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Synthesis characterisation and Biological Activities of Co(II), Ni(II) & Cu(II) Complexes with DFMPM and glycine

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ABSTRACT

Schiff base complexes derived from DFMPM and glycine with Co(II), Ni(II) and Cu(II) were synthesized and characterized by IR, UV-Vis, ¹HNMR, elemental analysis, molar conductance, powder XRD and SEM. The IR results indicated the tetradentate binding mode of the ligand involving azomethine nitrogen and carboxylato oxygen. The electronic spectral results indicate that Cu(II) complex has Tetragonally distorted octahedral geometry, while Co(II) and Ni(II) complexes are 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 lambda-DNA using gel electrophoresis in the presence of H₂O₂. The DNA cleavage activity of complexes is greater than the ligand. The in-vitro anticancer studies reveal that the Co(II) complex had the moderate anticancer activity against HT-29 (Colon Carcinoma) cell with IC₅₀ value of 86.76 μM and Cu(II) complex had IC₅₀ value of 83.24 μM.

Key words: DFMPM, glycine, XRD, SEM, DNA cleavage, HT-29 (Colon Carcinoma)

INTRODUCTION

A large number of Schiff bases and their complexes have been investigated for their interesting and important properties, such as their ability to reversibly bind oxygen, catalytic activity in the hydrogenation of olefins photochromic properties and complexing ability towards some toxic metals. Schiff bases are a special class of ligands with a variety of donor atoms exhibiting interesting coordination modes towards various metals[1-7]. The azomethine linkage in Schiff bases is responsible for the biological activities such as antitumor, antibacterial, antifungal and herbicidal activities. A large number of Schiff base (N₂O₂) complexes have been reported so far, and their catalytic and biological properties have been studied intensively. The present study was undertaken to throw more light on the chelation behavior of Schiff base (L) towards some transition elements, which may help in more understanding of the mode of chelation of the new Schiff base towards metals.

MATERIALS AND METHODS

Cardanol was obtained from M/S Satya Cashew, Chennai, India. Formaldehyde (37% solution) hydrochloric acid, epichlorohydrin, glycine, sodium hydroxide and other chemicals used were of GR 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. ^1H NMR 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, Pune.

2.1 Synthesis of Schiff base ligand

The synthesis of Schiff base ligand was carried out by reported methods[10]. Ethanolic solution of DFMPM and glycine 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 recrystallised from 50% absolute alcohol. Yield = 61% Melting point = 28°C.

2.2 Synthesis 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 1:1 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 = 61 – 65%.

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.

3.1 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 1:1 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 | | | Molar conductance $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ |
|--|---------|------------|--|---------|------------------|--------------------|--------------|--------------|---|
| | | | | | | C | H | N | |
| Ligand L $\text{C}_{51}\text{H}_{76}\text{N}_2\text{O}_6$ | 61 | Brown | $\text{C}_{51}\text{H}_{76}\text{N}_2\text{O}_6$ | 812 | 228 | 75.1 (75.3) | 9.5 (9.3) | 3.3 (3.4) | - |
| CoL (H_2O) ₂ | 63 | Purple | $\text{CoC}_{51}\text{H}_{80}\text{N}_2\text{O}_8$ | 906.9 | >250 | 67.3 (67.4) | 8.5 (8.8) | 3.1 (3.0) | 12 |
| NiL (H_2O) ₂ | 65 | Pale green | $\text{NiC}_{51}\text{H}_{80}\text{N}_2\text{O}_8$ | 906.7 | >250 | 67.2 (67.4) | 8.4 (8.8) | 3.2 (3.0) | 11 |
| CuL | 64 | Green | $\text{CuC}_{51}\text{H}_{76}\text{N}_2\text{O}_6$ | 875.5 | >250 | 69.8 (69.9) | 8.5 (8.6) | 3.3 (3.1) | 13 |

3.2 IR Spectra

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 3009 cm^{-1} , 2926 cm^{-1} , 1596 cm^{-1} , 1452 cm^{-1} and 1700 cm^{-1} which are attributed to the stretching frequencies of C-H, O-C, asym COO-, sym COO- and HC = N (azomethine) respectively. The IR Spectrum of the free ligands was compared with the spectra of metal complexes. The characterized absorption bands 3441 cm^{-1} , 3487 cm^{-1} and 3534 cm^{-1} range were attributed to -OH group of the lattice water or the coordinated water. The absorption bands in the range 2925 cm^{-1} , 2927 cm^{-1} and 2926 cm^{-1} were assigned to stretching frequencies of C-H. The absorption bands in the range 2854 cm^{-1} , 2854 cm^{-1} and 2853 cm^{-1} were assigned to stretching frequencies of O- C group. The absorption bands in the range 1599 cm^{-1} , 1598 cm^{-1} and 1597 cm^{-1} , 1493 cm^{-1} , 1492 cm^{-1} and 1495 cm^{-1} were assigned to asym COO- sym COO- stretching frequencies. The absorption bands in the range 1690 cm^{-1} , 1690 cm^{-1} and 1690 cm^{-1} were assigned to HC = N stretching frequencies. 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. Another absorption bands at 773 cm^{-1} , 773 cm^{-1} & 772 cm^{-1} is assigned to M-N bond and 450 cm^{-1} , 450 cm^{-1} & 475 cm^{-1} is assigned M-O bonds[8].

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

| Ligand/ Complex | $\nu_{\text{O-H}}$ | $\nu_{\text{C-H}}$ | $\nu_{\text{O-C}}$ | $\nu_{\text{C=N}}$ | $\nu_{\text{asym COO}^-}$ | $\nu_{\text{sym COO}^-}$ | $\nu_{\text{M-N}}$ | $\nu_{\text{M-O}}$ | λ_{max} (nm) | | |
|--|--------------------|--------------------|--------------------|--------------------|---------------------------|--------------------------|--------------------|--------------------|-----------------------------|-----|------|
| | | | | | | | | | | | |
| $\text{C}_{51}\text{H}_{76}\text{N}_2\text{O}_6$ | - | 3009 | 2926 | 1700 | 1596 | 1452 | - | - | 239 | 278 | 801 |
| $[\text{CoL}(\text{H}_2\text{O})_2]$ | 3441 | 2925 | 2854 | 1690 | 1599 | 1493 | 773 | 450 | 222 | 272 | ~350 |
| $[\text{NiL}(\text{H}_2\text{O})_2]$ | 3487 | 2927 | 2854 | 1690 | 1598 | 1492 | 773 | 450 | 228 | 272 | ~333 |
| $[\text{CuL}]$ | 3534 | 2926 | 2853 | 1690 | 1597 | 1495 | 772 | 475 | 225 | 279 | ~400 |

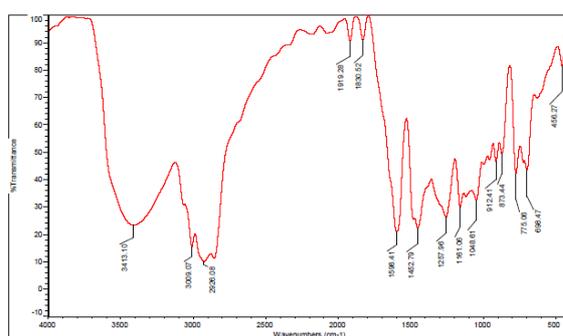


Figure 1 : FTIR Spectrum of Ligand

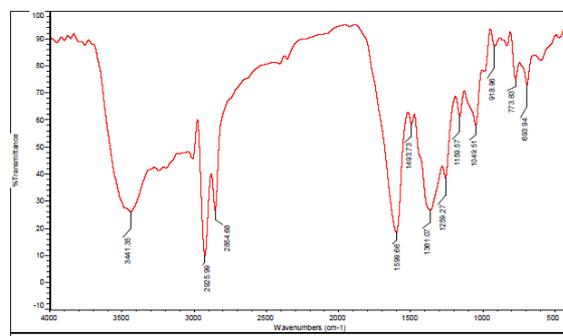


Figure 2 : FTIR Spectrum of Co (II) Complex

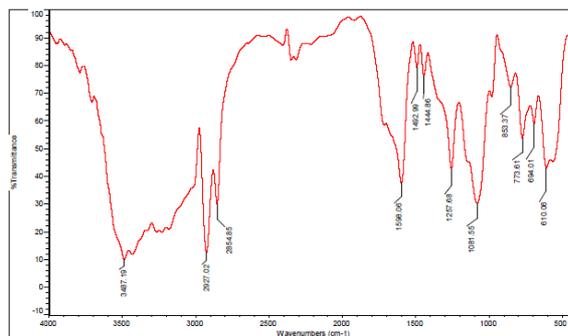
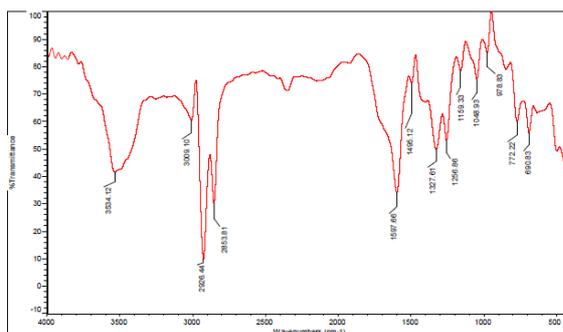
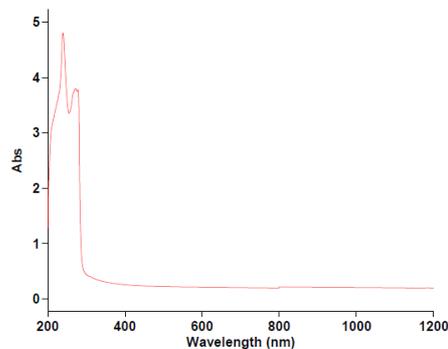
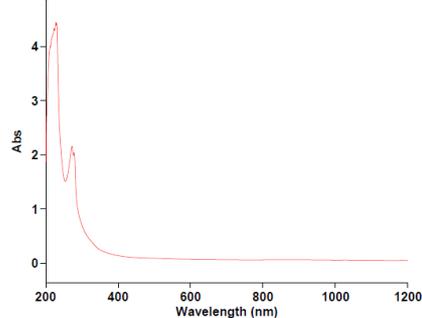
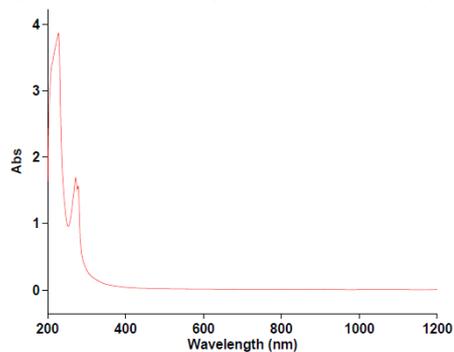


Figure 3 : FTIR spectrum of Ni (II) complex

**Figure 4 : FTIR spectrum of Cu (II) complex**

3.3 Electronic spectra

The electronic spectral data of Schiff base and its metal complexes are given in table 2. The electronic spectra of and its metal complexes are given in Fig. 4-7. The ligand shows a broad band at 239 nm which is assigned to $\pi \rightarrow \pi^*$ transition of the C = N chromophore[17]. In octahedral environment of the ligand fields, d orbitals of Co (II) show the transition $4T_{1g}(F) \rightarrow 4T_{1g}(P)$ with the electronic absorption bands at 550 - 385 nm. The electronic spectrum of Ni (II) complex shows an intense band at 333nm. This corresponds to the transition $3A_{2g}(F) \rightarrow 3T_{1g}(P)$ which is the characteristic of octahedral geometry. The electronic transition and its absorption bands in the tetragonally distorted octahedral environment around Cu(II) is $2B_{1g} \rightarrow 2E_{1g}$ 470nm-450nm.

**Figure 5 : Electronic spectrum of ligand****Figure : 6 Electronic spectrum of Co(II) complex****Figure 7 : Electronic spectrum of Ni(II) Complex**

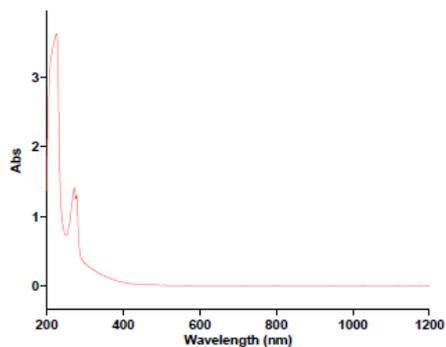
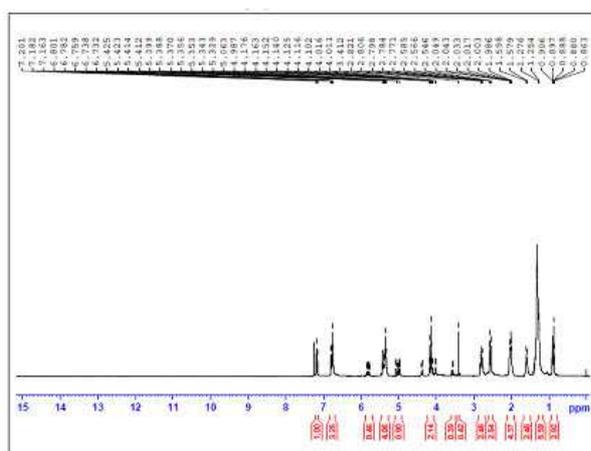
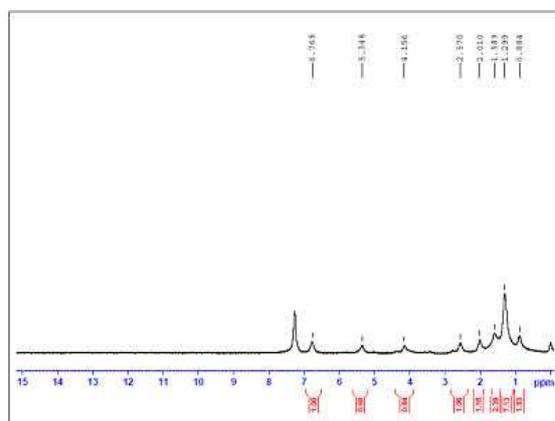


Figure 8 : Electronic spectrum of Cu(II) Complex

3.4 ¹H NMR spectra

On examining the ¹H NMR spectrum of ligand (fig.9) it exhibited a multiplet signal at $\delta = 7.16$ ppm - 7.18 ppm for aromatic protons. The presence of HC = N group is indicated by the singlet at $\delta = 7.2$ ppm. A signal at $\delta = 6.73$ ppm - 6.78 ppm, $\delta = 4.10$ ppm - 4.17 ppm, 3.32 ppm - 3.39 ppm and 1.2 ppm - 2 ppm indicate the presence of olefinic, -CH₂-COO, O-CH₂ and -CH₂- protons.

The ¹H NMR spectrum of Co (II) complex gave the signals for aromatic protons ($\delta = 7.2 - 7.5$ ppm) olefinic protons ($\delta = 6.76$ ppm), CH₂ COO protons (4.156 ppm), O-CH₂- protons ($\delta = 3.4$ ppm), -CH₂- protons ($\delta = 1.2 - 2$ ppm) and CH = N proton ($\delta = 7.7$ ppm). There is a down field shift of imine proton after complex formation than in the ligand. Thus ¹H NMR study also confirmed the structural information than in the ligand.

Figure 9 : ¹H NMR Spectrum of LigandFigure 10 : ¹H NMR Spectrum of Co(II) Complex

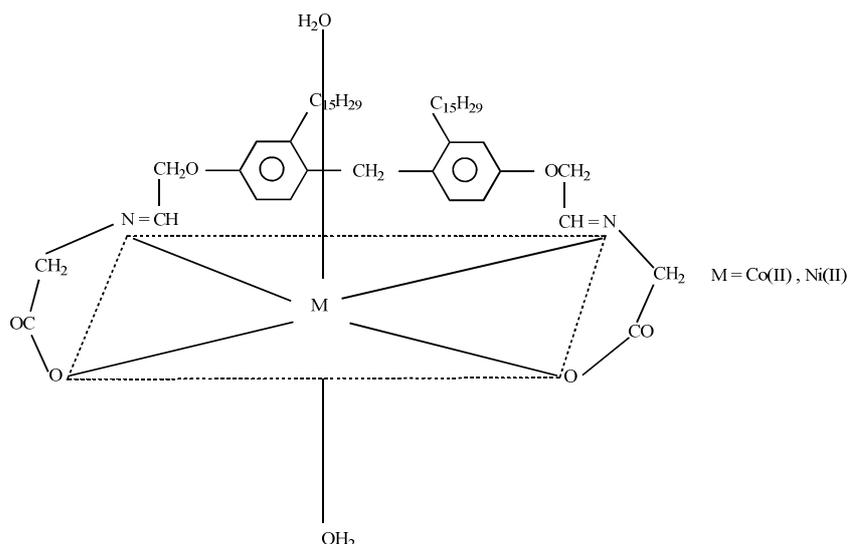


Figure .11 Structure of Co(II) & Ni (II) Complex

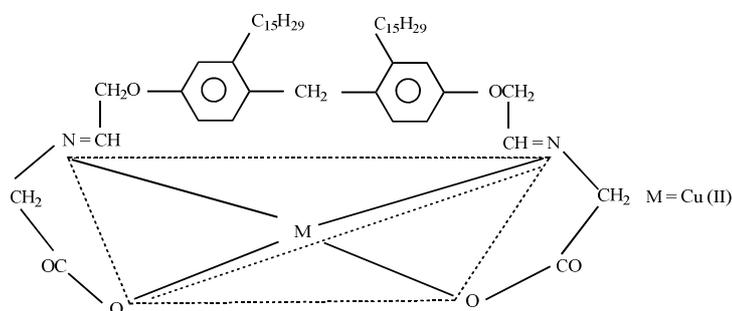


Figure .12 Structure of Cu(II) Complex

3.5 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. 13. From the fig. 13 pitted and rough surface is observed in the complex. The particle size of the Cu (II) complex was in the diameter range of few microns[11-13].

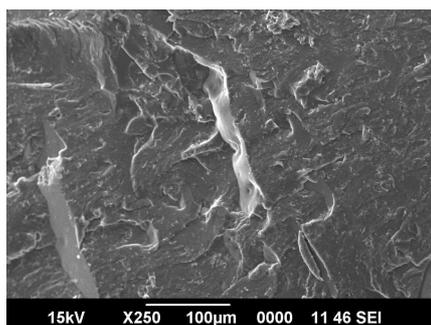


Figure 13: SEM image of Schiff base complex of Cu(II)

3.6 Powder 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[13-18]. XRD shows that Cu(II) complexes have the average crystallite size of 4.5 nm suggesting the complexes to be nano crystalline (Fig14).

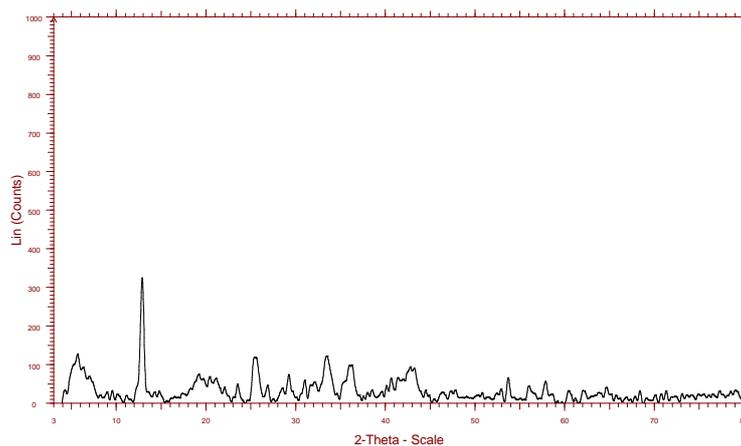


Figure 14: XRD spectrum of Cu(II) complex

3.7 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*, *Streptococcus mutans*, *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) | | | |
|------|----------------------|---------------------|-------------------------|---------------|-----------------------------|-----------------|
| | | | <i>Klebsiella</i> sps | <i>E.coli</i> | <i>Streptococcus mutans</i> | <i>S.aureus</i> |
| 1 | $C_{51}H_{76}N_2O_6$ | Mueller Hinton Agar | 6.0 | 6.0 | 6.0 | 6.0 |
| 2 | $[CoL(H_2O)_2]$ | | 12.0 | 6.0 | 6.0 | 11.0 |
| 3 | $[Ni L(H_2O)_2]$ | | 6.0 | 11.0 | 6.0 | 6.0 |
| 4 | $[Cu L]$ | | 6.0 | 6.0 | 10.0 | 6.0 |
| 5 | PC(Gentamicin) | | 25.0 | 26.0 | 24.0 | 25.0 |
| 6 | NC | | 6.0 | 6.0 | 6.0 | 6 |

Table: 4 Antimicrobial Activity for Fungi

| S.No | Samples | Media | Zone of Inhibition (mm) | |
|------|----------------------|---------------------|-------------------------|------------------------|
| | | | <i>Candida</i> sps | <i>Aspergillus</i> sps |
| 1 | $C_{51}H_{76}N_2O_6$ | Mueller Hinton Agar | 6.0 | 6.0 |
| 2 | $[CoL(H_2O)_2]$ | | 13.0 | 6.0 |
| 3 | $[Ni L(H_2O)_2]$ | | 6.0 | 12.0 |
| 4 | $[Cu L]$ | | 6.0 | 6.0 |
| 5 | PC(Nystatin) | | 25.0 | 26.0 |
| 6 | NC | | 6.0 | 6.0 |

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 centres of cell constituents [23] resulting in interferences with the normal cell process.

3.8 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 .[24-27] The results of DNA cleavage are given in Fig16. 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 Ni(II)>Co(II)>Cu(II) with lamda - DNA . The oxidative DNA cleavage by singlet oxygen is likely to proceed via oxidation of guanine nucleobase.

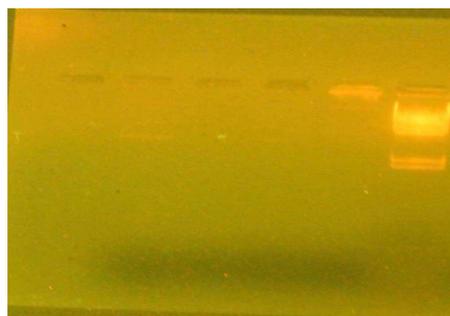


Figure 15: Gel electrophoresis diagram of the Schiff base metal complexes.

| | | | |
|----------|--|----------|--|
| Lane 1 : | DNA + L + H ₂ O ₂ | Lane 2 : | DNA + CoL ₂ + H ₂ O ₂ |
| Lane 3 : | DNA + [Ni L ₂ (H ₂ O) ₂] | Lane 4 : | DNA + CuL ₂ + H ₂ O ₂ |
| Lane 5 : | Control DNA | Lane 6 : | Marker |

3.9 Anticancer Activity

The result of anticancer activities are presented in table 5 & 6. The colon carcinoma (HT-20) cells, were sensitive to the Co (II) complex with an IC₅₀ value of 86.76 μ M. The Cu (II) complex had the better activity with an IC₅₀ value of 83.24 μ M. 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[28]. 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. (fig 16, & fig 17)

Table 5 : % viability of Co(II) complex

| Sample Concentration (μ g/ml) | Average OD at 570nm | Percentage Viability |
|------------------------------------|---------------------|----------------------|
| Control | 0.654 | |
| 6.25 | 0.627 | 95.81 |
| 12.5 | 0.557 | 85.16 |
| 25 | 0.516 | 78.89 |
| 50 | 0.426 | 65.13 |
| 100 | 0.278 | 42.5 |

IC₅₀ = 86.76 μ M

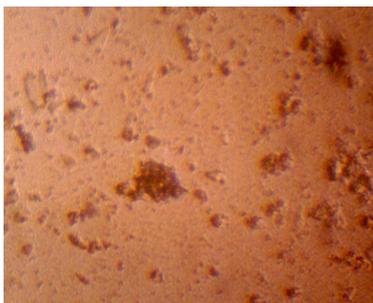
Co(IV) Complex at 100 μM

Figure 16 : Microscopic observation of anticancer activity

Table 6 : % viability of Cu(II) complex

| Sample Concentration ($\mu\text{g/ml}$) | Average OD at 570nm | Percentage Viability |
|---|---------------------|----------------------|
| Control | 0.703 | |
| 6.25 | 0.689 | 98 |
| 12.5 | 0.611 | 86.91 |
| 25 | 0.513 | 72.97 |
| 50 | 0.443 | 63.01 |
| 100 | 0.304 | 43.24 |

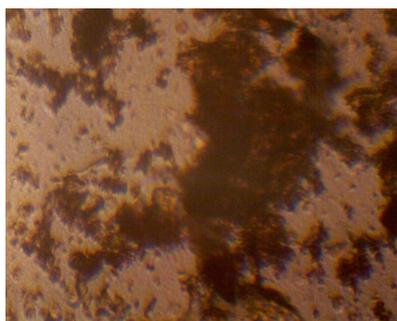
IC₅₀ = 83.24 μM Cu(II) Complex at 100 μM

Figure 17 : Microscopic observation of anticancer activity

CONCLUSION

Schiff base complexes of Co(II), Ni(II) and Cu(II) were synthesized from DFMPM using glycine were clearly described and characterized on the basis of analytical and spectral data. The SEM & XRD analysis indicate that the crystals of Schiff base metal complexes are nano crystalline in nature. Anti microbial study showed that the complexes have higher anti microbial activity than the ligand. The DNA cleavage studies show that the Co(II) Ni(II) and Cu(II) complexes cleave DNA more efficiently. The in-vitro anti cancer studies reveal that the Cu(II) complex had better anticancer activity against HT-29(colon carcinoma) cell with IC₅₀ value of 83.24 μM .

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