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***Research Paper***

# Synthesis and Characterization of Bioactive Transition Metal Complexes of Cu(II), Co(II) and Ni(II) using DFMPM and 1,4-Diamino Butane

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***Abstract:*** Cardanol ,a naturally occurring aliphatic chain substituted phenol derived from cashewnut shell liquid (CNSL) wasused for the preparation of Schiff base ligand using 1,4 diamino butane . The Schiff base complexes of Cu(II) Co(II) and Ni(II) of diαformylmethoxybis(3pentadecenylphenyl)methane (DFMPM) and 1,4-Diamino butane were synthesized in three stages. The ligand and complexes were characterized by UV-visible,IR Spectroscopy.The elemental analysis, melting pointmolar conductance,magnetic susceptability, SEM,XRD, antibacterial, antifungal activity and DNA cleavage were also studied. The conductance measurements indicate that all the complexes of non-electrolytes. The result indicate that the complexes of Cu(II),Co(II) and Ni(II) metal ion intake indicates the ligand can be used for the removal of these metals from water . The SEM and XRD studies revealed the nano crystalline nature of the complexes.

**Keywords:** Cardanol , Formaldehyde , Epichlorohydrin , 1,4 diamino butane, Schiff base.

## Introduction

Schiff base of azomethine nitrogen donar hetrocyclic ligands are well known due to their wide range of applications in pharmaceutical and industrial fields and have been found to act as antimicrobial,DNAcleavage and anticancer activity [1-7.] Schiff base can accomadate different metal centres involving various coordination methods there by allowing successful synthesis of homo and hetro metallic complexes with varied stereo chemistry [8,9]. Metal complexes of nitrogen oxygen chelating agents derived from 1,4-diaminobutane have been studied [10,11]. The present study deals with the synthesis, characterization and biological studies of the Schiff base complexes of Cu(II),Co(II) and Ni(II) derived from DFMPM and 1,4-diamino butane.

## Materials and Methods

Cardanol was obtained from M/S Satya Cashew, Chennai, India. Formaldehyde (37% solution) hydrochloric acid, epichlorohydrin, 2-aminothiophenol, sodium hydroxide and other chemicals used were of AR grade quality obtained from Merk Chemicals. All the solvent used was purified by standard methods[12,13]. The micro analytical data (C, H, N) were collected using Perkin Elmer 2400 instrument. The metal ion intake were estimated by standard methods. 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.

### Preparation of Schiff base Ligand(L) with DFMPM with 1,4-diaminobutane

The Schiff base Ligand(L) was prepared by mixing equimolar ethanolic solution of DFMPM and 1,4-diaminobutane in ethanol and refluxed for 2 hours and then cooled the mixture and Schiff base was separated and recrystallized from ethanol and washed thoroughly with diethyl ether.[14] [figure 1)

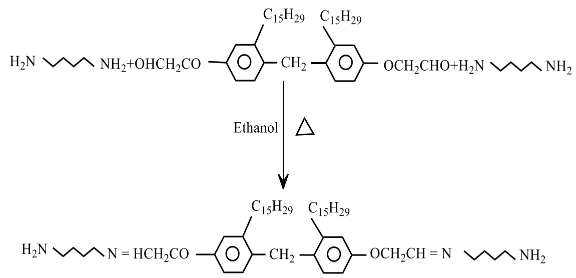


**Figure 1: Preparation of Schiff base ligand**

## Preparation of Schiff base metal complexes

Schiff base complexes of Ligand(L) were prepared by the addition of hot ethanolic solution (60°c) of Ligand(L) and aqueous solution nitrates of Cu(II), Co(II) and Ni(II) ions in drop by drop in 2:1 molar ratio The mixture was stirred for 12 hours the resulting solution was concentrated on a water bath and allowed to cool. The solid product formed was separated by filtration and washed thoroughly by ethanol and then by diethyl ether. The solid complexes were dried in vacuum.

## Results and discussion

All the metal complexes prepared above are coloured and are stable towards air and have high melting point. The complexes are insoluble in water and common organic solvents but are soluble in DFM and DMSO.

## Elemantal analysis

The analytical data suggested that all the complexes are mononuclear with the ligand coordinated to the central metal atom. The metal to ligand ratio in all the complexes was 1:2. The details are given in table 1 and shown the (Figure 2).

**Table 1: Physical characteristics and analytical data of the Ligand and its complexes**

| Ligand/ Complexes | Yield % | colour | Mol. formula | Mol.wt | Melting point | Elemental analysis | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | N |
| Ligand(L) | 62 | brown | $C_{55}H_{108}N_4O_2$ | 856 | 220 | 77.10 (77.09) | 12.61 (12.53) | 6.54 (6.42) |
| $[Cu(L_2)(NO_3)_2]$ | 57 | Light green | $CuC_{110}H_{216}N_{10}O_4$ | 1803.54 | >250°c | 73.18 (73.10) | 11.97 (11.89) | 7.76 (7.64) |
| $[Co(L_2)(NO_3)_2]$ | 55 | Brown | $CoC_{110}H_{216}N_{10}O_4$ | 1798.9 | >250°c | 73.37 (73.25) | 12.00 (12.05) | 7.78 (7.64) |
| $[Ni(L_2)(NO_3)_2]$ | 58 | Light green | $NiC_{110}H_{216}N_{10}O_4$ | 1798.69 | >250°c | 73.38 (73.25) | 12.00 (11.98) | 7.78 (7.65) |

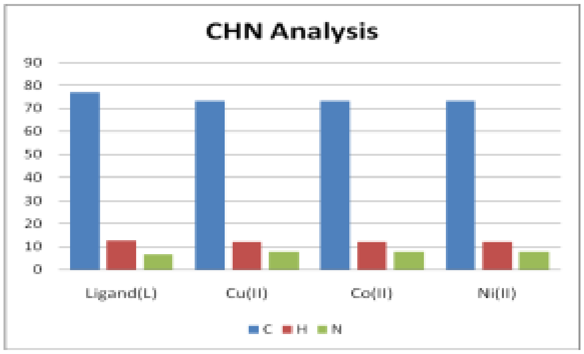


**Figure 2: Elemental Analysis**

## Molar conductivity measurements

The molar conductivity values are given in Table 2 and shown the [Figure 3.] The molar conductivity value were in the range of 15-23 ohm$^{-1}$ cm$^2$ mol$^{-1}$. It showed that all the complexes are non-electrolytes because their conductivity values were low.[15]

**Table 2: Molar conductance data of ligand and its complexes**

| Ligand/ complexes | Molar conductance Ohm$^{-1}$ cm$^2$ mol$^{-1}$ | Nature |
|---|---|---|
| Ligand(L) | 23 | non-electrolyte |
| $[Cu(L)(NO_3)_2]$ | 15 | non-electrolyte |
| $[Co(L)(NO_3)_2]$ | 17 | non-electrolyte |
| $[Ni(L)(NO_3)_2]$ | 15 | non-electrolyte |

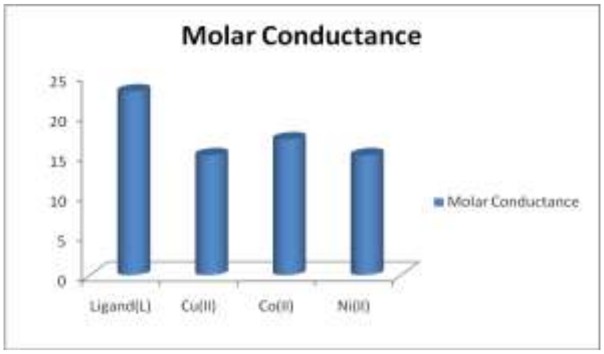


**Figure 3: Molar conductance data of the complex**

### IR spectral studies of ligand and complexes

The IR spectrum of the free ligand (figure .4) is characterized mainly the strong bands at 3317.56cm$^{-1}$, 2924.09cm$^{-1}$, 2854.65cm$^{-1}$ and 1581.63cm$^{-1}$ which are attributed to the stretching frequencies of $\vartheta_{O-H}$, $\vartheta_{C-H}$, $\vartheta_{C-O}$, $\vartheta_{C=N}$ respectively [16].

The FT-IR spectrum of the ligand is compared with the spectra of the complexes. The characterization of absorption bands 3448.72cm$^{-1}$- 3402.43cm$^{-1}$ range were attributed to -OH group of the lattice water or the coordinated water [17] in the IR spectrum of the complexes (Fig.ure 5 ,6, 7). The absorption bands in the range 2924.9cm$^{-1}$ – 2931.8cm$^{-1}$ were assigned to $\vartheta_{C-H}$ stetching frequencies. The absorption band in the range 2854.65cm$^{-1}$ – 2399.45cm$^{-1}$ were assigned to $\vartheta_{C-O}$ stretching frequencies. The $\vartheta_{C=N}$ bands for the free ligand in the spectra of the complexes 1627.92cm$^{-1}$ – 1589.34cm$^{-1}$ suggesting coordination of both the nitrogen atoms to metal [18,19]. The absorption band in the range of 3054.31cm$^{-1}$ – 3089cm$^{-1}$ were assigned to the amino nitrogen to the metal ions.The imine peak in the metal complexes showed change in schiffs compared to the ligand indicating coordination of the imine nitrogen atom to the metal ion due to coordination. The assigned to the coordinate nitro group with the central metal atom 779.24cm$^{-1}$ – 725.53cm$^{-1}$ is assigned to M-N bond and 509.21cm$^{-1}$ – 617.22cm$^{-1}$ is assigned to M-O bond region respectively. Selected IR spectral data for the ligand and its complexes are given in table 3.

**Table 3: Selected FITR frequencies (cm$^{-1}$) of the ligand and its complexes**

| Ligand/complexes | $\vartheta_{O-H}$ | $\vartheta_{C-H}$ | $\vartheta_{O-C}$ | $\vartheta_{C=N}$ | FreeNH$_2$ | $\vartheta_{M-N}$ | $\vartheta_{M-O}$ |
|---|---|---|---|---|---|---|---|
| Ligand(L) | 3317.56 | 2924.09 | 2854.65 | 1581.63 | 3008.95 | - | - |
| $[Cu(L)(NO_3)_2]$ | 3448.72 | 2924.09 | 2854.65 | 1627.92 | 3054.31 | 779.24 | 509.21 |
| $[Co(L)(NO_3)_2]$ | 3456.44 | 2931.8 | 2399.45 | 1620.21 | 3078.39 | 725.53 | 516.92 |
| $[Ni(L)(NO_3)_2]$ | 3402.43 | 2931.8 | 2376.3 | 1651.07 | - | 740.67 | 617.22 |

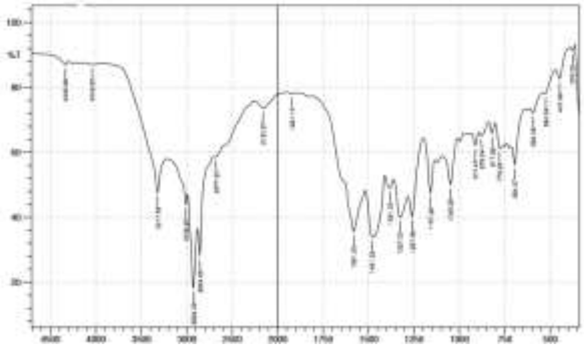


**Figure 4 : FT-IR spectrum of Schiff base Ligand(L)**

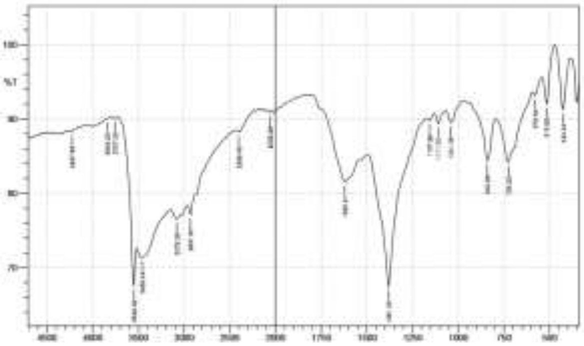


**Fig .6: FT-IR spectrum of Co(II) complex of Ligand(L)**

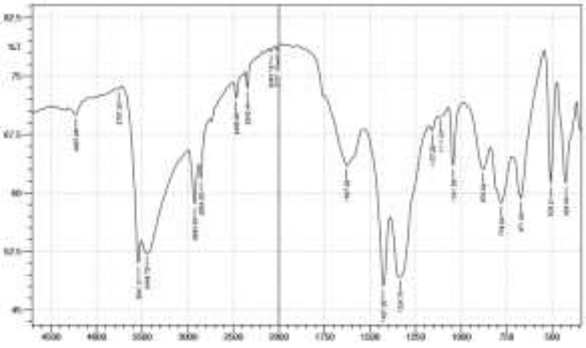


**Figure 5: FT-IR spectrum of Cu(II) complex of Ligands(L)**

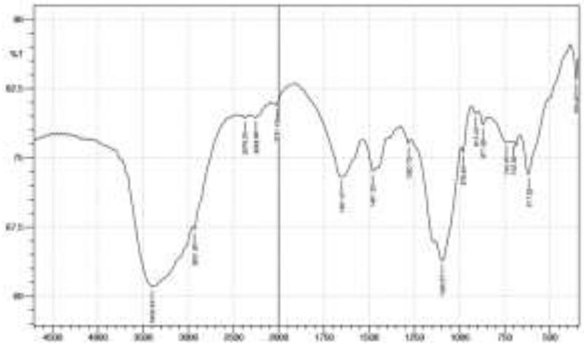


**Figure.7: FT-IR spectrum of Ni(II) complex of LIgand(L)**

**Magnetic susceptibility measurements (BM)**

The magnetic susceptibility values of the complexes of given in table 4 Cu(II) complex showed magnetic moment of 2.04 BM indicating distorted octahedral nature of the complexes . The Co(II) complex showed magnetic moment in the range of 5.07 Bm typically for octahedral complexes. The Ni(II) complex had a magnetic moment value 2.87 BM, closely related to the value experted for octahedral complexes .

**Table 4: Magnetic susceptibility**

| Complex | Magnetic susceptibility (BM) |
|---|---|
| $[Cu(L)(NO_3)_2]$ | 2.04 |
| $[Co(L)(NO_3)_2]$ | 5.07 |
| $[Ni(L)(NO_3)_2]$ | 2.87 |

**Electronic Spectra of Ligand and its complexes**

The electronic spectral data are given in table 5 The uv-visible spectra of Ligand (L) shows the peak around at 325nm, 340nm, 328nm which are assigned to n-π* transitions respectively [19]. The uv-visible spectra of the Cu(II) complex showed absorption band at 316nm, 318nm, 357nm region which are assigned to$^2Eg \rightarrow ^2T_{2g}$ transition indicating the complex to have distorted octahedral geometry [20].The uv-visible spectra of the Co(II) complex showed absorption bands at 350nm which are assignable to the overlap of transitions $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$ indicating an octahedral environment .The uv-visible spectra of the Ni(II) complex showed absorption bands at 226nm,224nm,274nm attributed to $^3A_{2g}(F) \rightarrow ^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$ transition suggesting an octahedral geometry [21,22]

**Table 5: Electronic spectral data of the ligand and its complexes**

| Ligand/complex | λmax(nm) |
|---|---|
| Ligand(L) | 325,340, 328 |
| $[Cu(L)(NO_3)_2]$ | 316, 318, 357 |
| $[Co(L)(NO_3)_2]$ | 350 - |
| $[Ni(L)(NO_3)_2]$ | 226, 224, 274 |

**$^1$HNMR Spectra**

The $^1$HNMR spectra of Schiff base Ligand(L) (fig 8) exhibit a multiplet signal at (δ=7.140ppm-7.178ppm) is due to substituted aromatic ring protons . The O-$CH_2$ protons (δ=3.026ppm – 3.961ppm), olefinic protons of the side chain (δ = 6.704ppm – 6.798ppm). The presence of -CH=N group is assigned by the signal (δ = 7.178ppm) respectively [16].

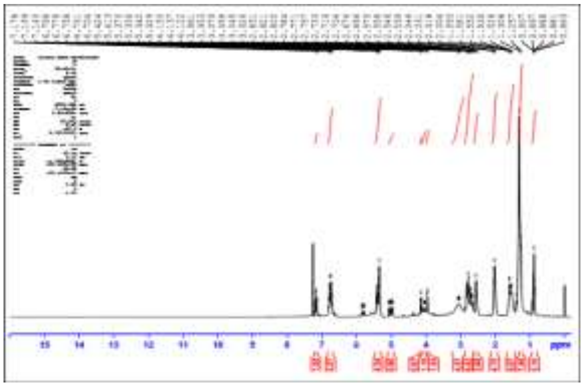


**Figure 8: $^1$HNMR Spectrum of Schiff base Ligand(L)**

$^1$HNMR spectrum of the Cu(II) complex also shows Fig(9) the multiplet signals for aromatic protons (δ = 6.800ppm – 6.740ppm). A multiplet at (δ = 2.551ppm – 2.586ppm) is due to substituted H-C-C=O group and a singlet at (δ = 2.013ppm) due to substituted –$CH_2$-NH group. The presence of azomethine of –CH=N proton is assigned by the signal at (δ=7.571ppm) is respectively group of both ligand and its complexes. Thus $^1$HNMR study also confirmed both functional groups and other structural information of ligand and complexes[16-18].

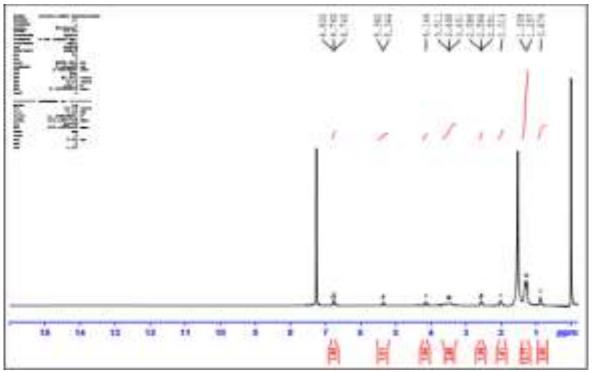
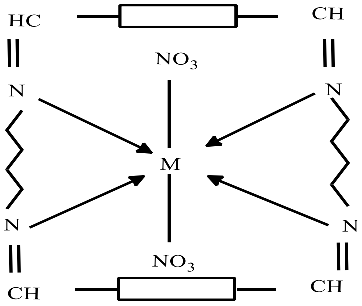


**Figure 9: $^1$HNMR Spectrum of Cu(II) complex of Ligand(L)**

Based on the observations in elemental analysis IR Spectra, uv-visible spectra and $^1$HNMR spectral studies, the proposed structure of metal- Schiff base complexes $[M(L_2(NO_3)_2]$ are given in (Figure 10)



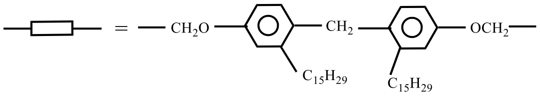


**Figure 10 : General structure of Schiff base complexes of Cu(II), Co(II) and Ni(II)**

M = Cu(II), Co(II) and Ni(II)

### X-ray Powder Diffraction Analysis

The powder XRD pattern of the metal complexes Cu(II) were performed. The difractogram is given in [fig 11].The grain size of the complexes was calculated using scherer's formula . The metal complexes possess sharp crystalline peaks indicate their crystalline calculated using Scherer's equation has the average grain size value of 3.40nm suggesting that the complexes are in nanocrystalline nature.

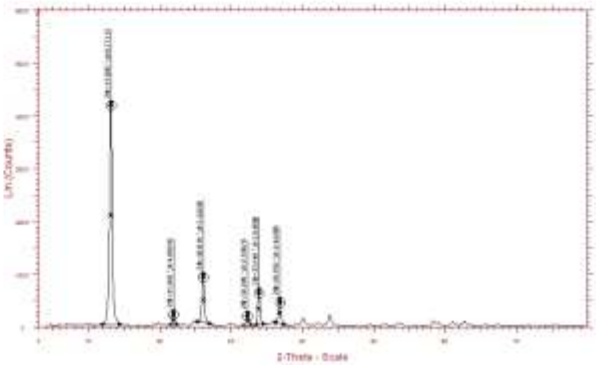


**Figure 11: XRD Spectrum of Cu(II) complex of Ligand(L)**

### SEM Analysis

The surface morphology of Cu(II) complexe of Ligand(L) have been studied using SEM and the respective images are given in (Figure 12 ). SEM study reveals that all the complexes are crystalline in nature. The crystals are very clear, rough and pitted needle shaped surface are also seen. The particle size of the complexes is microcrystalline in nature but under high magnification the grain like appearance disappears[19].

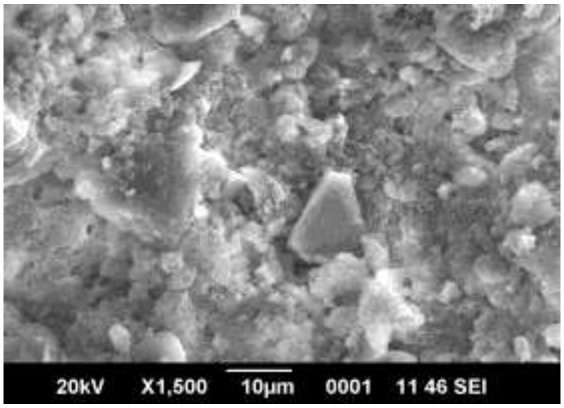


**Figure 12: SEM image of Cu(II) complex of Ligand(L)**

### Biological Screening

### Antibacterial activity

The antibacterial activity of the ligand and complexes are presented in Table 9 and shown the (Figure 13) The antibacterial activity were estimated based on the size of inhibition zone in the discs under identical conditions the Schiff base complexes of Cu(II), Co(II), Ni(II). have moderate antibacterial activities against bacteria.The test drug solution of (100g) of each compound was prepared by dissolving 1mg of each compound separately in 1ml of DMSO. *Staphylococus aureus, Streptococus mutans, E. Coli, Klebsilla pneumonia*. The antibacterial activity was estimated based on the size of inhibition zone in the discs. The results of antibacterial activity substanticate the findings of earlier researchers. That biologically inactive compounds become active and less biologically active compounds become more active upon coordination. Such enhancement in biological activity of metal complexes can be explained on the basis of overtones concept and chelation theory according to overtones concept of cell permeability, the lipid membrane that surrounds the cell favours the passage as only lipid soluble materials due to which liposolubilty is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π – electron over the whole chelated ring end enhanced lipophilicity of the complex. This enhanced the lipophilicity in turn enhances the penetration of the complexes in to lipid membranes and blocking of metal binding sites on the enzymes of the microorganisms[20-22]. The results were also expressed by means of activity index.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of standard}}$$

From the results it was found that the Ni(II) complex exhibit maximum antibacterial activity against E.Coli. But Cu(II) and Co(II) shows the less activity towards the Klebsilla pneumonia and Staphylococus mutans. It showed that the maximum activity against Ni(II) complex when compared to other complexes.

**Table 9: Antibacterial activities of Ligand(L) and its complexes**

| Ligand/ Complexes | Media | Antibacterial activity(mm) | | | |
|---|---|---|---|---|---|
| | | Staphylococus aureus | Streptococus mutans | E.coli | Klebsilla pneumonia |
| Ligand(L) | Muller Hinton Agar Bacteria | 7 | 7 | 7 | 7 |
| $Cu(L)(NO_3)_2]$ | | 7 | 7 | 7 | 7 |
| $Co(L)(NO_3)_2]$ | | 7 | 7 | 7 | 7 |
| $[Ni(L)(NO_3)_2]$ | | 7 | 14 | 20 | 8 |

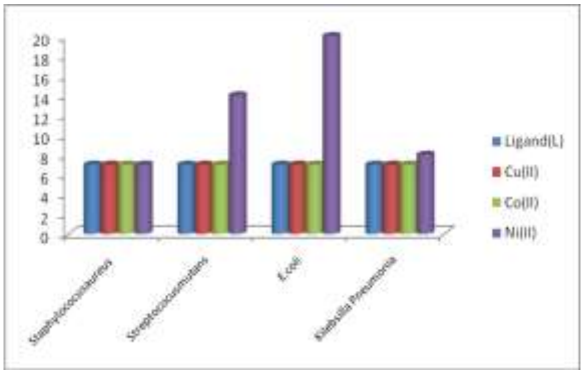


**Figure 13: Antibacterial activity data of ligand and complexes**

**Antifungal activity**

The antifungal activity of the ligand and complexes are presented in Table .10 and shown the Fig 14. The antifungal activity were estimated on the size of inhibition zone in the disc under identical conditions the Schiff base complexes of Cu(II), Co(II), Ni(II), Zr(IV) and Th(IV) have moderate antifungal activity against fungi. The biological activities of the synthesized Schiff base and its complexes were studied for antifungal properties by the agar diffusion method respectively in DMSO solvent against *Aspergillus niger* and *Aspergillus flavus*[23]

**Table 10: Antifungal activity data of ligand and its complexes**

| **Ligand/ complexes** | **Media** | **Antifungal activity(mm)** | |
|---|---|---|---|
| | | ***Aspergillus niger*** | ***Aspergillus flavus*** |
| Ligand | Antimytotic Agar for Fungi | 7 | 7 |
| $[Cu(L_2)(NO_3)_2]$ | | 9 | 7 |
| $[Co(L_2)(NO_3)_2]$ | | 7 | 7 |
| $[Ni(L_2)(NO_3)_2]$ | | 7 | 10 |

The results of antifungal activity substanticate the findings of earlier researchers. That biologically inactive compounds become active and less biologically active compounds become more active upon coordination. Such enhancement in biological activity of metal complexes can be explained on the basis of overtones concept and chelation theory according to overtones concept of cell permeability, the lipid membrane that surrounds the cell favours the passage as only lipid soluble materials due to which liposolubilty is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π – electron over the whole chelated ring end enhanced lipophilicity of the complex. This enhanced the lipophilicity in turn enhances the penetration of the complexes in to lipid membranes and blocking of metal binding sites on the enzymes of the microorganisms. The results were also expressed by means of activity index.

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of standard}}$$

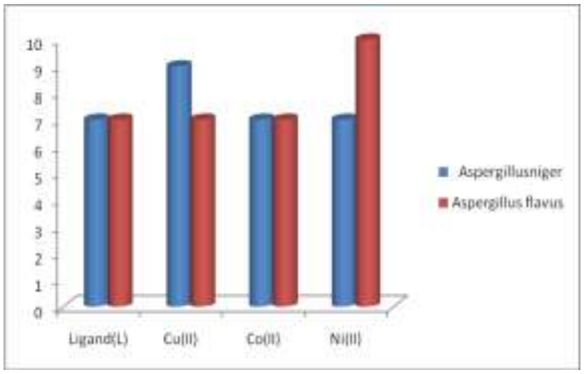


**Figure 14: Antifungal activity data of ligand and complexes**

From the results it was found that then Ni(II) complex exhibit maximum antifungal activity against *Aspergillus flavus*. It showed that the maximum activity against Ni(II) complex when compared to other complexes.

### DNA Cleavage studies

The DNA cleavage activities of Schiff base ligand and its metal complexes at a 1µM concentration were studied using pUC18 DNA (2µg) in $H_2O_2$ (10µL) in 30 mm Tris Buffer (pH 7.4) and upon radiation with uv light. The reaction is modulated by metallo complexes bound hydroxyl radical or a peroxo species generated from the co-reactant $H_2O_2$. It is evident from Fig 10, that the Ni(II) complex cleave DNA more efficiently in the presence of an oxidant than the ligand and Ni(II) complex. This may be hydroxyl free radicals, which can be produced by metal ions reacting with $H_2O_2$ to produce the diffusible hydroxyl radical or molecular oxygen, which may damage DNA through Fenton type chemistry. This hydroxyl radical participates in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of sugar- phosphate back bone [24]. Further, the presence of a smear in the gel diagram indicated the presence of radical cleavage.

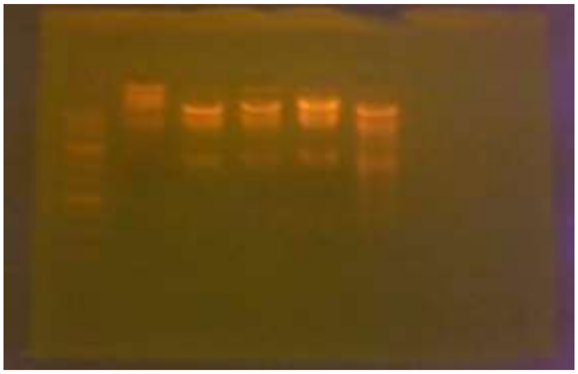


**Figure 15: DNA Cleavage of the complexes**

### Conclusion

Schiff base metal complexes of Cu(II), Co(II), Ni(II), Zr(IV) and Th(IV) were synthesized from cardanol using DFMPM and 1,4- diamino butane were clearly described and characterized on the basis of analytical and spectral data. From the spectral and stoichiometric analysis of octacordinated Cu(II), Co(II) , Ni(II) was assigned for the metal complexes. The XRD and SEM studies reveal that the complexes are nano crystalline state. . Antibacterial study showed that the complexes of Cu(II), Co(II), Ni(II),. The more active than the complex of Ni(II) complexes. Antifungal activity showed that the complexes of Cu(II), Co(II). The more active than the complex of Ni(II)when compared to other complexes . DNA cleavage studies showed that the complex cleave more efficiency.

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