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Synthesis ,Characterisation And Anti Cancerus Studies of Schiff Base Complexes of Co(II),Cu(II) And Ni(II) Using DFMPM And L-Histidine

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ABSTRACT

Cardanol a naturally occurring C_{15} unsaturated aliphatic chain substituted phenol derived from cashew nut shell liquid (CNSL) was used for the preparation of Schiff base ligand using Histidine. di-α-formylmethoxybis (3-pentadecenylphenyl) methane is prepared from cardanol using standard methods. The Schiff base complexes Cu(II), Co(II) and Ni(II) of DFMPM and Histidine were synthesized in three stages . The ligand and complexes were characterized by IR, UV-visible, ^{1}HNMR and elemental analysis, melting point, metal ion intake, SEM, XRD antibacterial, antifungal, anticancerus, DNA clevage and anti inflammatory activities were studied. The conductance measurements indicate that all the complexes are of non-electrolytes. The result indicate that the complexes of Cu(II), Co(II) and Ni(II) are hexa co-ordinated and have moderate antibacterial, antifungal activity anti cancer, DNA cleavage, larvacidal and anti inflammatory. The metal ion intake indicates the ligand can be used for the removal of these metals from water.

Keywords: Cardanol, Formaldehyde, Epichlorohydrin, Histidine, Schiff base

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I. INTRODUCTION

Schiff bases are versatile ligands synthesized from the condensation of an amino compound with carbonyl compounds and these coordinate to metal ions via azomethine nitrogen Schiff's base and their complexes possess remarkable properties as catalysts in various biological systems, polymers, dyes, antimicrobial activities, antifungal activities, antitumor and cytotoxic activities, plant growth regulator, enzymatic activity and pharmaceutical fields. A variety of Schiff's base and its complexes have been studied extensively[1]. Cardanol is the main component obtained by vaccum distillation of roasted cashew nut shell liquid (CNSL) and was used for the preparation of bioactive transition Schiff base metal complexs using standard methods [2-3]. The ligand and complexes were characterised by UV-visible, FTIR, ^{1}HNMR, elemental analysis, melting point, conductivity, metal ion intake, anti bacterial, anti fungal, anti cancer, DNA cleavage, larvacidal and anti inflammatory activity were studied . The result indicate that the complexes of Cu(II), Co(II) and Ni(II) were bioactive and also used for the removal of such ions from water the nano crystalline nature of complexes were confirmed by SEM and XRD studies.[4-5]

II. MATERIALS AND METHODS

Cardanol was obtained from M/S Sathya Cashew Chennai India, Formaldehyde (37% solution) , hydrochloric acid, epichlorohydrin, L-histidine, sodium hydroxide and other chemicals were used of GR/AR grade quality obtained from Merk chemicals. All the solvents used were purified by standard methods[6] . Elemental analysis (C,H,N) were performed by using Elementar Vario EL III at STIC, CUSAT, Cochin. The IR spectra were recorded in KBr pellets using Shimadzu FTIR spectrometer (4000 – 400 cm-1). The UV-Visible spectra (200 – 800 nm) were recorded using Lab India 3000+ double beam spectrophotometer. The metal ion intake were estimated by standard methods[7] .

Synthesis of Schiff base ligand with DFMPM and L-glutamine

The Schiff base ligand was prepared by the reported methods[8-9].Equimolar ethanolic solution of DFMPM and L-histidine were mixed and refluxed for about an hour. Pour the reaction product in ice,(1+2) Schiff base ligand was obtained[10]. The precipated yellow compound was filtered washed with water and dried over unhydrous calcium chloride. The crude sample was recrystalised from 50% absolute alcohol yield=62%. Melting point =223^{0}C.

Synthesis of Cu(II), Co(II) and Ni(II) Schiff base metal complexes

All the metal complexes were prepared by mixing ethanolic solution of Schiff base ligand with the corresponding aqueous metal salt solution of Cu(II) nitrate, Co(II) nitrate and Ni(II) nitrate in 1:1 molar ratio. The resulting mixture was refluxed for about twelve hours at 70-80^{o}c[11], a coloured solution appeared on standing. The complexes were filtered, washed with ethanol, diethyl ether, acetone and hot water and finally dried under vacuum at 90^{o}C. yield=60%

Estimation of metal ion intake

The filtrates obtained in the above method were collected. The collections were used for the estimation of Cu(II), Co(II) and Ni(II) intake for complexation using standard methods[12].

III. RESULT AND DISCUSSION

The analytical data of the complexes, together with their physical properties are mentioned in Table 1. The data suggested that the complexes are in ML composition. The metal complexes of Cu(II), Co(II) and Ni(II)are coloured solids, stable towards air and have high melting points above (250^{o}c). The complexes are insoluble in water and common organic solvents but are soluble in DMF, $CDCl_{3,}$ DMSO. Analytical data suggest that the metal to ligand ratio in all the complexes to be 1:2[13] (Table 1). Conductivities of solutions of the complexes are non electrolytes because their conductivity value were in the range 12-15 ohm^{-1}cm^2 mol^{-1}.However the conductivity value is higher than expected for non electrolytes probably due to partial solvolysis of the complexes in DMF medium.

Table 1Physical characteristics and analytical data of complexes

Complex / Ligand	Yield	Colour	Molecular formula	Mol. weight	Melting point	Elemental Analysis C	H	N
Ligand L	60	brown	$C_{59}H_{98}N_6O_4$	954	229	72.52 (74.213)	8.94 (10.27)	7.11 (8.80)
[CuL $(NO_3)_2$] H_2O	57	green	$C_{118}H_{196}N_{14}O_{14}$ Cu	2095	>250	65.28 (67.58)	7.96 (9.355)	8.10 (9.355)
[CoL $(NO_3)_2$] H_2O	56	black	$C_{118}H_{196}N_{14}O_{14}$ CO	2091	>250	66.93 (67.718)	8.58 (9.37)	7.84 (9.37)
[NiL $(NO_3)_2$] H_2O	58	grey	$C_{118}H_{196}N_{14}O_{14}$ Ni	2090	>250	65.15 (67.75)	7.64 (9.37)	8.06 (9.37)

IR Spectra

The diagnostic IR frequencies of the ligand and its complexes are compiled in Table 2. The IR spectrum of free ligand is compared with that of complexes in order to determine the co-ordination sites that may have involved in chelation. Characteristic peaks in the spectra of the ligand and complexes were considered and compared. The selected IR spectral data are given in (Table2). The IR spectrum of the ligand (L) shows characteristic bands at 2856 cm^{-1}, 2923 cm^{-1},1606 cm^{-1} due to the ν_{O-C},ν_{C-H}, $\nu_{C=N}$ respectively[14].The IR spectra of the complexes exhibited ligand bands with the appropriate shifts due to complex formation. The IR broad bands of metal complexes in the range of 3200 cm^{-1} to 3400cm^{-1} indicate the presence of co-ordinated or lattice water molecule[15]. The ν_{C-O} phenolic stretching frequency is observed around 2348 cm^{-1} to 2200cm^{-1} which get shifted to lower or higher frequency region indicating co-ordination of phenolic oxygen. Band at 2905 cm^{-1} to 2900cm^{-1} and 1651 cm^{-1} to 1623 cm^{-1} were assigned to C-H and C=N respectively[16-17].Bands at 1450cm^{-1} to 1400cm^{-1} and 3580 cm^{-1} to 3250 cm^{-1} were assigned to Free-COOH and free –NH groups .The imine peaks in the metal complexes showed changes in the ligand indicating co-ordination of the imine nitrogen atom to the metal ion due to co-ordination. Another absorption bands at 986 cm^{-1} to 875 cm^{-1} is assigned to M-N bond and 758 cm^{-1} to 750cm^{-1} is assigned to M-O bond[18-19].

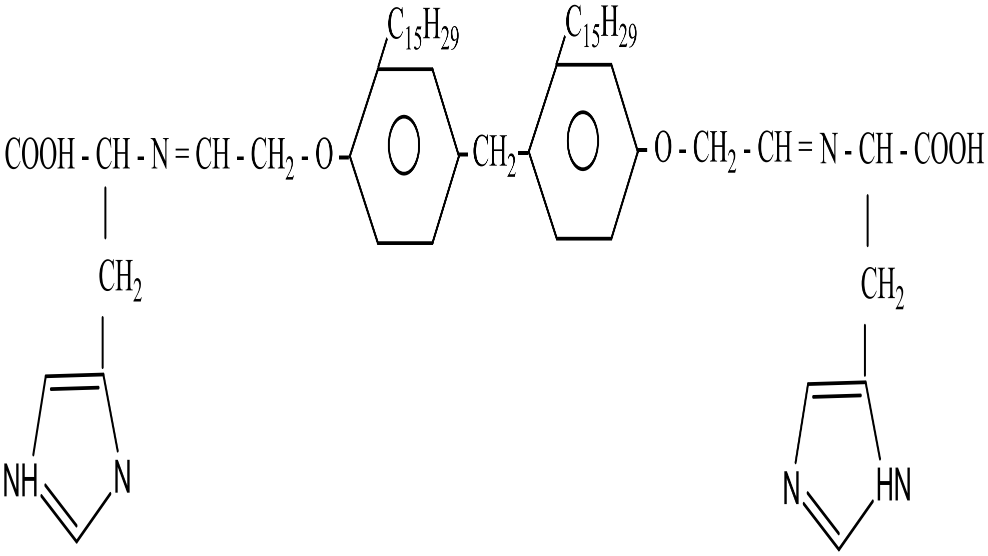

Figure 1: structure of ligand

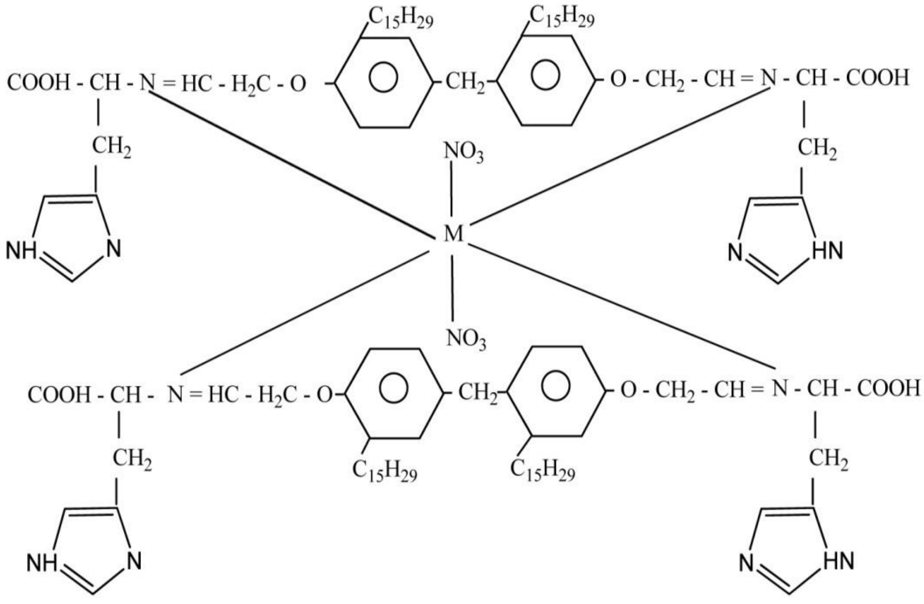

Figure 2: structure of Schiff base complexes, M=Cu(II),Co(II) and Ni(II)

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

Ligand/ Complexe	ν_{O-H}	ν_{O-C}	ν_{C-H}	$\nu_{C=N}$	$\nu_{C=O}$	free - COOH	free - NH	ν_{M-N}	ν_{M-0}	λ max(nm)
Ligand L	3350	2856	2923	1606	1489	1455	3007	780	702	448,607,620
$[CuL(No_3)_2]$ H_2O	3400	2348	2905	1634	1416	1400	3580	875	750	402,584,601
$[CoL(No_3)_2]$ H_2O	3200	2200	2900	1623	1305	1450	3250	819	758	408,596,616
$[NiL(No_3)_2]$ H_2O	3200	2265		1651		1433	3300	986		404,580,602

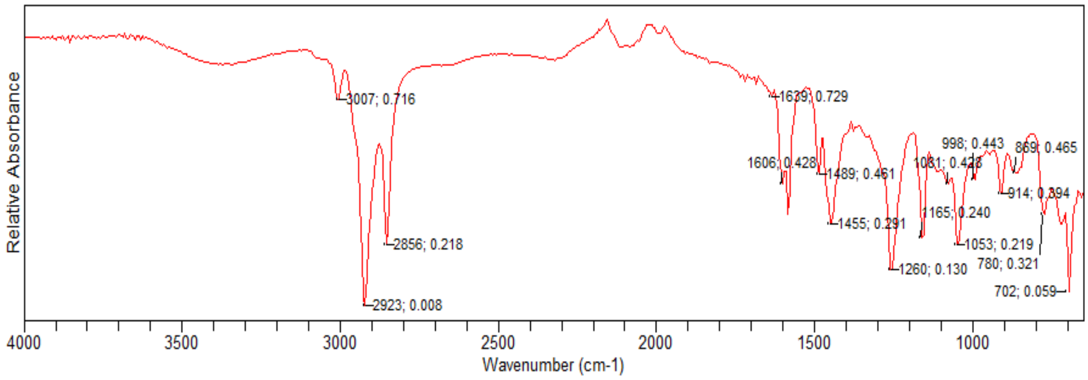

Fig. 3: FTIR Spectrum of ligand (L)

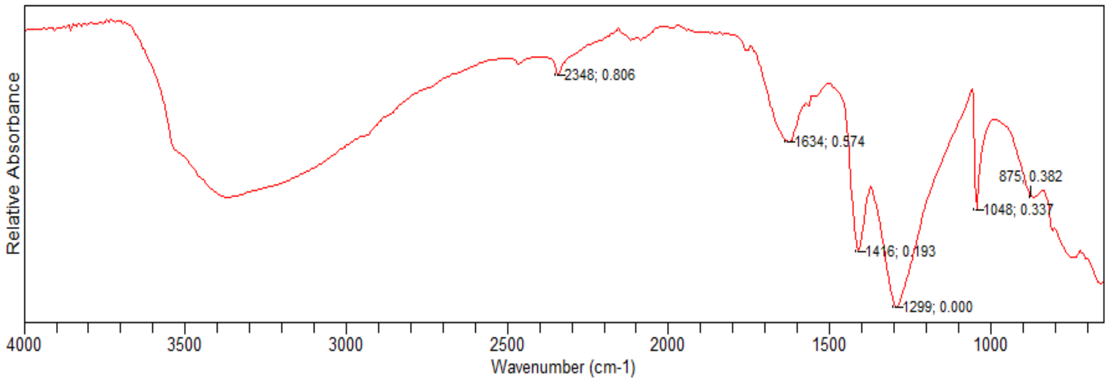

Fig.4: FTIR Spectrum of Cu(11) complex

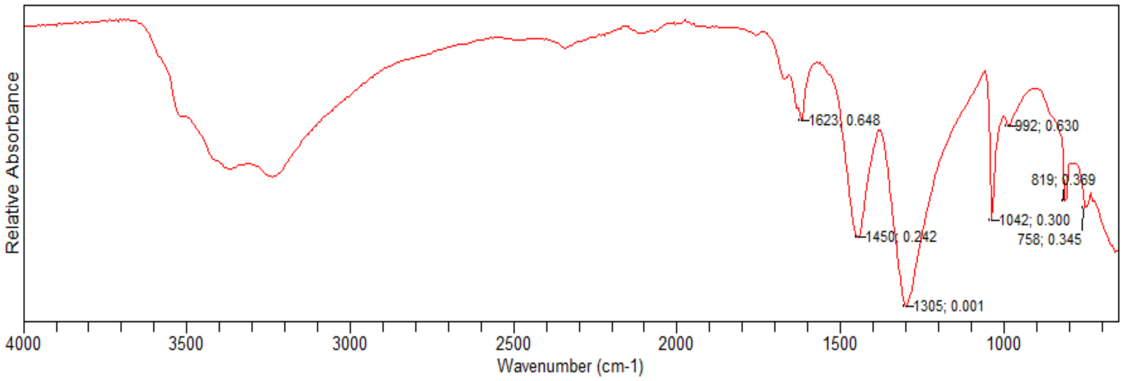

Fig.5: FTIR Spectrum of Co(11) complex

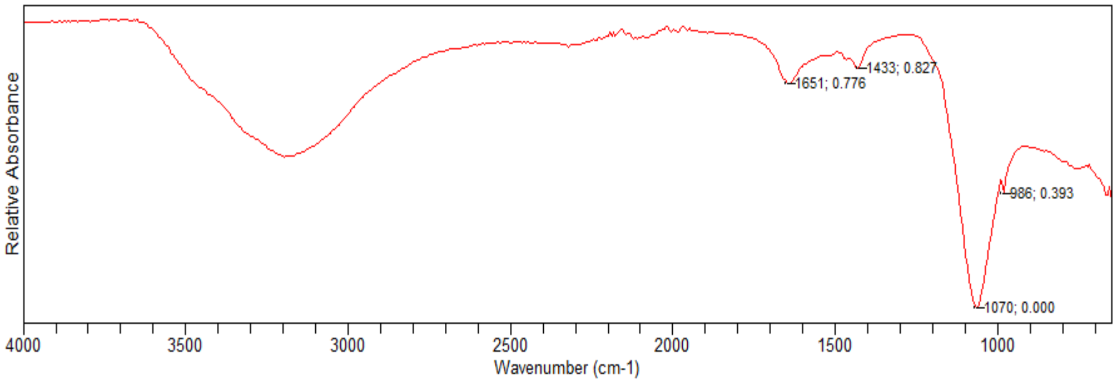

Fig.6:FTIR Spectrum of Ni(11) complex

UV visible spectra

The UV visible spectra are often very helpful in the evaluation of results furnished by other methods of structural investigation.The ligand showed a broad band at 448nm which is assigned to π-π* transition of the C=N chromophore.On complexation this bond was shifted to lower wave length suggesting the co-ordination of imine nitrogen with central metal ion. The UV spectrum of ligand showed three absorption bands at 448, 607, 620nm giving an octahedral geometry with field transition ^2B_1g->^2A_1g,^2B_1g->^2B_2g and ^2B_1g->^2E_2g,respectively The UV spectrum of Cu(11) complex showed three absorption bands at 402,584,601nm [19-20]. The UV spectrum of Co(11) and Ni(11) complexes showed absorption bands at 616 to 408nm and 602 to 404nm respectively suggesting octahedral geometry[20].

^{1}HNMR Spectra

On examining the ^{1}HNMR spectrum of ligand (Figure 11), it exhibited a multiplet signed at δ=7.004 ppm –7.029 ppm is due to substituted aromatic ring protons[16]. The presence of H – C = N- group is indicated by the singlet at δ = 7.023ppm. The multiplet at δ 6.537 ppm - δ = 6.639 ppm and δ = 5.168 ppm – δ = 5.691 ppm were due to the olifinic protons of the side chain and – O-CH_2 -group of the ligand respectively[21-22] .

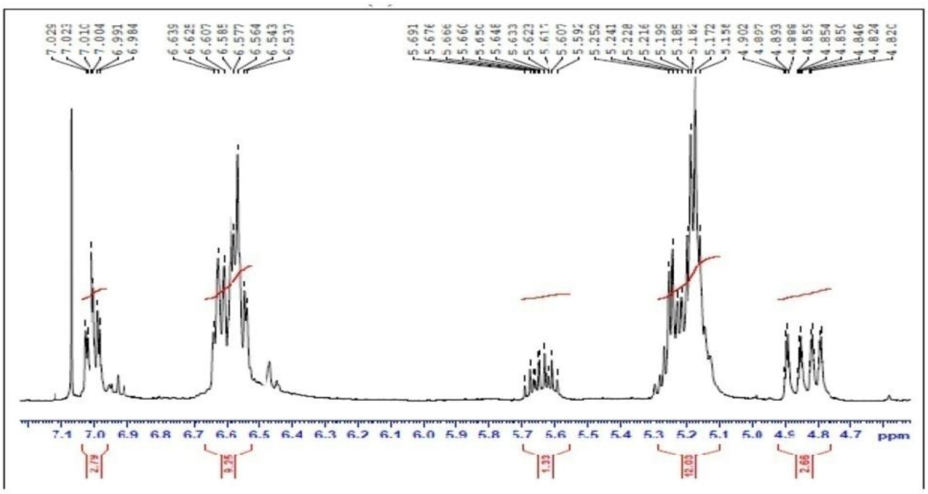

Fig.11: ^{1}HNMR specrum of ligand

The ^{1}HNMR spectrum of the Cu(II) complex (Figure 12), the presence of H – C = N- group is indicated by the singlet at δ = 7.120 ppm it is due to the olifinic protons of the side chain and – O-CH_2 -group. A multiplet at δ =2.163 ppm - δ =2.736 ppm is due to substituted H-C-C=O- group and a singlet at δ = 1.150ppm due to substituted -CH_2-NH group.. Thus ^{1}HNMR study also confirms the binding of metal with Schiff base ligand through pyridine nitrogen and azo methane nitrogen.

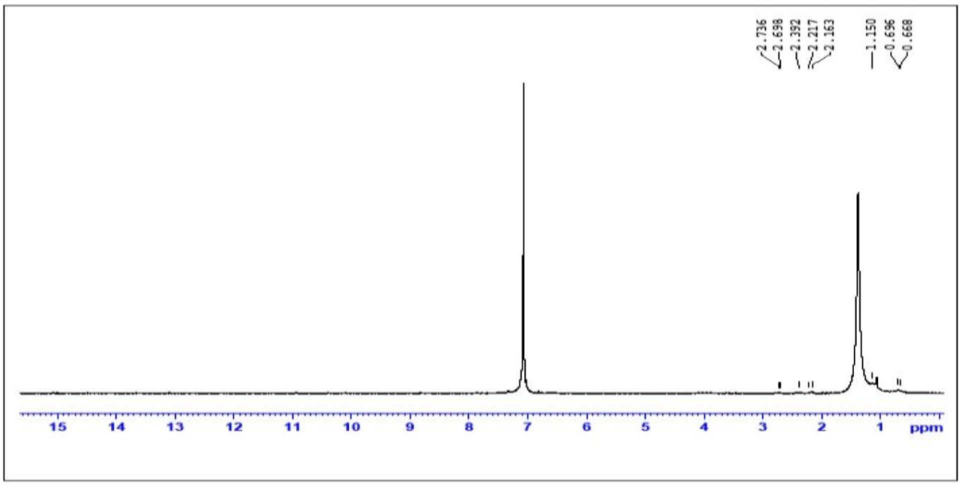

Fig.12: ^{1}HNMR specrum of Cu(II) complex

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The ^{1}HNMR spectrum of the CO(II) complex (Figure 13), the presence of H – C = N- group is indicated by the singlet at δ = 7.250 ppm it is due to the olifinic protons of the side chain and – O-CH_2 -group. A multiplet at δ =2.102 ppm - δ =2.564ppm is due to substituted H-C-C=O- group and a doublet at δ = 1.238ppm - δ =1.261ppm due to substituted CH_2-NH group. Thus ^{1}HNMR study also confirms the binding of metal with Schiff base ligand through pyridine nitrogen and azo methane nitrogen.

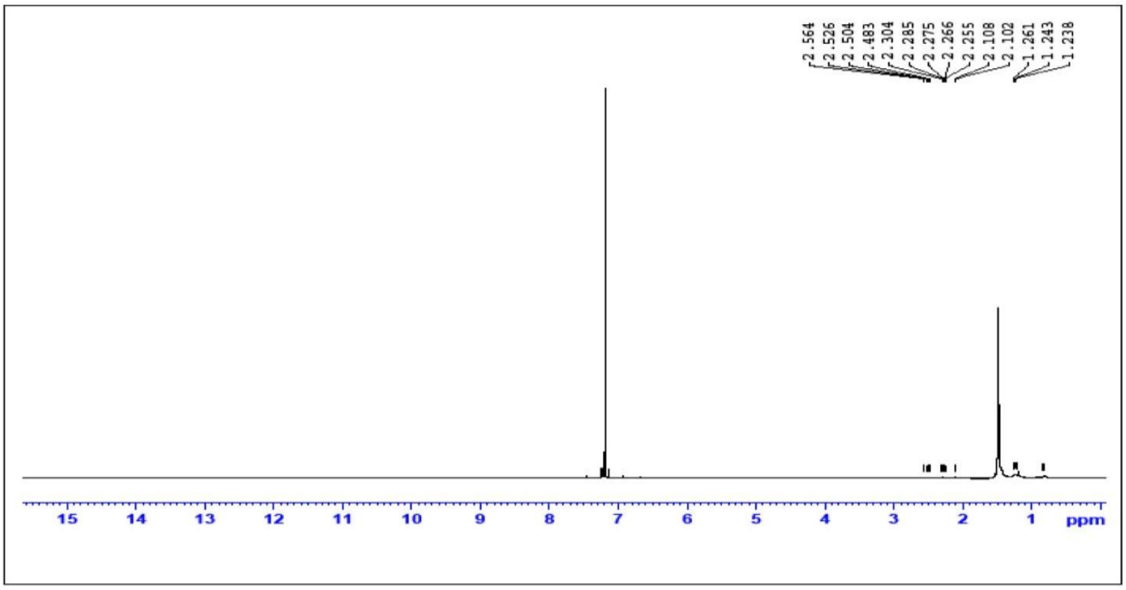


Fig.13: ^{1}HNMR specrum of Co(II)

The ^{1}HNMR spectrum of the Ni(II) complex (Figure 14), the presence of H – C = N- group is indicated by the singlet at δ = 7.220 ppm due to the olifinic protons of the side chain and – O-CH_2 -group. A singlet at δ =2.582 ppm is due to substituted H-C-C=O- group and a multiplet t at δ = 1.145ppm- δ =1.260ppm due to substituted CH_2-NH group. Thus ^{1}HNMR study also confirms the binding of metal with Schiff base ligand through pyridine nitrogen and azo methane nitrogen

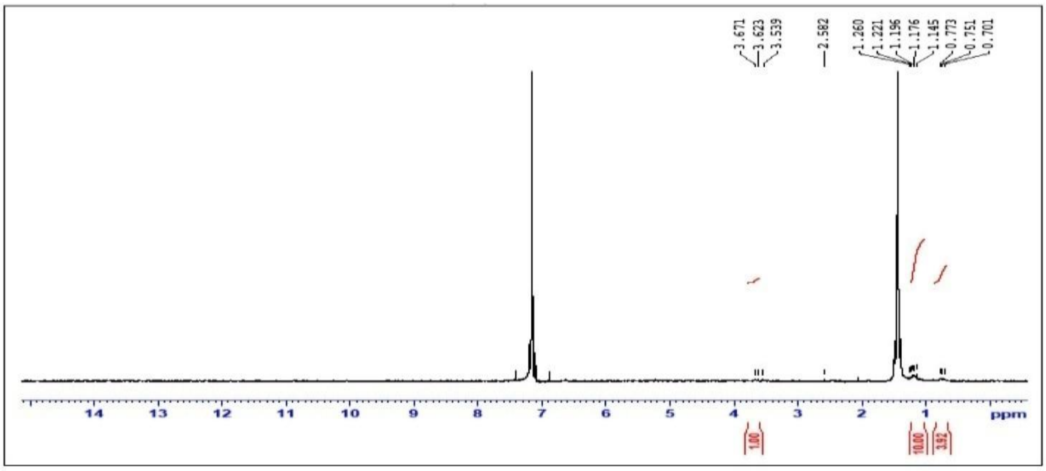
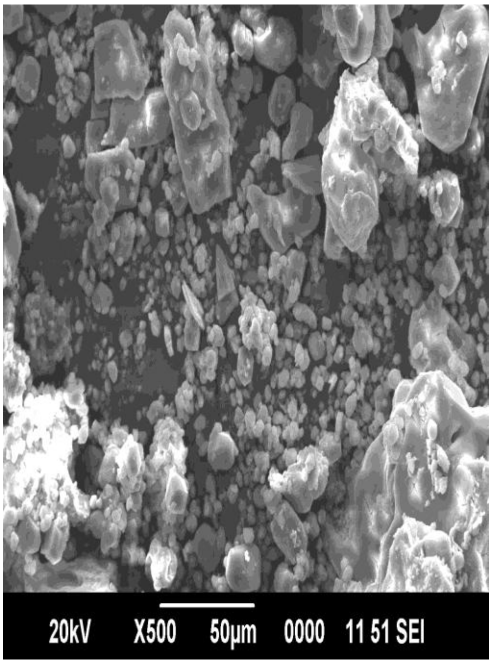
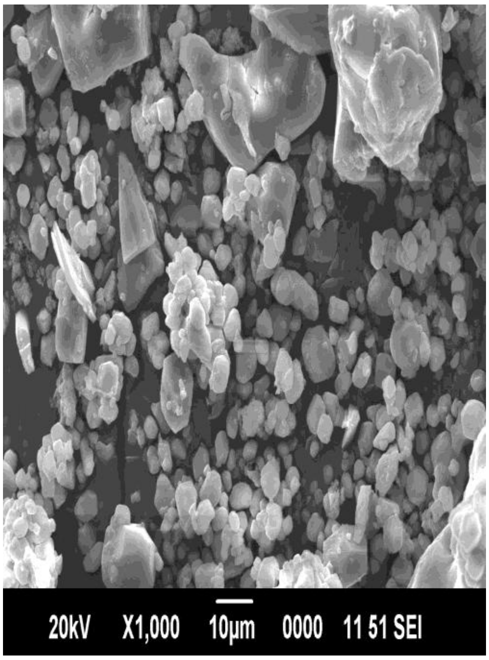


Fig.14: ^{1}HNMR specrum of Ni(II) complex

SEM analysis

The surface morphology of the complexes has been examined using scanning electron microscope. The SEM image of Ni(II) complex is given below. The SEM images showed that the complex is micro crystalline in nature. Careful examination of single crystal, clearly indicate the nanoscale size of the single crystal of the complexes[23]. Lower magnification showed grain like appearance.





X-Ray Diffraction Analysis

The powder XRD for the Cu(II) was performed. The diffractogram is given in Fig.17. It is evident that the strong and broad peaks confirm the complex formation and the appearance of large feeble peaks indicate micro crystalline. The grain size of the complexes was calculated using Scherrer`s formula. The calculated grain size of the complexes is in the range of 0.1341nm. These values suggested that the complexes are in nano crystalline size[24].

Table:3 Grain size of the Cu(II) complexes

Complex	Grain size(nm)
$[CuL(NO_3)_2]\ H_2O$	0.1341

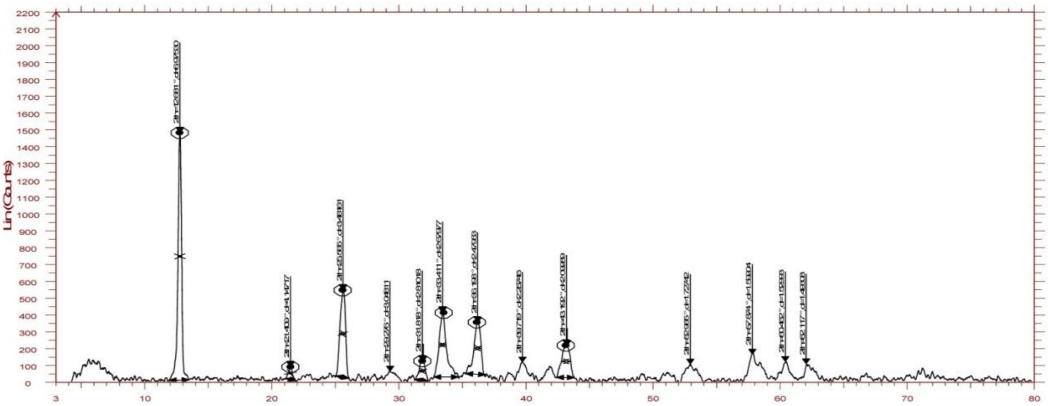


Figure 17 : X-Ray Diffraction Analysis Cu(II) complex

Metal ion intake

The complexation behaviour of cardanol based Schiff base was affected by structural parameters [25]. This study indicates that the metal ion intake decreased [26] (Table.4).This order can be explained by Pearson’s proposal, hard acid preferred to combine with hard base and soft acid prefered to combine with soft base[27]. It was found that the interaction of Ni(II) is normally more intense than other divalent metal ion with Schiff base ligand [28]. Nature of the ligand and the chelate effect were the factors involved in the environmental chemistry and technological interest [29].

Table:4 Metal ion intake

Complexes	**Metal Ion intake meq/g**
$[CuL_1(NO_3)_2]H_2O$	0.4532
$[CoL_1(NO_3)_2\ ]H_2O$	0.6201
$[NiL_1(NO_3)_2]H_2O$	0.6720

Antibacterial activity

Schiff bases are characterized by an imine group –N=CH-, which helps to clarify the mechanism of transamination and racemizationreaction in biological system. 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 various cellular constituents, resulting in interference with normal cellular processes. Heterocyclic compounds play important role in regulating biological activities. This is further evidenced when histidine based Schiff base metal complexes shows good antibacterial activity as they contains heterocyclic group [30] .The antibacterial activity of V.cholerae shows activity in Co(II),Cu(II) and Ni(II) complexes.

Organisms	**Diameter of zone of inhibition (mm)**			
	Name of the sample			
	Identity	**$[CuL(NO_3)_2]$ H_2O**	**$[CoL(NO_3)_2]$ H_2O**	**$[NiL(NO_3)_2]H_2O$**
E.coli	Positive	33	33	33
	F_1	8	-	13
	F_2	11	7	17
V.cholerae	Positive	35	35	33
	F_1	11	7	13
	F_2	13	10	16
S.typhi	Positive	35	36	36
	F_1	8	-	12
	F_2	12	-	16
S.aureus	Positive	39	37	39
	F_1	9	-	11
	F_2	11	9	14

Anti-inflammatory activity

In vitro anti-inflammatory study, $[NiL(NO_3)_2]H_2O$ was performed to evaluate the effect of HRBC membrane stabilization. Diclofenac sodium was used as standard and distilled water was used as control. The percentage inhibition of haemolysis by Diclofenac sodium and $[NiL(NO_3)_2]H_2O$ samples increases with increase in concentration. Diclofenacsodium and $[NiL(NO_3)_2]H_2O$ samples exhibit statistically significant anti-inflammatory activity.

Anti-inflammatory activity of $[NiL(NO_3)_2]H_2O$ by HRBC membrane stabilization method

Sl No.	**Drug**	**Concentration [µg]**	**% Inhibition of haemolysis**
1	Diclofenac	100	96.01
		300	97.09
		500	99.67
2	$[NiL(NO_3)_2]H_2O$	100	83.5
		300	86.29
		500	90.74

Brine shrimp lethality bioassay (larvacidal)

The brine shrimp lethality bioassay was carried out in $[NiL(NO_3)_2]H_2O$. Dried cysts were hatched (1 g cyst per litre) in a hatcher at 28–30°C with strong aeration, under a continuous light regime. Approximately 12 h after hatching the phototropic Artemia nauplii were collected with a pipette from the lighter side and concentrated in a small vial. Ten brine shrimp were transferred to each well using adequate pipette. Each test consisted of exposing groups of 10 Artemia nauplii aged 12 h to various concentrations of the drug extract. The toxicity was determined after 12 h, 24 h and 48 h of exposure. The numbers of survivors were counted and percentages of deaths were calculated. Larvae were considered dead if they did not exhibit any internal or external movement during several seconds of observation.

The percentage of mortality (% M) = percentage of survival in the control - percentage of survival in the treatment. Each concentration was tested in triplicate, giving a total of 15 test-tubes. A control containing 5 ml of DMSO alone was used. The final volume of the solution in each test-tube were made up to 5 ml with sea water immediately after adding shrimp larvae.

Brine Shrimp Cytotoxic assays

Brine shrimp cytotoxic assay of $[NiL(NO_3)_2]H_2O$ was performed to evaluate its cytotoxic activity. Samples exhibited very high cytotoxic activity**.** Sample$[NiL(NO_3)_2]H_2O$ 400 µg and 600 µg showed 100% mortality, 50 µg and 200 µg showed mortality percentage in the range of 70% and 90% respectively.

Brine Shrimp lethality assay of $[NiL(NO_3)_2]H_2O$

Concentration (µg/ml)	**50 µg**	**200 µg**	**400 µg**	**600 µg**	**Control**	**-ve control**
Number of brine shrimp per test sample	10	10	10	10	10	10
Average number of survivors	3	1	0	0	10	10
Average number of deaths	7	9	10	10	0	0
Percentage mortality	70	90	100	100	0	0

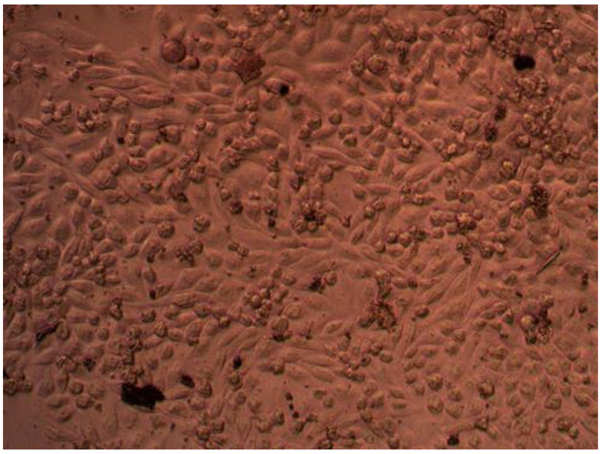
MTT ASSAY

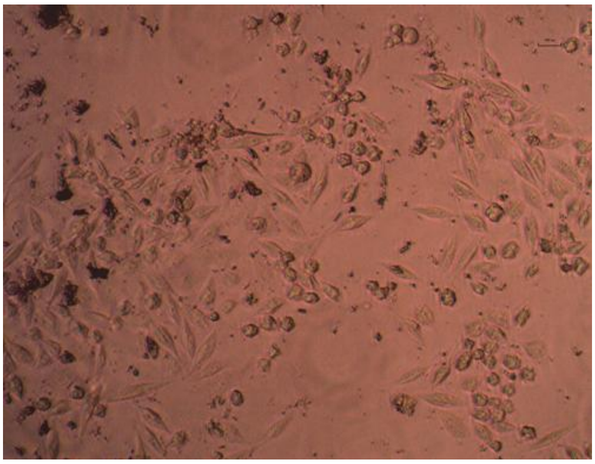
Determination of Invitro Antiproliferative effect of $[NiL(NO_3)_2]H_2O$ extracts on cultured hela cells

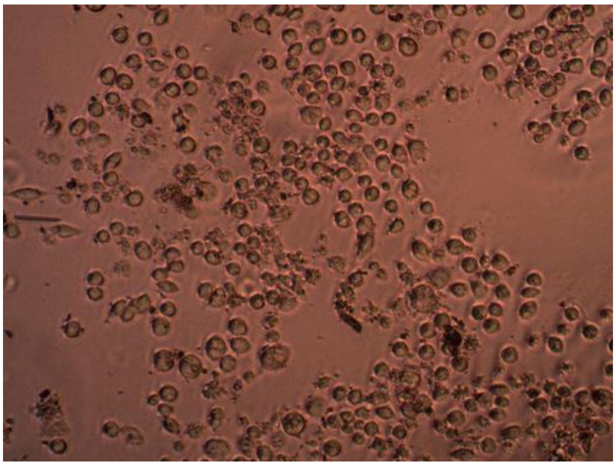
MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilisedformazan product was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The cells was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 37^{o}C for 3 hours. MTT was removed by washing with 1x PBS and 200µlof DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a microplate reader (ELISASCAN, ERBA). % viability = (OD of Test/ OD of Control) X 100[31-32] .

From this the volume of the samples increases the percentage viability of the assay decreases.

Sample volume (µl)		**Average Absorbance @ 540nm**	**Percentage Viability**
CONTROL		0.5149	
$[NiL(NO_3)_2]H_2O$	6	0.4703	91.33812
$[NiL(NO_3)_2]H_2O$	12	0.3163	57.05962
$[NiL(NO_3)_2]H_2O$	24	0.2938	54.80676







IV. CONCLUSION

The Schiff base of Cu(II),Co(II) and Ni(II) were synthesized from cardanol using L-Histidine .The ligands and complexes are insoluble in water and common organic solvents, but are soluble in ethanol, acetone and DMSO. The ligands and their complexes were characterized using spectral and analytical data. From the spectral and stoichiometric analysis, a hexa coordinated nature was assigned for the metal complexes. The nitrate group is present inside the coordination sphere. The XRD and SEM studies reveal that the complexes are nano crystalline. Antimicrobial activity and nuclease activity were studied. The antimicrobial study showed that Ni(II) have more antibacterial activity than other two complexes. Anti cancerous study shows that when of $[NiL(NO_3)_2]H_2O$ decreases with increase in % viability. Larvacidal studies shows $[NiL(NO_3)_2]H_2O$ exhibited very high cytotoxic activity.

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