obtained from Merck Chemicals. All the solvent used was purified by standard methods[8, 9]. The micro analytical data (C, H, N) were collected using Perkin Elmer 2400 instrument. IR spectra were obtained using PE IR spectrum instrument model: system 2000. ^{1}HNMR spectra were obtained using AMX–300 MHz, FT NMR Spectrometer. Conductance measurements were obtained using systronics–305 conductivity meter. Electronic spectra of the ligands and its complexes was obtained using Perkins Elmer Lamda–25 UV-visible spectrometer in the range of 200-1100 nm. Surface morphological studies was obtained using JSM–5610 scanning electron microscope. HT 29 (colon cancer) cell line was initially procured from National Centre for cell sciences.

Synthesis of Schiff base ligand

The synthesis of Schiff base ligand was carried out by reported methods[10]. Ethanolic solution of DFMPM and n-propylamine were taken in RB flask in 1:2 molar ratios and refluxed for an hour. The reaction mixture was poured in ice, an yellow compound of Schiff base ligands was obtained[10]. The precipitated yellow compound was filtered, washed with water and dried over anhydrous calcium chloride. The crude sample was recrystalised from 50% absolute alcohol. Yield = 60% Melting point = 228°C.

Preparation of Schiff base metal complexes

The metal complexes were prepared by adding aqueous solution of Cu(II) nitrate, Ni(II) nitrate, Co(II) nitrate to the ligand in ethanol in 1:2 molar ratio and refluxed for about twelve hours at 80°C[10]. The precipitated solids were filtered, washed with ethanol, diethyl ether and hot water and finally dried under vacuum at 90°C. Yield = 53 – 61%.

Results and Discussion

All the metal complexes are coloured solids, stable towards air and have high melting points (above 250°C). The complexes are insoluble in water and common organic solvents, but are soluble in DMF, $CDCl_3$ and DMSO.

Elemental analysis

The analytical data suggest that all the complexes are mono nuclear with the ligands coordinated to the central metal atom. The metal to ligand ratio in all complexes was 1:2 and their formulae have been computed and given in table 1. Conductivities of solution of the complexes in DMF showed that all the complexes are non – electrolytes because their conductivity values were low. However, the conductivity value is higher than expected for non-electrolytes probably due to partial solvolysis of complexes in DMF medium.

Table 1: Physical Characteristics and analytical data of the complexes

Compound	Yield %	Colour	Mol. formula	Mol. Wt.	Melting point °C	Elemental Analysis C	H	N	Molar conductance ohm^{-1}cm^2 mol^{-1}
Ligand L $C_{53}H_{86}N_2O_2$	61	Brown	$C_{53}H_{86}N_2O_2$	782	220	81.76 (81.33)	10.57 (10.9)	3.35 (3.58)	-
COL_2	56	Purple	$CoC_{106}H_{172}N_4O_4$	1620.9	>250	78.55 (78.47)	10.3 (10.61)	3.6 (3.45)	14
$[NiL_2(H_2O)_2]$	53	Pale green	$NiC_{106}H_{172}N_4O_4$ $2H_20$	1658.7	>250	76.68 (76.68)	10.68 (10.37)	3.18 (3.37)	12
CuL_2	60	Green	$CuC_{106}H_{172}N_4O_4$	1609.5	>250	79.6 (79.03)	10.94 (10.68)	3.37 (3.44)	15

IR Spectrum

Selected IR spectral bands for the ligand and its complexes are given in Table 2. The IR spectrum of the free ligand is characterized mainly by the strong bands at 2856 cm^{-1} 2923 cm^{-1} and 1606 cm^{-1} which are attributed to the stretching frequencies of O – C, C – H and C = N (azomethine) respectively [10] (Figure 1). The IR spectrum of the free ligands was compared with the spectra of metal complexes. The characterization absorption bands 3454 cm^{-1}, 3454 cm^{-1} and 3700 cm^{-1} range were attributed to – OH group of the lattice water or the coordinated water[11]. The absorption bands in the range 2736 cm^{-1} , 2900 cm^{-1} and 2900 cm^{-1}, 2933 cm^{-1}, 2933 cm^{-1} and 2850 cm^{-1} and 1662 cm^{-1} , 1662 cm^{-1} and 1650 cm^{-1} were assigned to O – C, C – H and C = N respectively[12, 13]. The imine peak in the metal complexes showed change in shifts compared to the ligand indicating coordination of the imine nitrogen atom to the metal ion due to coordination. Another absorption bands at 509, 750, 740 cm^{-1} is assigned to M – N bond and 450, 468, 450 cm^{-1} is assigned to M – O bond[14-16] (Figure 2-4).

Table 2: Selected UV and FTIR frequencies (cm^{-1}) of the ligand and complexes

Ligand/ Complex	ν_{O-H}	ν_{O-C}	ν_{C-H}	$\nu_{C=N}$	ν_{M-N}	ν_{M-O}	λ_{max} (nm)		
$C_{53}H_{86}N_2O_2$	-	2856	2923	1606	-	-	-	272	354
[Co L_2]	3454	2900	2933	1662	509	450	322	357	550
[Ni L_2 (H2O)$_2$]	3700	2736	2850	1650	750	468	323	356	366
[Cu L_2]	3454	2900	2933	1662	740	450	332	510	640

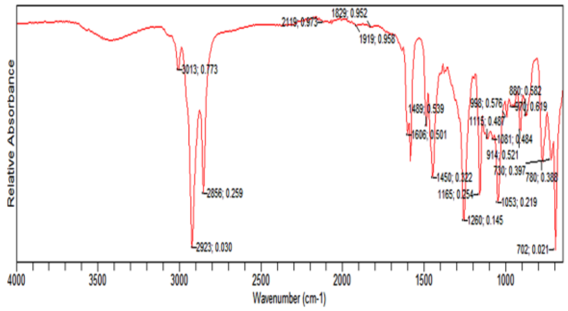

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Figure 1: FTIR Spectrum of Schiff base ligand

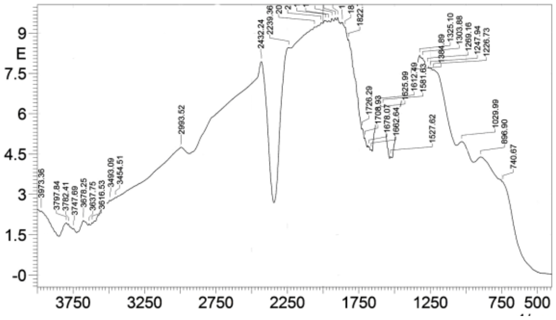

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Figure 2: FTIR spectrum of Co(II) complex

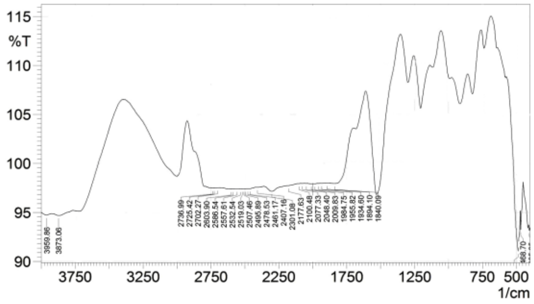

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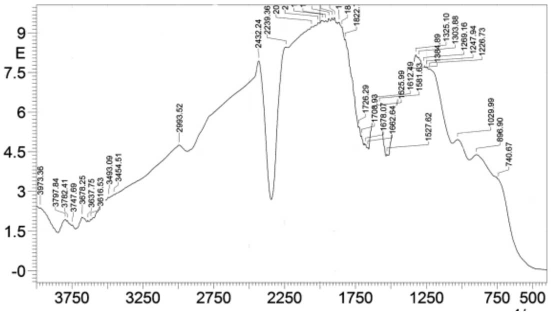
Figure 3 : FTIR spectrum of Ni (II) complex



ν

Figure 4 : FTIR spectrum of Cu(II) complex

Electronic spectra

The electronic spectral data of Schiff base and its metal complexes are given in table 2. The electronic spectra of Schiff base and its metal complexes are given in Figure 5-8. The ligand shows a broad band at 272 nm which is assigned to $\pi \rightarrow \pi^*$ transition of the C = N chromophore[17]. The low spin square planar complexes of Co(II) show a strong absorption maxima in the visible region at ~ 500 nm due to the transition ${}^4A_{1g} \rightarrow {}^4B_{1g}$. It also shows an intense peak at ~ 380 nm. The electronic spectrum of Ni (II) complex shows an intense band at 366 nm. This corresponds to the transition ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$ which is the characteristic of octahedral geometry. The transition of the Cu(II) d-orbitals in square planar geometrical environment of ligands and their corresponding absorption maxima[17] are given below. $dxy \rightarrow dx^2\text{-}y^2$ but normally the square planar complexes of Cu(II) give a broad absorption band between 600 and 700 nm due to Jahn–Teller distortion and the peak at ~510nm merges with the broad band [17].

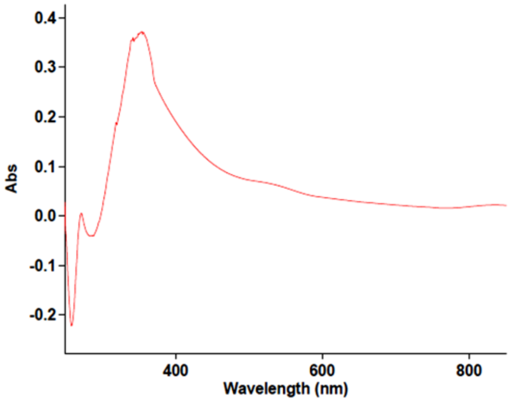


Figure 5: Electronic spectrum of ligand

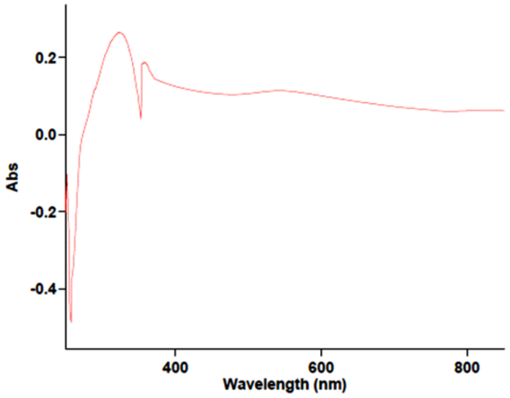


Figure 6: Electronic spectrum of cobalt complex

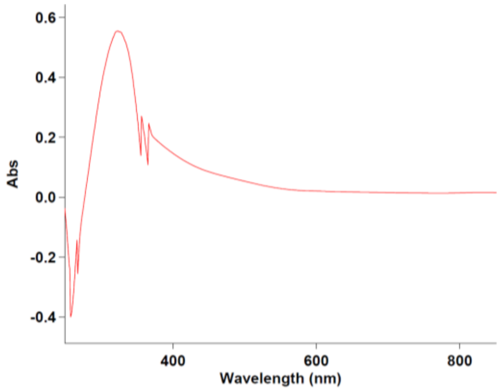


Figure 7: Electronic spectrum of nickel complex

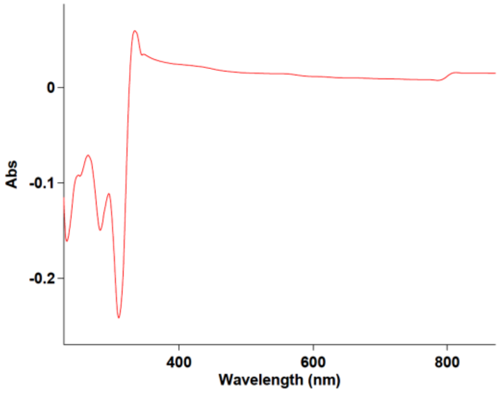


Figure 8: Electronic spectrum of copper complex

^{1}H NMR

On examining the ^{1}H NMR spectrum of ligand, (Figure : 9) it exhibited a multiplet signal at □ = 7.135 ppm -7.174 ppm is due to substituted aromatic ring protons. The presence of H-C=N group is indicated by the singlet at □ = 8.3 ppm. A signal at □ = 1.263 ppm – 1.529 ppm indicate the presence of -CH$_2$- protons. The multiplet □ = 6.731 ppm – 6.751 ppm and □ = 3.838 ppm-3.988 ppm were due to the olifinic protons of the side chain and O-CH$_2$ group of the ligand respectively.

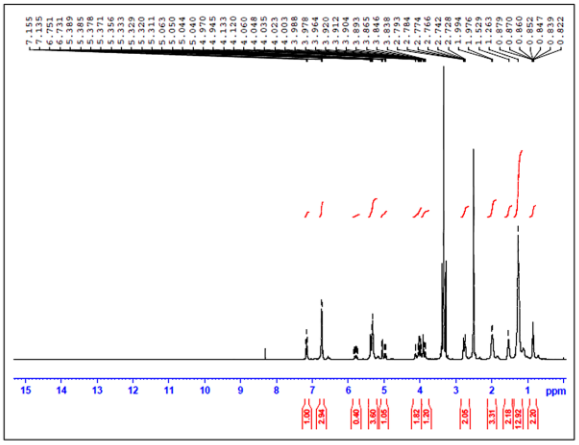


Figure 9: H^{1}NMR Spectrum of ligand

^{1}H NMR of Co (II) complex

The ^{1}H NMR spectrum of the Co (II) complex (Fig. 10) gave the signals for atomatic protons (δ = 7.136 - 7.176 ppm) , O-CH2 protons (δ = 3.836 – 3.920 ppm), olifinic protons of the side chain (δ =6.713 – 6.753 ppm) and H-C=N proton (δ = 8.34 ppm). There is a down field shift of imine proton after complex formation than in the ligand. Thus ^{1}H NMR study also confirmed the structural informations of both ligand and its complexes [18].

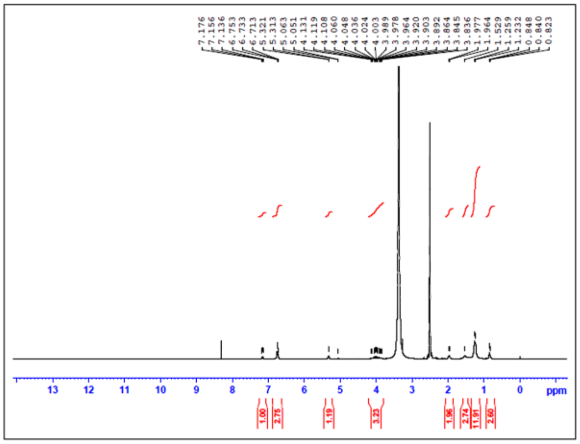


Figure 10: H^1 NMR spectrum of cobalt complex

Based on the present study the structure of the ligand and complexes may be given as follows (figure 11-13)

Figure 11: Structure Schiff base

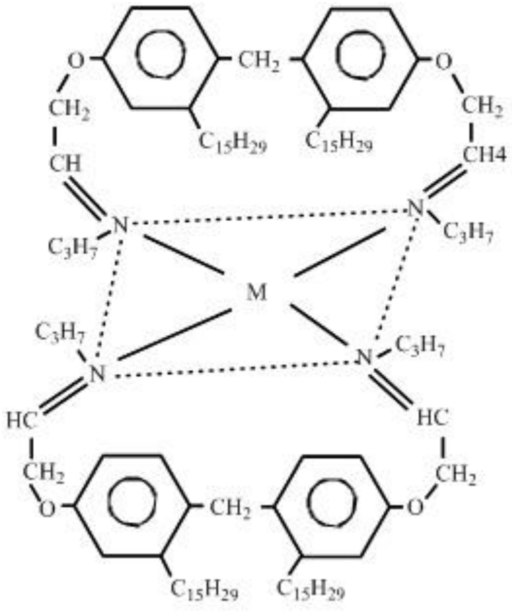


Figure 12: Structure of Co (II) and Cu (II) complex

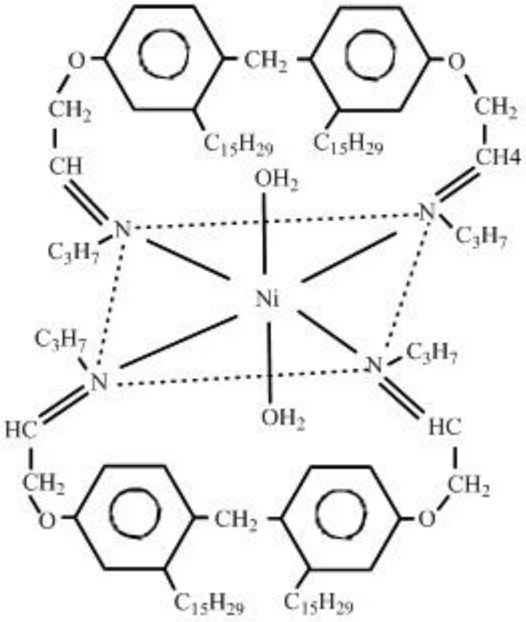


Figure 13: Structure of Nickel complex

SEM Analysis

Scanning electron micrography is used to evaluate morphology of the Schiff base metal complexes. The SEM picture of Cu(II) complex is shown in fig. 14. From the fig. 14 pitted and rough surface is observed in the complex. The particle size of the Cu (II) complex were in the diameter range of few microns.

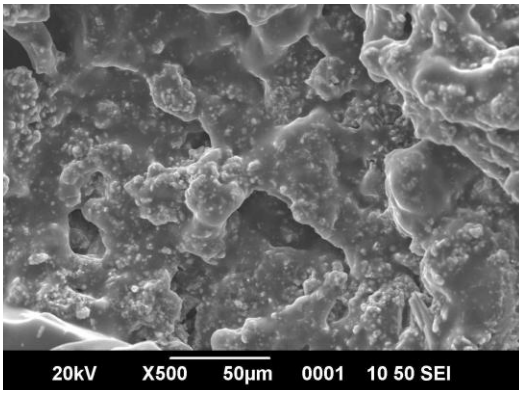


Figure 14: SEM image of Schiff base complex of Cu(II)

XRD

The XRD pattern of Cu(II) complexes show well defined crystalline peaks indicating that the samples are crystalline in nature . The above complexes have specific 'd' values which can be used for its characterization . The crystallite size of the complexes d_{XRD} could be estimated from XRD patterns by the Scherre's formula. XRD shows that Cu(II) complexes have the average crystallite size of 27.36 nm suggesting the complexes to be nano crystalline (Figure 15).

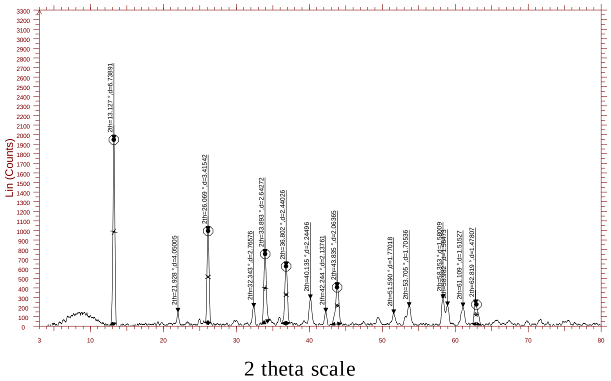


Figure 15: XRD spectrum of Cu(II) complex

Antimicrobial study

The biological activities of synthesized Schiff base and its metal complexes have been studied for their antibacterial and antifungal activities by disc diffusion method , and the stock solution (0.001 mol) was prepared by dissolving the compounds in DMSO and the antimicrobial activity was estimated based on the size of inhibition zone in the discs [19-21].

Four bacterial stains Klebsiella sps , *E. Coli*, *P. aeruginosa* , *S. aureus* were incubated for 24h at 37°C and Fungal stains Candida sps , Aspergillus sps were incubated for 48h at 37°C.

Table 3: Antimicrobial Activity for Bacteria

S. No.	Samples	Media	Zone of Inhibition (mm)			
			Klebsiella sps	*E. coli*	*P. aeruginosa*	*S. aureus*
1	$C_{53}H_{86}N_2O_2$	Mueller Hinton Agar	6.0	6.0	6.0	6.0
2	$[COL_2]$		6.0	12.0	10.0	6.0
3	$[NiL_2 . (H_2O)_2]$		6.0	12.0	6.0	10.0
4	[CuL2]		6.0	6.0	12.0	6.0
5	PC(Chloramphenicol)		25.0	26.0	24.0	25.0
6	NC		6.0	6.0	6.0	6.0

Table 4: Antimicrobial Activity for Fungi

S. No.	Samples	Media	Zone of Inhibition (mm)	
			Candida sps.	Aspergillus sps.
1	$C_{53}H_{86}N_2O_2$	Mueller Hinton Agar	6	6
2	$[COL_2]$		11	10
3	$[NiL_2 . (H_2O)_2]$		10	12
4	[CuL2]		12	11
5	PC(Nystatin)		25	26
6	NC		6	6

The antimicrobial activity results (Table 3 & 4) reveal that , the higher the activity of the metal complexes may be owing to the effect of metal ions on the normal cell membrane [22]. Metal chelates bear polar and nonpolar properties together, this makes them suitable for permeation to the cells and tissues. In addition, chelation may enhance or suppress the biochemical potential of bioactive organic species. Further, lipophilicity, which controls the rate of entry of molecules into the cell, is modified by coordination, so the metal complex can become more active than the free ligand. Therefore, the metal complexes show greater antimicrobial activities than the uncoordinated ligand and free metal ion which in fact is in agreement with the literature. The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group (>C=N) with the active centers of cell constituents[23] resulting in interferences with the normal cell process.

DNA cleavage studies

The DNA cleavage ability of the complexes is monitored by gel electrophoresis. All the metal complexes are able to convert super coiled DNA into open circular DNA. The results of DNA cleavage are given in Figure 16. [24-28]

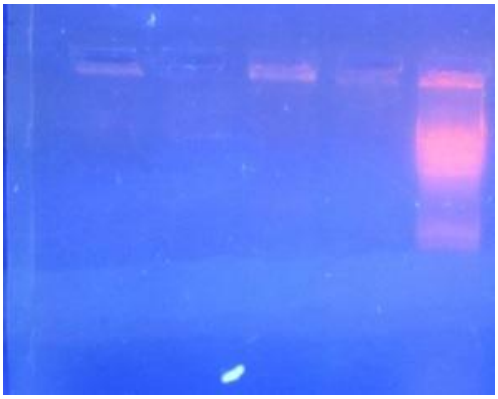


Figure 16: Gel electrophoresis diagram of the Schiff base metal complexes

Lane 1 : DNA + L + H_2O_2
Lane 2 : DNA + CoL_2 + H_2O_2
Lane 3 : DNA + $[Ni L_2(H_2O)_2]$
Lane 4 : DNA + CuL_2 + H_2O_2
Lane 5 : Control DNA

The greater cleavage efficiency of complexes compared to that of the control experiments is due to their efficient DNA-binding ability. Control experiments using DNA alone do not show any significant cleavage of lamda - DNA even after a longer exposure time. This result revealed the damage of DNA in Co(II), Ni(II), Cu(II) complexes could be attributed to the cleavage of DNA. The

DNA cleavage activity of complexes is in the order Co(II)>Cu(II)>Ni(II) with lamda-DNA. The oxidative DNA cleavage by singlet oxygen is likely to proceed via oxidation of guanine nucleobase.

Invitro anticancer activity determination by MTT assay

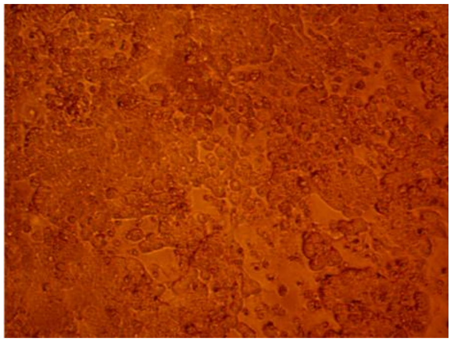
Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator. The viability of cells were evaluated by direct observation of cells by Inverted phase constrast microscope and followed by MTT assay method.

Anticancer Activity

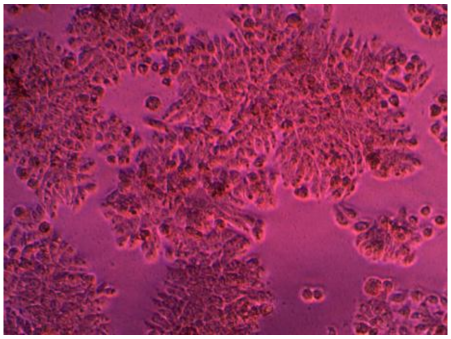
The result of anticancer activities are presented in table 5 & 6. The colon carcinoma (HT-29) cells, were sensitive to the Co (II) complex with an IC_{50} value of 100 µM. The Ni (II) complex had the better activity with an IC_{50} value of 37.5 µM. The enhancement of cytotoxic activity may be assigned to that the positive charge of the metal increased the acidity of coordinated ligand that bears protons, leading to stronger hydrogen bonds which enhanced the biological activity[29-32]. It seems that changing the anion, coordination sites, and the nature of the metal ion has a pronounced effect on the biological behavior by altering the binding ability of DNA. Gaetke and Chow had reported that metal has been suggested to facilitate oxidative tissue injury through a free radical mediated pathway analogous to the Fenton reaction. (Figure 16, a, b, c, d & e)

Table 5 : % viability of Co(II) complex

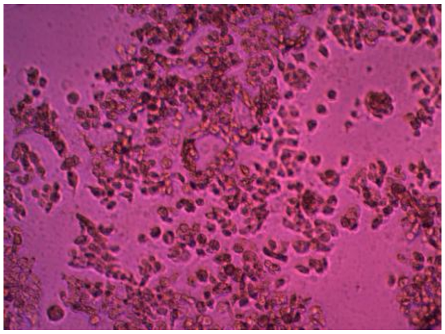
Sample concentration (µg / ml)	Average OD at 540 nm	Percentage Vilability
Control	0.7689	
6.25	0.5553	72.22
12.5	0.5844	76.00
25	0.4932	64.14
50	0.4263	55.44
100	0.3793	49.33
IC_{50}value = 100 µM		



(a) Control

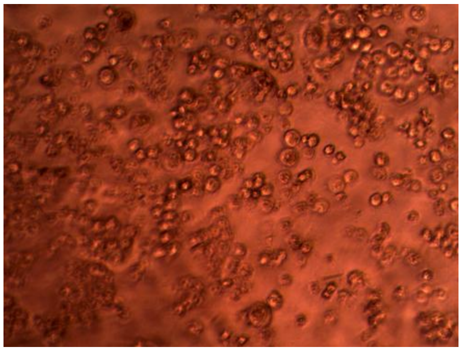


(b) Co (II) Complex at 50 µM

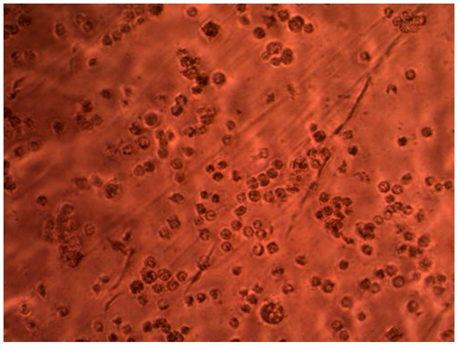


(c) Co (II) complex at 100 µM

Figure 17: Microscopic observation of anticancer activity



(d) Ni (II) at 50 µM



(e) Ni (II) at 100 µM

Table 6: % viability of Ni(II) complex

Sample concentration (µg/ml)	**Average OD at 540 nm**	**Percentage Vilability**
Control	0.4955	
6.25	0.4616	93.15
12.5	0.3494	70.51
25	0.335	67.60
50	0.1644	33.17
100	0.1329	26.82
IC_{50} value = 37.5 µ M		

Conclusion

Schiff base complexes of Cu (II), Ni (II) and Co (II) with DFMPM and n-propyl amine were synthesized and characterized. The UV-visible, IR, NMR studies revealed the geometry of the complexes of which Ni (II) complex is hexa coordinated. Cu (II) and Co (II) complexes are tetra coordinated. The antimicrobial activity results show that the metal complexes were found to be more active than the ligand. The DNA cleavage activity of complexes is in the order Co (II) > Cu (II) > Ni (II). The in-vitro anticancer studies reveal that the Co (II) complex had the moderate anticancer activity against HT-29 (Colon Carcinoma) cell with IC_{50} value of 100 µM and Ni (II) complex had IC_{50} value of 37.5 µM.

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