IN-VITRO ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF TRANSITION METAL COMPLEXES DERIVED FROM 4-((3, 5-DIBROMO-2-HYDROXY BENZYLIDENE) AMINO) –N-(PYRIMIDIN-2- YL) BENZENE SULFONAMIDE

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Abstract

This paper enlightens synthesis, characterization and biological activities of ligand (L4) 4-((3, 5-dibromo-2-hydroxy benzylidene) amino)-N-(pyrimidin-2-yl) benzene sulfonamide derived from 3,5 – dibromo salicylaldehyde and sulfadiazine and its metal complexes [Cd(II), Ni(II), Cu(II), Zn(II), Co(II) and Mn(II)]. The entire compounds were characterized by elemental analysis, spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR, mass spectra, UV-Visibleible, EPR, Powder XRD) and thermal analysis. The bidentate coordination of ligand through phenolic oxygen and imine nitrogen (-CH=N-) was confirmed using spectral analysis data. Number of water molecules in coordination sphere of complexes was predicted with the help of thermograms. EPR spectrum was used to propose the geometry of complexes. Mass spectral fragmentation and molecular ion peaks have been used to confirm the proposed formula mass. Crystalline nature of the compounds was obtained using powder XRD analysis. *In-vitro* antimicrobial activity of compounds L4, L4-Cd, L4-Cu, and L4-Zn was evolved with bacterial and fungal strains. *In-vitro* anticancer activity toward HeLa (human cervical cancer cell) cell line was carried out for compounds L4, L4-Cd, L4-Cu, L4-Zn and L4-Mn. The results indicate moderate activity against HeLa cell line.

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Introduction:

In this paper enlightens synthesis, characterization and biological activities of ligand (L4) 4-((3, 5-dibromo-2-hydroxy benzylidene) amino)-N-(pyrimidin-2-yl) benzene sulfonamide derived from 3,5 – dibromo salicylaldehyde and sulfadiazine and its metal complexes [Cd(II), Ni(II), Cu(II), Zn(II), Co(II) and Mn(II)]. The entire compounds were characterized by elemental analysis, spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR, mass spectra, UV-Visibleible, EPR, Powder XRD) and thermal analysis. The bidentate coordination of ligand through phenolic oxygen and imine nitrogen (-CH=N-) was confirmed using spectral analysis data. Number of water molecules in coordination sphere of complexes was predicted with the help of thermograms. EPR spectrum was used to propose the geometry of complexes. Mass spectral fragmentation and molecular ion peaks have been used to confirm the proposed formula mass. Crystalline nature of the compounds was obtained using powder XRD analysis. *In-vitro* antimicrobial activity of compounds L4, L4-Cd, L4-Cu, and L4-Zn was evolved with bacterial and fungal strains. *In-vitro* anticancer activity toward HeLa (human cervical cancer cell) cell line was carried out for compounds L4, L4-Cd, L4-Cu, L4-Zn and L4-Mn. The results indicate moderate activity against HeLa cell line.

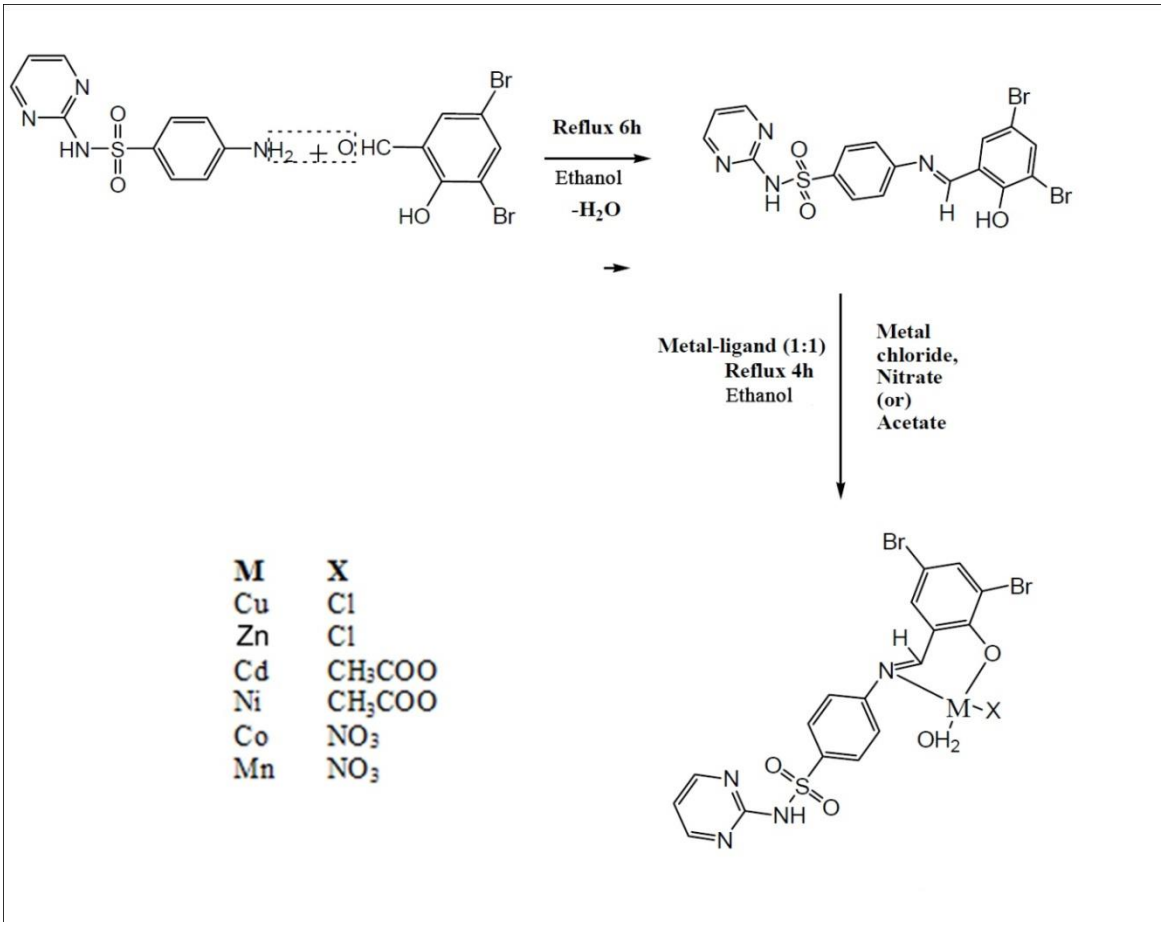
Synthesis:

Synthesis of 4-((3, 5-dibromo-2-hydroxy benzylidene) amino)-N-(pyrimidin-2-yl) benzene sulphonamide (L4)

The ligand L4 was synthesized by refluxing 3, 5-dibromosalicylaldehyde (0.02 mol) with sulfadiazine (0.02 mol) in ethanol (50 mL) in minimum quantity of dimethyl formamide for 6 h. The solvent was removed by evaporation in vacuum. The resultant product was filtered, dried, washed several times with hot methanol and recrystallized. The recrystallized product was dried under vacuum over anhydrous $CaCl_2$.

Synthesis of L4 – metal complexes (L4-Zn, L4-Cd, L4-Ni, L4-Cu, L4-Co and L 4-Mn)

Ethanolic (50 ml) solution of (0.0015 mol) ligand (L4) with little amount of DMF was mixed with metal chloride, nitrate, or acetate (0.0015 mol) in ethanol (50 ml) solution keeping metal-ligand ratio 1:1. The mixture was refluxed for 4 h, and the precipitated solid product on cooling was collected by filtration and washed with hot methanol until the washing becomes colorless. The product was dried in vacuum over $CaCl_2$. All the complexes were kept in desiccators until used. The synthesized metal complexes are colored and stable to air and moisture. The synthetic routes are schematically represented in Scheme 1.



Scheme 1: synthetic route of 4-((3, 5-dibromo-2-hydroxy benzylidene) amino)-N-(pyrimidin-2-yl) benzene sulpfonamide and its metal complexes

CHARACTERISATION TECHNIQUES

The carbon, hydrogen and nitrogen contents of the synthesized ligands and their metal complexes were performed by using Perkin Elmer 240 (USA) analyzer. Molar conductivity is defined as the conductivity of an electrolyte solution divided by the molar concentration of the electrolyte and so measures the efficiency with which a given electrolyte conducts electricity in solution. Its units are siemens per meter per molarity, or siemens meter-squared per mole. The usual symbol is a capital lambda, Λ, or Λ_m. From its definition, the molar conductivity is given by

$$\Lambda_m = \frac{\kappa}{c}$$

Where κ is the measured conductivity, *c* is the electrolyte concentration.

Conductance values of the complexes were obtained on a Systronics Model-304 digital conductivity meter using DMSO as solvent. To find out the electrolytic nature of the complexes, the conductance values were compared with the standard values from literature [3]. IR spectra of ligands and their metal complexes were recorded in the range of 4000 to 400 cm^{-1} on an Avatar 330 FT-IR, spectrophotometer as KBr discs. The electronic absorption spectra of the ligands and their metal complexes were recorded on a Shimadzu UV-1650 PC double beam spectrophotometer in the wavelength range of 200-800 nm, using DMSO as

solvent. The ^{1}H NMR spectra of ligands and their zinc, cadmium and mercury complexes in DMSO-d_6 were recorded on Bruker Magnet System (400 MHz/54 mm) Ultra shield plus using DMSO/$CDCl_3$ as a solvent and TMS as internal standard. Mass spectra of all the compounds were recorded by using JEOL GCMATE II GC-MS with data system is a high resolution, double focusing instrument with 6000 maximum resolution . X-ray diffraction spectra of the ligands and their metal complexes were carried out with powdered compounds using XPERT-PRO diffractometer system at 25° C in Cu anode material [K-Alpha1 (Å) 1.54060, K-Alpha 2 (Å) 1.54443 K-Beta (Å) 1.39225] and the generator settings as 30 mA, 40 kV. Thermo gravimetric (TG) measurements were carried out on a TA instrument, Model: Q 600 SDT TG/DTA thermal analyzer. The heating rate was 20° C per minute with a sample mass of approximately 35 mg in nitrogen atmosphere. Each run was repeated to check the reproducibility and was found to be good. The instrument was standardized for mass and temperature against standard reference materials. The analyses were carried out from room temperature to 1200° C. The X-band EPR spectra of the Cu (II) complexes in DMSO solution at room temperature (300 K) and liquid nitrogen (77 K) conditions were recorded on a Varian E112 spectrometer using tetracyanoethylene (TCNE) as g marker.

Antibacterial activity

Newly synthesized compounds were screened for their *in vitro* antibacterial activity by agar well diffusion method [1] against bacterial strains (*E. faecalis, S. aureus, P. fluorescens, E. coli, Klebsiella.sp, K. pneumonia,*) Ampicillin and streptomycin were used as reference. The bacteria were cultured for 24 h at 37° C in an incubator. Stock solutions of the compounds were prepared by dissolving (2 mg/ml) it in DMSO solvent. The Muller Hinton agar medium was prepared (33.9 g of agar in 1000 ml of distilled water) and autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto a pre-sterilized 100mm petri dish (25-30ml/plate). Nutrient broth was also prepared (13g of commercial nutrient medium/1000 ml of distilled water) and sterilized by autoclaving. Petri dishes containing nutrient Muller Hinton medium were seeded with 24 h culture of bacterial strains using sterile L-rod. Wells were punched using a sterile cork borer and 20 μgmL^{-1} of the compound was added from stock solution. The inoculated plates were incubated for 24 h at 37° C. After incubation, the diameter of the inhibition zone was measured and the results were recorded in millimeters (mm). Each solvent extracts were tested separately in triplicates.

The antimicrobial screening concentrations of the compounds were estimated from minimum inhibitory concentration (MIC) [2]. MIC is the lowest concentration of a compound that will inhibit the growth of microorganisms. The chemical compounds-broth medium at various concentration ranges from 20 μgmL^{-1} to 200 μgmL^{-1} (serial tube dilution) with each bacterium was incubated on a rotary shaker at 37° C for 18 h at 150 rpm [3]. The MIC of each compounds were recorded as the lowest concentration that did not give any visible bacteria growth (i.e. no turbidity).

Antifungal activity

The Schiff bases and their metal complexes were screened for their antifungal activity against fungal strain *Candidaalbicans, Fusarium.* sp, *Trichosporon.* Sp, *A. niger* according to the guidelines in NCCLS approved standard document M27-A2 [4] streptomycin and amphotericin B (AMB), were used as reference antifungal agent. MIC of the synthesized

compounds was determined by serial dilution tube method. The fungicidal effect of the compounds and reference can be assessed by the inhibition of growth of the fungus and was observed as a zone of inhibition near the wells. Potato dextrose agar medium (39 g of potato dextrose agar + 1000 ml of distilled water) were prepared, autoclaved and poured onto the petridishes. Each fungal inoculum was swab spread on the agar medium. Wells of 4 mm diameter were cut into agar plates, $20\mu gmL^{-1}$ of the compounds and the reference were added to each well. The inoculated plates were incubated for 3 days at 30° C. The growth inhibition zone was measured in millimeters. Tests were carried out in triplicates [5].

Anticancer activity

The anticancer activity was performed at Tata Memorial Centre Advanced for Treatment, Research and Education in Cancer (ACTREC), Khar, Navi Mumbai – 410210. (HeLa cell line) by SRB assay. The principle behind SRB assay is under acidic conditions, a bright pink aminoxanthine dye SRB binds dye to basic amino acid residues in TCA (Trichloro acetic acid) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude [6 - 8].

The cell count of trypsinized monolayer cell culture was adjusted to $0.5\text{-}1.0\times10^{-5}$ cells/ml using 10 % new born sheep serum medium. To each well of the 96 well micro titre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100 µl of different test compound concentrations were added to the cells in micro titre plates. Following compounds addition, plates were then incubated at 37° C for 72 h in 5 % CO_2, 95 % air and 100 % relative humidity. Every 24 h microscopic examination was carried out and results were recorded. After 72 h, 25 µl of 50 % trichloroacetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form overall concentration 10 % and incubated for 60 min. at 4° C. To remove traces of medium, sample and serum, the plates were flicked and washed five times with tap water and then air dried. The air-dried plates were strained with 100 µl SRB and kept for 30 min. at room temperature. After straining, the unbound dye was removed by rapidly washing four times with 1% acetic acid and then air dried. To solubilize the dye, 100 µl of 10 mM Tris base was then added and the plates were shaken vigorously for 5 min. The absorbance was measured using microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using following formula,

$$\% \text{ cell inhibition} = 100-\{(A_t-A_b)/(A_r-A_b)\} \times 100$$

Where, A_{te} = Absorbance value of test compound,

A_b = Absorbance value of blank,

eA_r = Absorbance value of reference.

Elemental analysis, molar conductance and UV-Visible spectra

The elemental analysis (C, H, N) results for the L4-M [M = Cd(II), Ni(II), Cu(II), Zn(II), Co(II) and Mn(II)] correspond well with the calculated values showing that the complexes have 1:1 metal ligand ratio. The observed molar conductance of the complexes in DMSO solution (1×10^{-3}M) at room temperature is consistent with the non electrolytic nature of the complexes [9].

The percentage composition of elements (C, H, N) in compounds and molar conductance values of entire compounds are summarized in table 1.

The UV-Visible spectra of entire compounds were recorded in DMSO at room temperature. The spectrum of the ligand (L4) exhibits two bands at 291 and 406 nm attributed to π-π* and n-π* transition within the ligand. In the spectra of complexes, π-π* band remains unaltered, whereas n-π* band is blue shifted between 286 and 376 nm region due to the polarization within the >C=N chromophore which caused by the formation of covalent metal-nitrogen bond [10]. The above observations confirm the charge transfer from ligand to metal charge transition (LMCT) [11].

Table . 1 Elemental analysis, molar conductance and physical constants of ligand and its metal complexes

Ligand/ Complexes	**Empirical formula**	**Colour**	**Mol. Wt**	**Yield %**	**Melting point/ ^{0}C**	**Elements found (calc) %**			**Λm Ohm^{-1} cm^{2}mol^{-1}**
						C	**H**	**N**	
L 4	$C_{17}H_{12}N_4SO_3Br_2$	Orange	512.166	90	232-234	39.88 (39.86)	2.41 (2.35)	10.97 (10.93)	6.42
L 4 - Cd	$C_{19}H_{18}N_4SO_7Br_2Cd$	Orange yellow	718.636	40	>300	32.84 (31.75)	2.67 (2.52)	8.02 (7.79)	5.61
L 4 - Ni	$C_{19}H_{18}N_4SO_7Br_2Ni$	Green	664.916	60	>300	34.64 (34.32)	2.82 (2.72)	8.71 (8.42)	5.31
L 4- Cu	$C_{17}H_{15}N_4SO_5Br_2CuCl$	Blackish brown	646.175	65	192-194	32.0 (31.59)	2.71 (2.33)	9.0 (8.67)	6.03
L 4-Zn	$C_{17}H_{15}N_4SO_5Br_2ZnCl$	Yellow	648.025	65	>300	31.66 (31.50)	2.38 (2.33)	8.71 (8.64)	5.38
L4-Co	$C_{17}H_{15}N_5SO_8Br_2Co$	Dark yellow	668.218	75	262-264	31.0 (30.55)	2.64 (2.26)	10.64 (10.48)	4.89
L4-Mn	$C_{17}H_{15}N_5SO_8Br_2Mn$	Blackish brown	664.118	70	>300	30.86 (30.74)	2.61 (2.27)	10.72 (10.54)	5.07

IR Spectra

Infrared spectroscopy is a simple and reliable technique widely used in both organic and inorganic chemistry. The important IR spectral bands of the ligand L4 and its metal complexes are given in table 3.4.2. A strong band observed in ligand spectrum at 1581 cm^{-1}, is characteristic of azomethine ν(C=N-) linkage [12]. Another peak appeared in the IR spectrum of the ligand at 1499 cm^{-1} can be assigned to (C=N-) group present in the pyrimidine ring [13]. The appearance of this band confirms the presence of two types of groups in the ligand, one due to azomethine linkage and another in the pyrimidine ring. A medium intensity band at 3433cm^{-1} is due to -NH group. The presence of SO_2 moiety in the Schiff base can be confirmed by the appearance of two bands at 1150 cm^{-1} and 1338cm^{-1} assigned to symmetric and asymmetric vibrations [14] respectively. Infrared spectra of the complexes when compared with that of Schiff base provide information concerning the mode of coordination. The band appearing at 1581 cm^{-1} due to azomethine linkage in the ligand is shifted to higher frequency after complexation. The shifting of this band indicates the participation of azomethine nitrogen in coordinating with metal ion [15].The coordination of azomethine nitrogen is further supported by the appearance of the non-ligand band in the region 432-468 cm^{-1} due to ν(M-N) in entire complexes [16]. The bands in the Schiff base due to ν_{sym} (SO_2) and ν_{asym} (SO_2) almost remain unchanged in the complexes indicating that this -SO_2 group is not participating in coordination [17]. All the metal complexes exhibit bands in the range (800-842 cm^{-1}) which indicates the presence of coordinated water molecules [18, 19].

Moreover L4-Cd and L4-Ni complexes showed the coordination of acetate group by the appearance of new bands due to ν_{asym}(COO) and ν_{sym} (COO) at 1522 cm^{-1} and 1431-1444 cm^{-1} respectively [20]. L4-Co and L4-Mn shows two new bands at 1382– 1386 cm^{-1} and 1201-1202 cm^{-1} due to the coordination of nitrato group in the coordination complex [21]. A new band appeared at lower frequency region 553 - 594 cm^{-1} are attributed to (M-O) coordination. A sharp band in ligand spectrum appeared at 1282 cm^{-1} [22] owing to (C-OH) stretching vibration of phenol is shifted to lower frequency in complexes (L4–Cu: 1267 cm^{-1}, L4-Cd: 1199 cm^{-1}, L4-Ni : 1263 cm^{-1}, L4-Co: 1261 cm^{-1}, L4-Mn: 1265 cm^{-1}, L4-Zn: 1269 cm^{-1}) indicates the coordination of phenolic oxygen with metal atoms [23]. The spectra obtained are shown in figure 1.

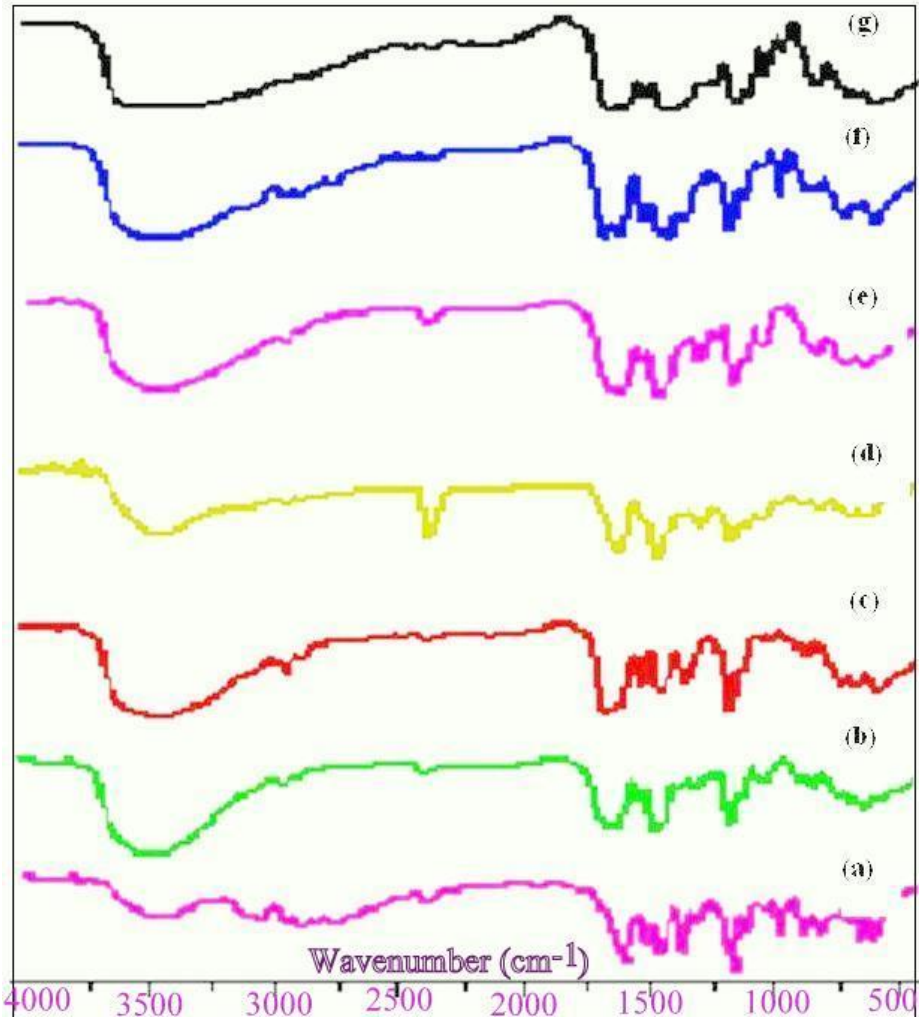


Fig :1 IR spectra of ligand and its metal complexes, (a) L4 - Zn (b) L4 - Mn (c) L4 - Co (d) L4 - Cu (e) L4 - Ni (f) L4 - Cd (g) Ligand

^{1}H and ^{13}C NMR spectra

NMR is a powerful analytical tool to establish the structural information by identifying the exact number of protons as well as carbon. ^{1}H NMR spectra of ligand (L4) and its diamagnetic complexes (L4-Zn and L4-Cd) were recorded in DMSO using tetramethylsilane (TMS) as internal standard, and all the three spectra are given in figures 2 – .4. ^{1}H NMR spectrum of ligand (L4) shows a signal at δ 12.739 ppm with integral value 1 is attributed to phenolic proton [24]. Aromatic protons are appeared as multiple signals between δ 8.080 and 6.987 ppm. The signal appeared at δ 8.445 ppm with an integral value one is assigned to imine proton (–HC=N–). Similarly the signal at δ 6.572 ppm with an integral value one is assigned to –NH protons [25]. The signal appeared for imine proton in free ligand at δ 8.445 ppm is shifted to up field in L4-Cd and L4-Zn complexes indicates the metal nitrogen coordination. A signal, which was observed at δ 12.739 ppm in ligand spectrum has been completely disappeared in L4-Cd and L4-Zn complex spectra, confirms the involvement of phenolic oxygen in coordination. Meanwhile a new signal appeared at δ 1.717 ppm is L4-Cd corresponding to three proton integrals attributed to coordination of acetate group with metal.

Taken together, ^{1}H NMR spectral observations support the bidentate nature of ligand. The observed spectral values are presented in table 2.

In ^{13}C NMR spectra of ligand, signals between δ 173.12 and 112.37 ppm are due to aromatic carbons. The signal due to imine carbon (–HC=N–) at δ164.18 ppm [26] is shifted to up field in the spectra of L4-Cd and L4-Zn complexes at δ161.18 ppm and δ162.11 ppm respectively, indicating the involvement of azomethine in complex formation. The ^{13}C NMR spectrum of complex L4-Zn shows no progressive change in aromatic carbon compared with free ligand. However, in L4-Cd complex, two signals appeared at δ 198.12 ppm and δ21.12 ppm, confirms the involvement of acetato group in coordination. The spectra are shown in figures .5 –7 and the spectral data are presented in table 3.

Table 2: ^{1}H NMR spectral data for ligand and its metal complexes (ppm)

Ligand/ Complex	OH	HC=N	Aromatic proton	NH	Aliphatic CH_3
L4	12.739	8.445	8.080 – 6.987	6.572	-
L4 Cd	-	8.384	8.188 – 6.439	6.585	1.171
L4 Zn	-	8.346	8.186-6.437	6.575	-

Table 3: ^{13}C NMR spectral data for ligand and its metal complexes (ppm)

Ligand/ complex	HC=N	Aromatic carbon	COO	Aliphatic CH_3
L4	164.18	112.37 – 173.12	-	-
L4-Cd	161.18	112.73 – 173.02	198.12	21.12
L4-Zn	162.11	112.73– 172.99	-	-

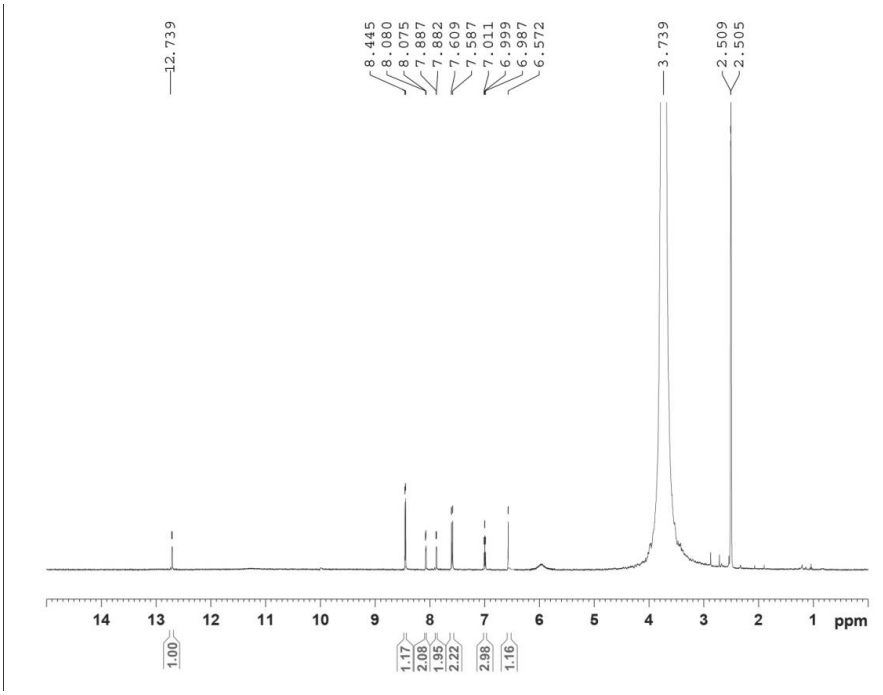


Fig. 2 ^{1}H NMR spectrum of ligand L4

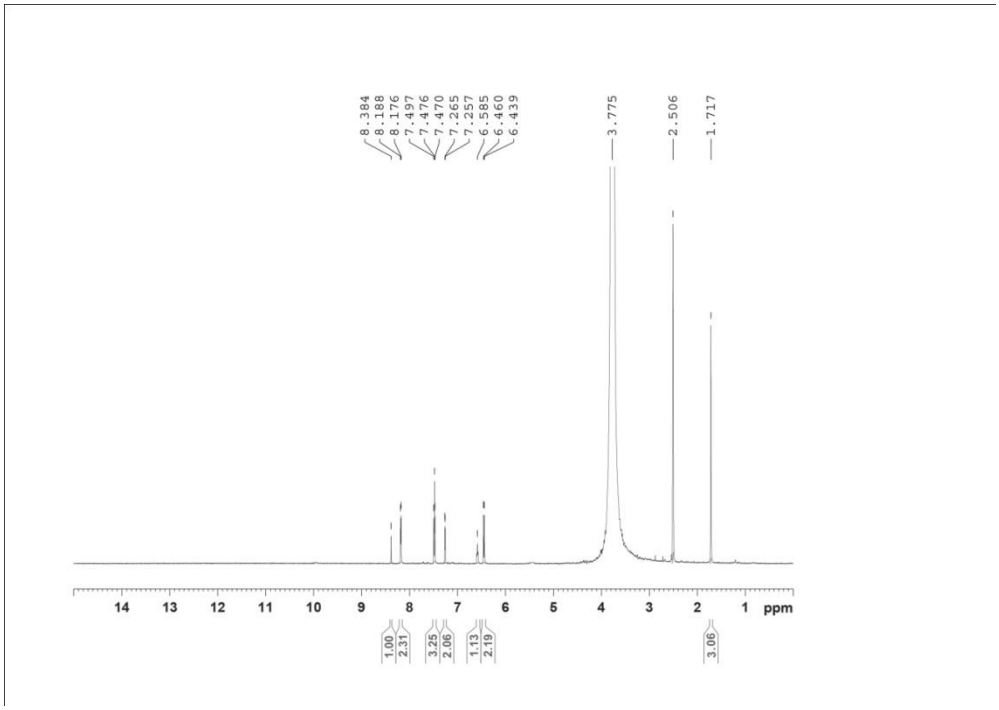


Fig. 3 ^{1}H NMR spectrum of metal complex L4-Cd

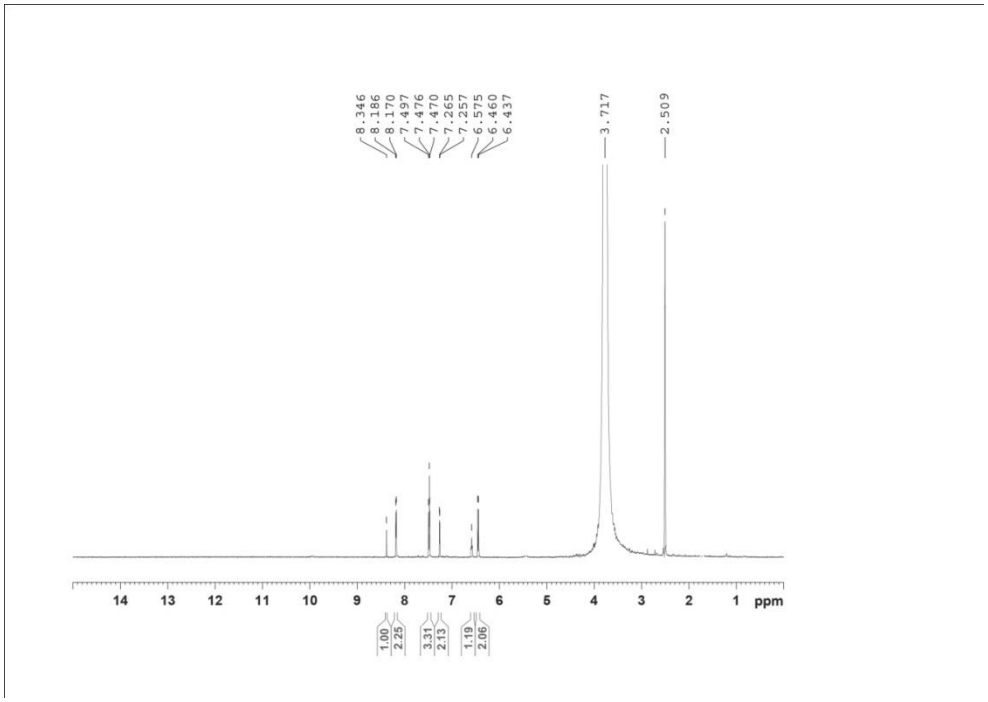


Fig..4 ^{1}H NMR spectrum of metal complex L4-Zn

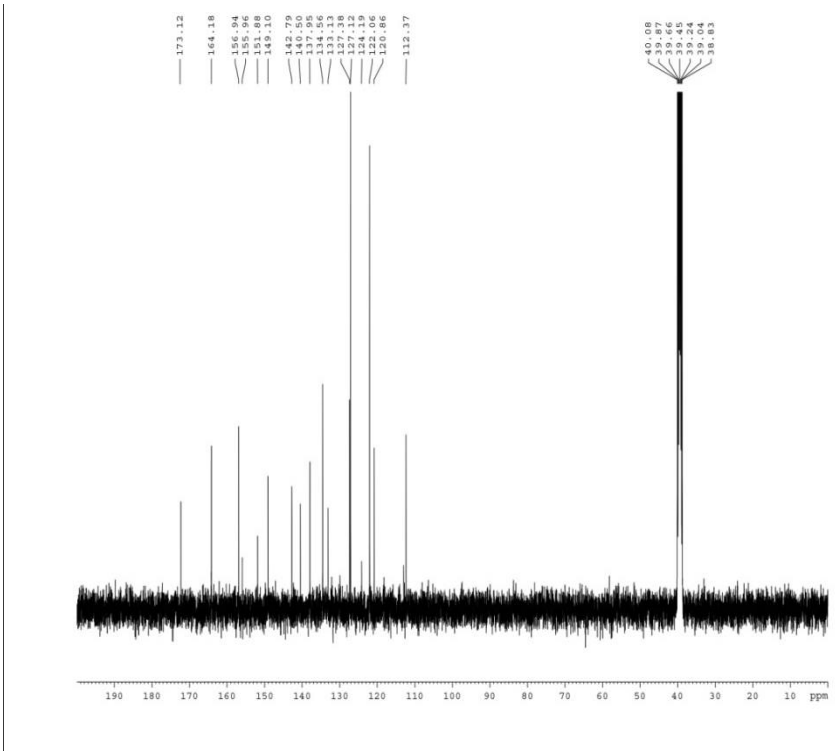


Fig. 5 ^{13}C NMR spectrum of ligand L4

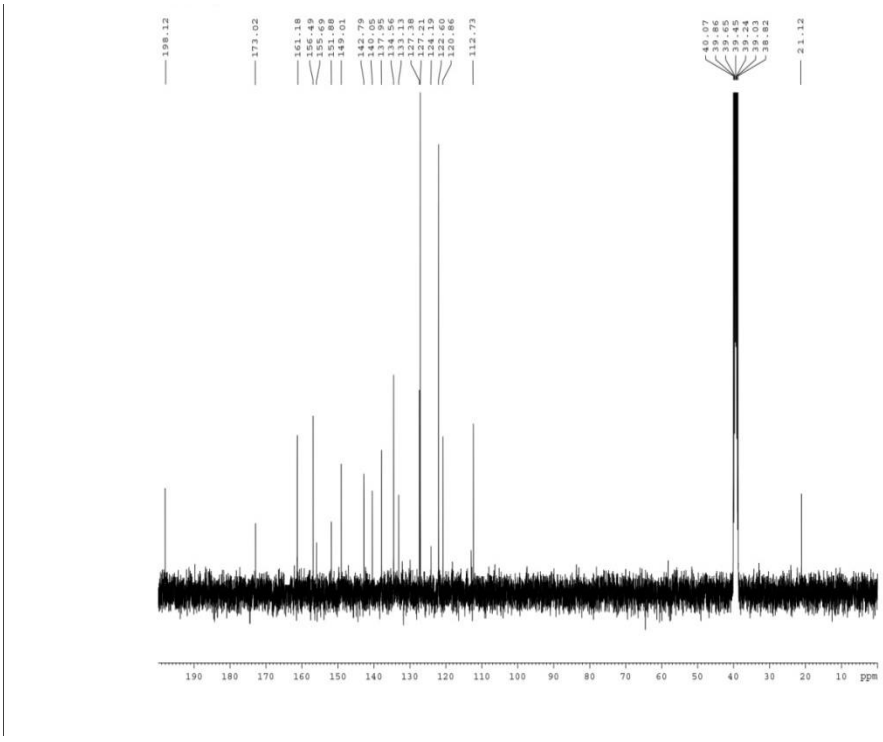


Fig. 6 ^{13}C NMR spectrum of metal complex L4-Cd

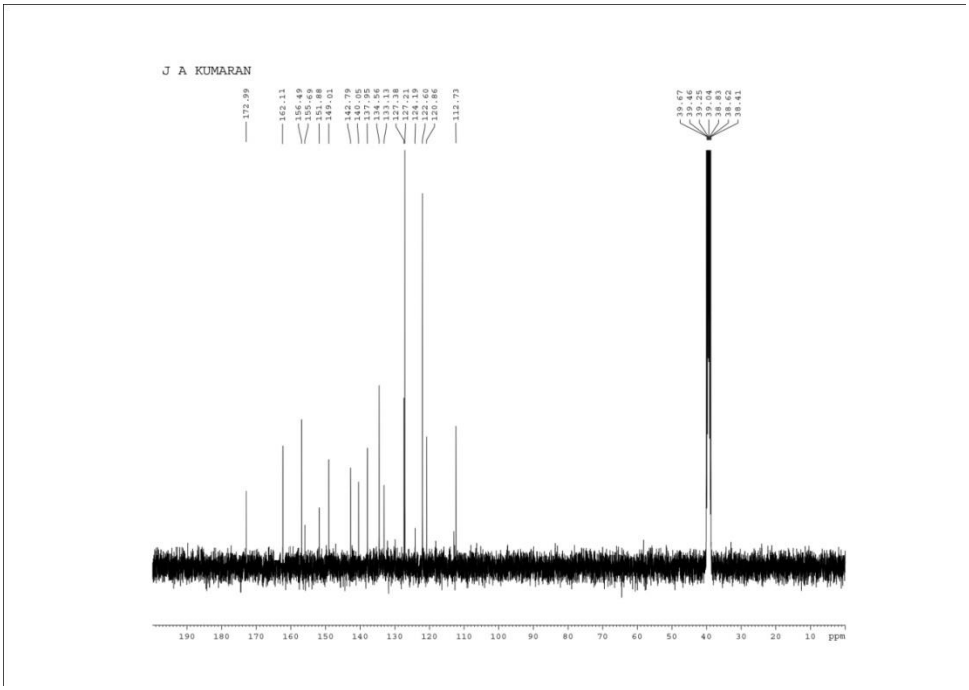


Fig. 7 ^{13}C NMR spectrum of metal complex L4-Zn

Powder X-ray diffraction

Powder X-ray diffraction was performed using XPERT-PRO diffractometer system at 25° C in which the copper X-ray source maintained at 40 kV and 30 mA to provide Cu Kα1(1.54060 Å) , Cu Kα2 (1.54443 Å) and Kβ (A)1.39225 radiation with an intensity weighted average of (Kαave) 1.54252 Å. An area detector or a scintillation detector was used for X-ray detection on the system. Data were collected on the area detector system with a source angle of 3.5° and a detector angle of 17° for 120 seconds. Data were collected on the scintillation counter system with a scan from 10 degrees 2θ to 80 degrees 2θ with a 0.05 step size and scan step time 10 s. Powder X-ray diffractometry of the samples was performed without any further treatment such as hand grinding. In addition, the experiments were performed in specially fabricated sample holders (of a modified polymer) to protect them from the ambient moisture. Among powder X-ray diffraction analysis of all the compounds, ligand L4 and its, Cu, Cd and Mn complexes display defined sharp peaks indicating their crystalline nature, whereas, the broadening of peaks in all other three complexes [Co, Ni and Zn] may reveals their amorphous nature. The respective spectra are given in figures.8 – 14. Amorphous materials do not depict any significant peak in diffraction pattern [27, 28]. Crystalline nature of the complexes was indicated by comparing the XRD pattern of the ligand and complexes. The $h^2 + k^2 + l^2$ values are determined for all the complexes by unit cell calculations. The result reveals that all the complexes are tetragonal geometry due to the presence of forbidden number 7 [29]. The structure of complexes could not be determined due to the unavailability of single crystals. Each complex has specific d values, which can be used in its characterization [30, 31]. The crystallite sizes (t) are calculated using the Scherrer equation [32].

$t = \kappa\lambda/\beta\ (\cos\theta)$

Where κ is Scherrer constant = 0.9, λ is the wavelength of X-ray, θ is the peak position measured in radian, and β is the integral breadth of reflections (in radians 2θ) located at 2θ. The calculated average crystallite size for ligand: 28.75 nm and its metal complexes: 31.64 nm (L4-Co), 34.94 nm (L4-Cd), 29.18 nm (L4-Cu), 31.3 nm (L4-Zn), 29.04 nm (L4-Mn) and 28.76 nm (L4-Ni).

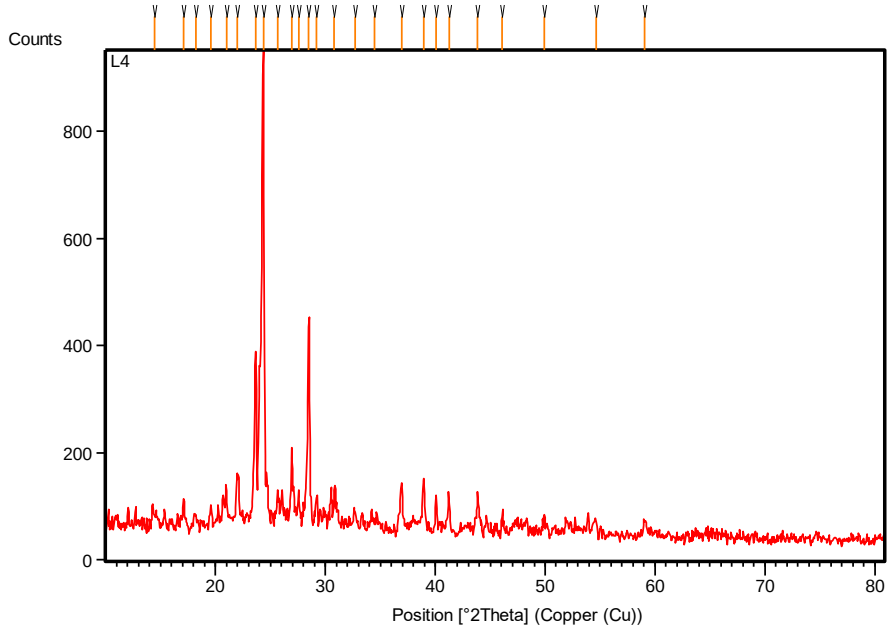


Fig. 8 Powder XRD of ligand (L4)

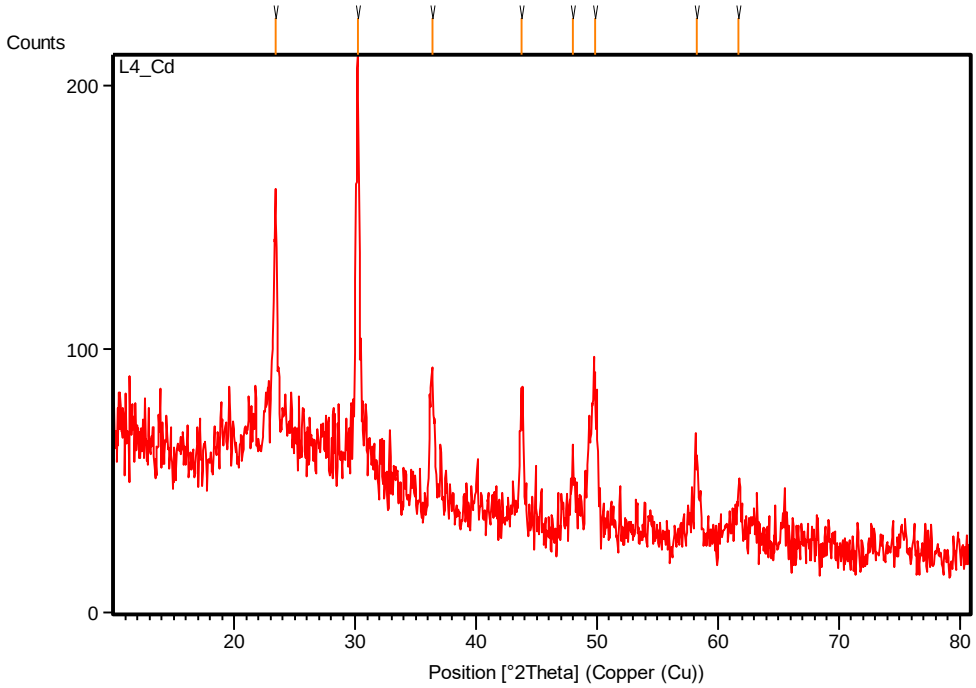


Fig. 9 Powder XRD of metal complex L4-Cd

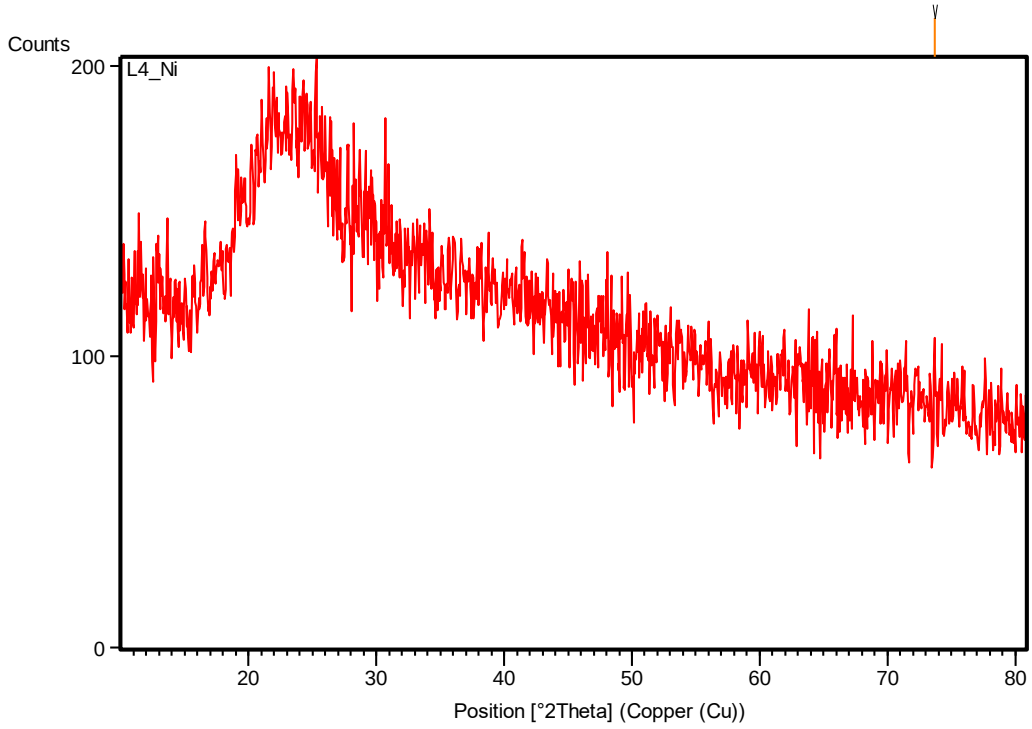


Fig. 10 Powder XRD of metal complex L4-Ni

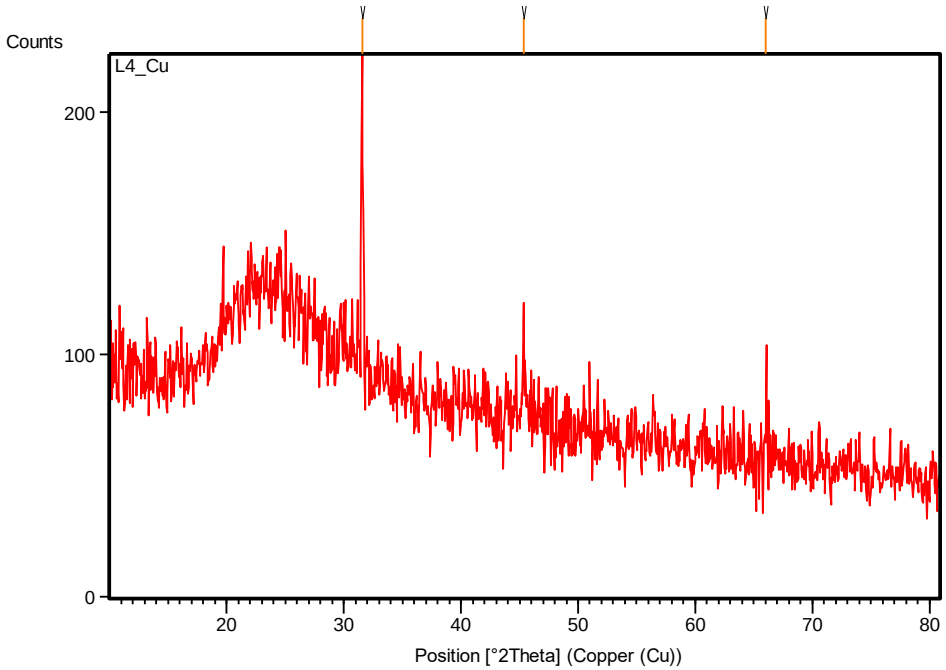


Fig. 11 Powder XRD of metal complex L4-Cu

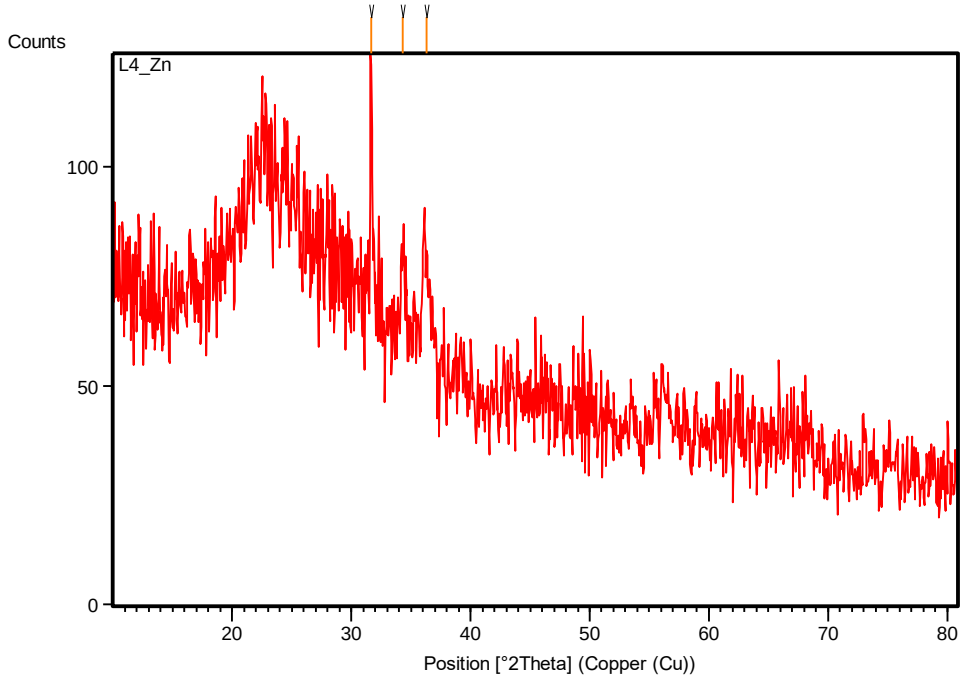


Fig. 12 Powder XRD of metal complex L4-Zn

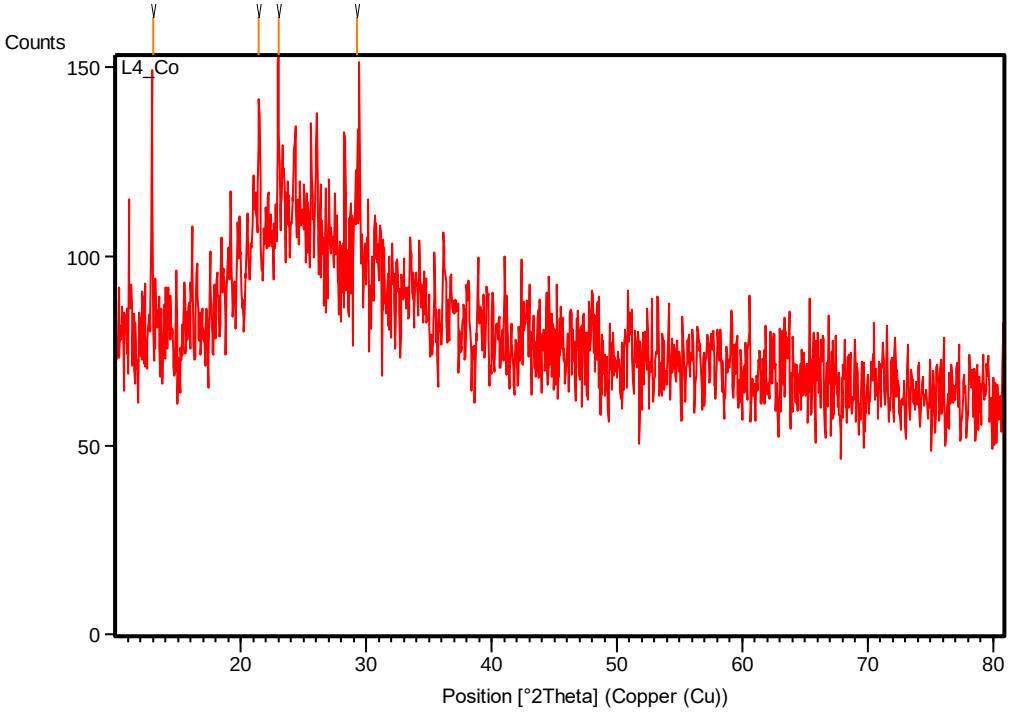


Fig. 13 Powder XRD of metal complex L4-Co

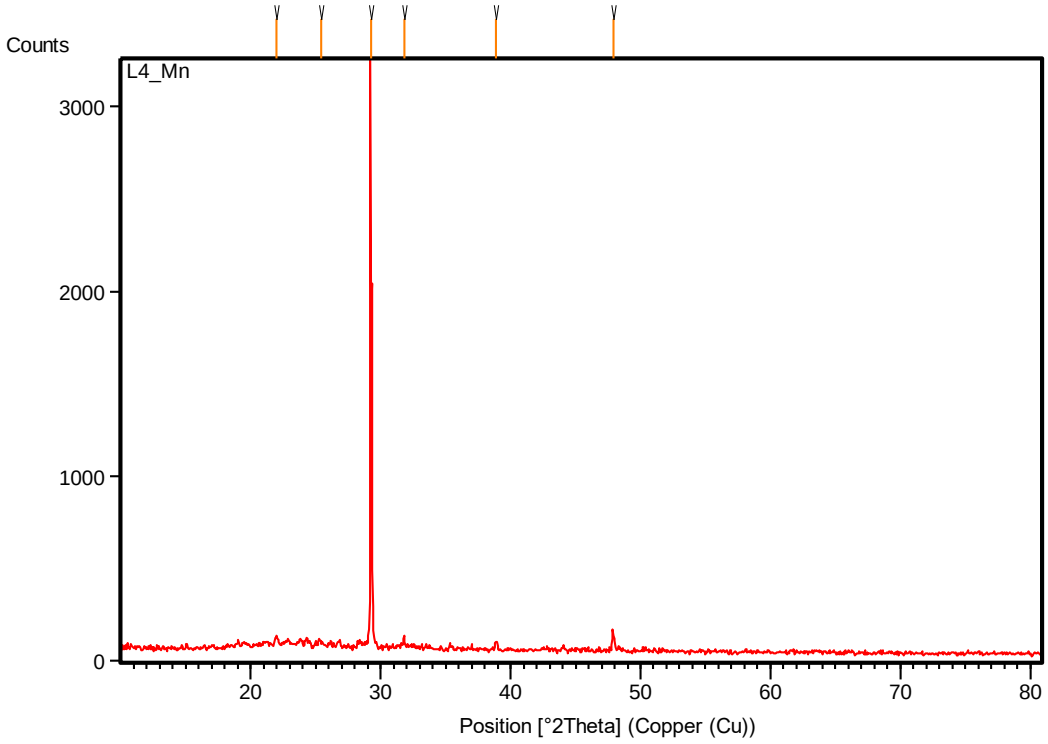


Fig. 14 Powder XRD of metal complex L4-Mn

Thermogravimetric Analysis

Thermogravimetric analysis of transition metal complexes was one of the useful techniques to identify the status of water molecules in complexes as well as to know the stability of the metal complexes. In the present study, heating rates were controlled at 20° C min^{-1} under nitrogen atmosphere and weight loss was measured up to 1000° C. The weight loss percentage at various stages are summarized in table 4 and the thermograms are shown in figure 14 – 19

In Cd complex, mass losses were occurred in three stages. The first stage at 20 – 370° C, corresponds to loss of SO_2, NH, C_4H_3, and one lattice and one coordinated water molecule (found : 27%, cal: 27.2%). The second stage decomposition with mass loss of 55.10% (cal: 54.8%) at 371 – 990 ^{0}C was attributed to the remaining organic molecule. Final decomposition of 18.0 % (cal: 17.9 %) mass loss was due to CdO.

For Ni complex, the first step of decomposition is in the temperature range of 20 – 410° C, with the mass loss of 30 %(calc: 29.20 %) may account for SO_2, NH, and C_4H_3 and two (one lattice and one coordinated) water molecules. The second step occurs within the temperature range 411 – 990° C which corresponds to the removal of organic molecule with a mass loss of 59.5 % (calc: 58.6 %). In the final step metal oxide was leaving as residue (found: 11.4 %, calc: 11.2 %)

The thermogram of Cu shows first step of decomposition in temperature range of 20 to 400° C with mass loss of 30.5 % (calc: 30 %) due to SO_2, NH, C_4H_3 and two water molecules. Lattice as well as co-ordinated water molecules was decomposed within this

temperature range. The remaining organic moiety has been decomposed from the molecule at 401 – 990° C (mass loss, found 58.0 % calc: 57.64 %). Last step was the removal of residue metal oxide with a mass loss of 12.0 % (calc: 12.30 %).

The first decomposition step in Zn complex at temperature range between 20 and 410° C is due to the removal of SO_2, NH, C_4H_3 and two water molecules (one lattice and one co-ordinated water molecule) with mass loss found 30.0 % (calc: 29.96 %). The second step (411-990° C) indicates the decomposition of $C_6H_3Br_2$ (found 57.7 %, calc: 57.47 %). Metal oxide is the stable residue at 1100° C (found 12.25 %; calc: 12.0 %).

In Co complex, the decomposition is characterized by steps at 20 -350° C, 351- 990° C and >990° C corresponds to the removal of lattice and co-ordinated water molecules, SO_2, NH, and C_4H_3 (found 29 %, calc: 29.05 %), remaining organic molecule (found 60 %, calc: 59.71 %) and metal oxide (found 11.0 %, calc: 11.20 %).

Similarly in Mn complex, the first step of decomposition was due to SO_2, NH, C_4H_3 and two water molecules (temp range 20 – 350° C) with mass loss of 30 % (calc : 29.23 %). The subsequent steps (351 – 990° C) correspond to the removal of organic part of the ligand (found 60.0 %, calc: 59.0 %) leaving metal oxide as residue

.Table. 4 Thermal analysis data for metal complexes

Complex	**Steps**			
		First Found (calc)	**Second Found (calc)**	**Third Found (calc)**
L4- Cd	Temp. θ° C	20-370	371-990	1000
	% Loss	27 (27.2)	55.10 (54.8)	18.0 (17.9)
L4 – Ni	Temp. θ° C	20-410	410-990	1000
	% Loss	30.0 (29.20)	59.5 (58.6)	11.4 (11.2)
L4 – Cu	Temp. θ° C	20-400	401-990	1100
	% Loss	30.5 (30)	58 (57.64)	12.0 (12.30)
L4 – Zn	Temp. θ° C	20-400	401-990	1100
	% Loss	30 (29.96)	57.7 (57.47)	12.25 (12.0)
L4 – Co	Temp. θ° C	20-350	351-990	1100
	% Loss	29 (29.05)	60 (59.71)	11 (11.20)
L4 - Mn	Temp. ° C	20-350	351-990	1100
	% Loss	30 (29.23)	60 (59)	11 (11.0)

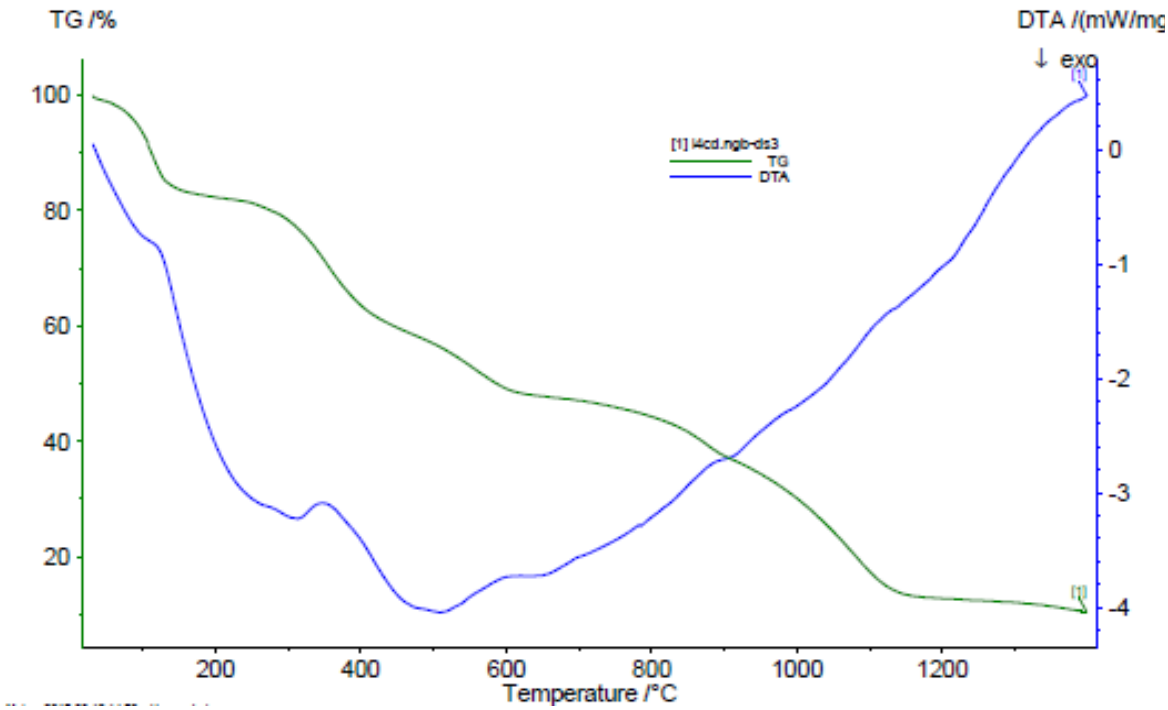


Fig. 15 Thermogram of metal complex L4-Cd

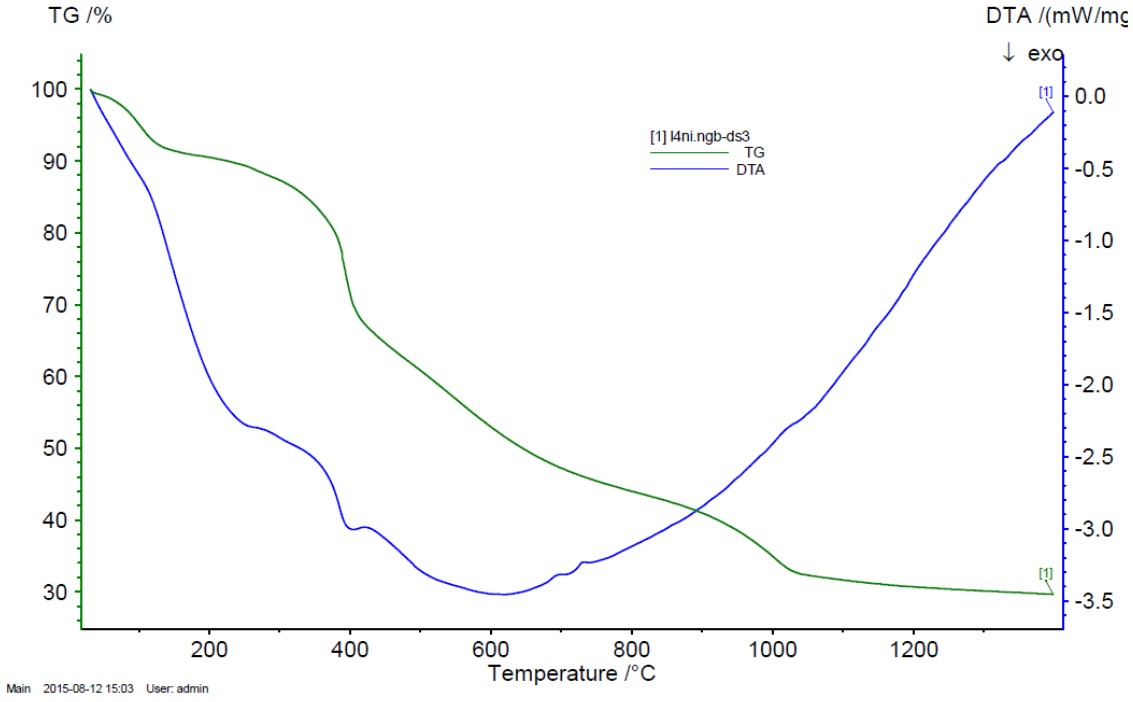


Fig. 16 Thermogram of metal complex L4-Ni

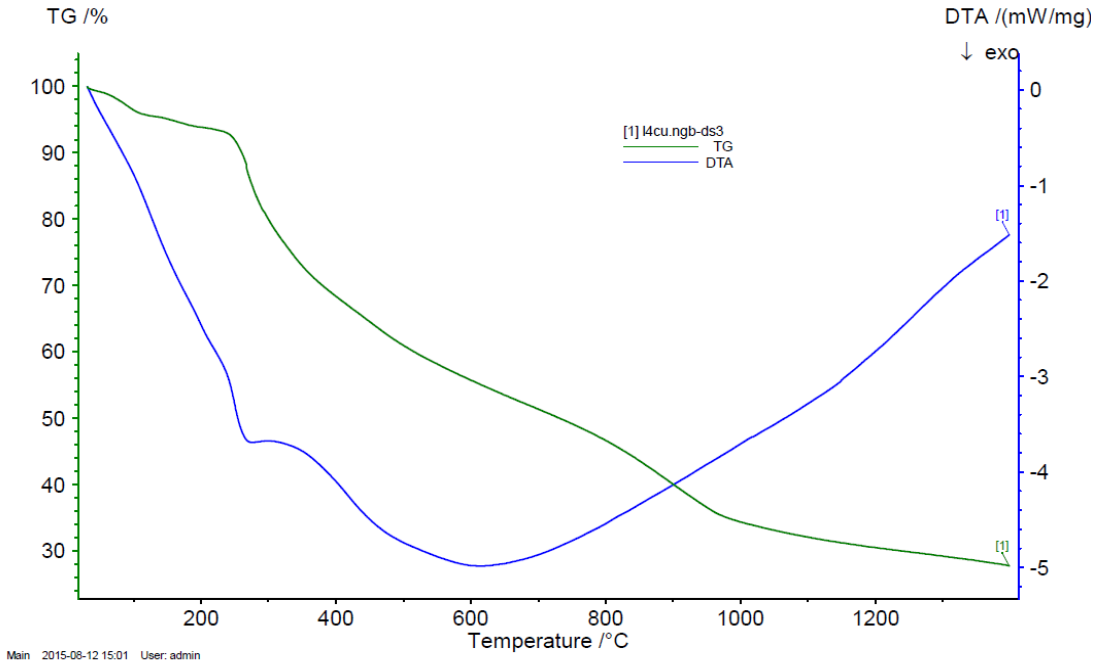


Fig. 17 Thermogram of metal complex L4-Cu

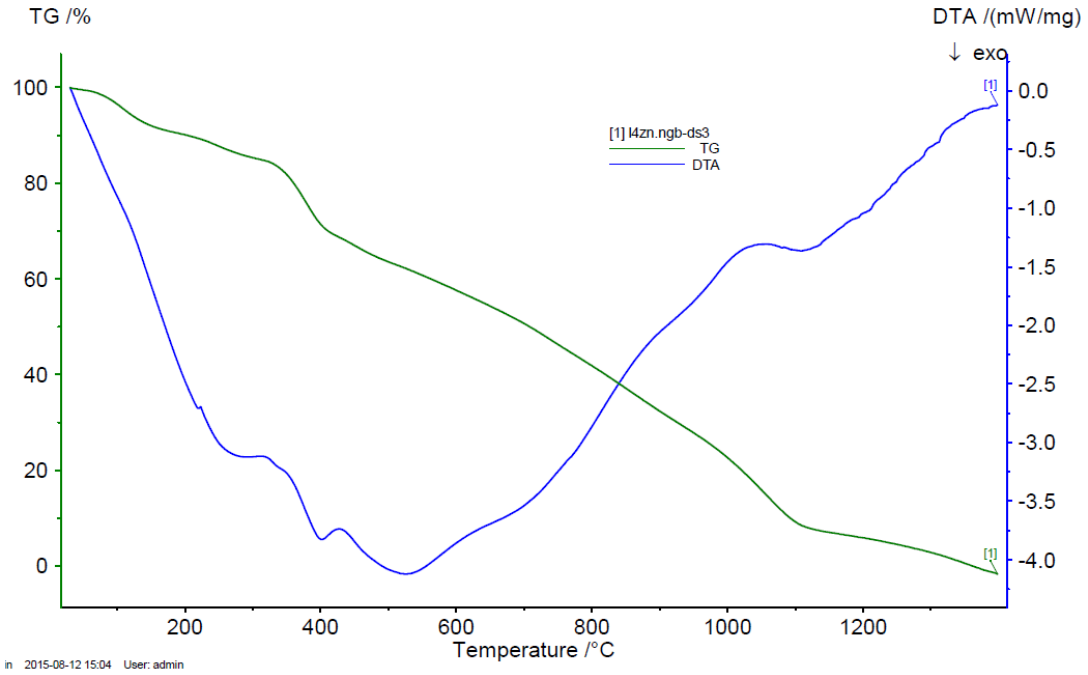


Fig. 18 Thermogram of metal complex L4-Zn

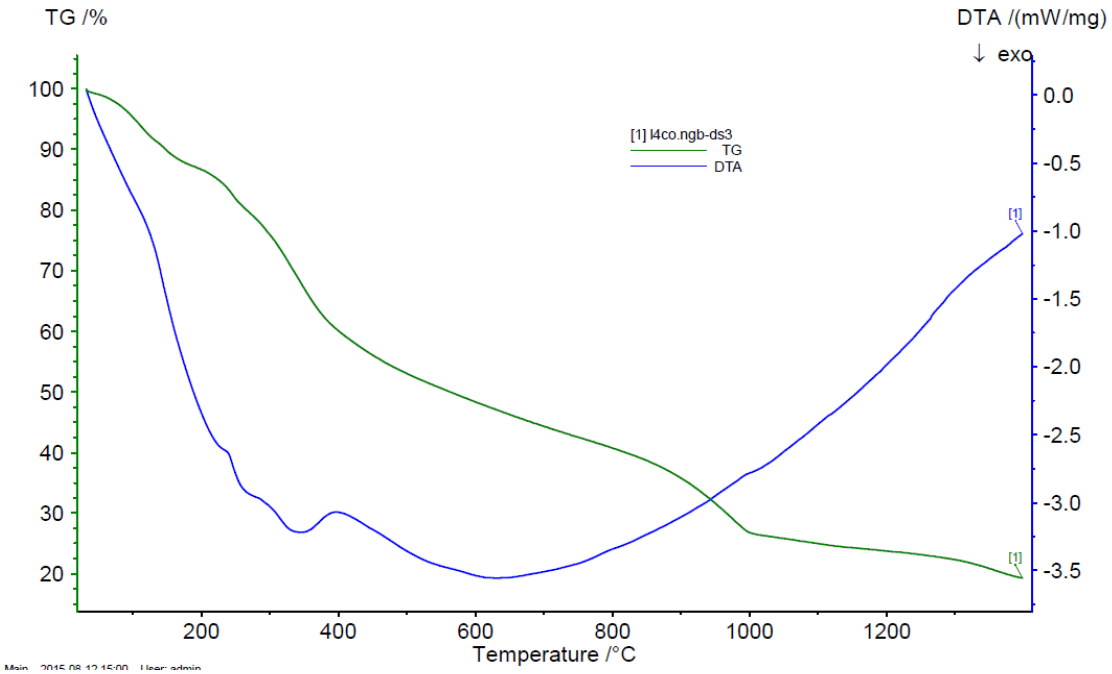


Fig. 19 Thermogram of metal complex L4-Co

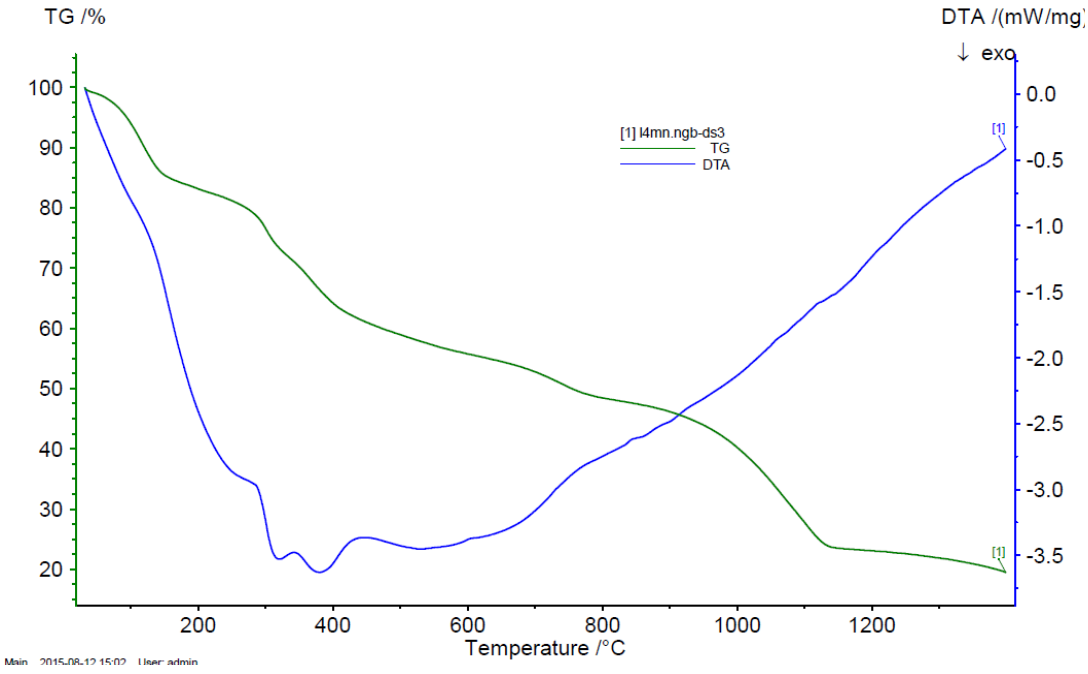


Fig. 20 Thermogram of metal complex L4-Mn

Electron paramagnetic resonance spectra

EPR studies of copper (II) complex were carried out on the X-band at 9.4 GHz under the magnetic field strength 3000 G. The spectrum was recorded in DMSO at room temperature. The spin Hamiltonian parameters for the copper complex are calculated from the spectrum. The explicit formulae for Cu (II) with a single electron in dx^2-y^2 are

$$Ax = Ay = [-\kappa + 2/7 + 11/14(g_{x,y} - g_e)] P \quad \rightarrow 1$$

$$Az = [- \kappa - 4/7 + (g_z - g_e)] P \quad \rightarrow 2$$

With P = energy unit for dipolar interaction ~ 0.035 cm^{-1} and □□= 0.23. The trend $g_{\|}$ > g⊥ > 2.0024 [($A_{\|}$ = 57 x 10^{-4} cm^{-1}> A⊥= 168 x 10^{-4} cm^{-1}; $g_{\|}$ = 2.3670 > g⊥= 2.0828] indicated that the copper the one unpaired electron is localized in dx^2-y^2 orbital of the Cu(II) ion and the spectral figures are characteristic for the axial symmetry tetragonal geometry [33] For the copper complex $g_{\|}$ = 2.36 which is in between 2.3 - 2.4. It shows that the complex having mixed copper-nitrogen and copper-oxygen bonds in its chelate [34,35]. The parameter G, determined as G=($g_{\|}$-2)/(g⊥-2), The Cu(II) complex reported in this paper gave the ‘‘G’’ values which are greater than 4 [i.e 4.43] indicating the exchange interaction is absent in solid complex [36]. The bonding parameters of this complex can be calculated with the help of optical spectra of the complex.[37]. The molecular orbital coefficients *viz.*, in-plane π – bonding (β^2) and in-plane σ – bonding (□^{2}) were calculated using following expressions [38]

$$□^2 = (A_{\|} / 0.036) + (g_{\|} - 2.0023) + 3/7 (g⊥ - 2.0023) + 0.004 \quad \rightarrow 3$$

$$\beta^2 = (g_{\|} - 2.0023)E / -8\lambda□^2 \quad \rightarrow 4$$

The spin-orbit coupling constant, λ value (– 514 cm^{-1}) calculated using the relations, g_{av} = 1/3[$g\|$ + 2g⊥] and g_{av} = 2(1–2λ/ 10Dq), is less than the free Cu(II) ion (–792 cm^{-1}) which also supports covalent character [39]. If the value of □^{2} = 0.5, it indicates complete covalent bonding, while the value of □^{2} = 1.0 suggests complete ionic bonding. The observed value of □^{2} (0.70) of the complex is less than unity, which indicates that the complex has some covalent character in the ligand environment. [40]. The spectra of Cu(II) complex is shown in figure . 21.

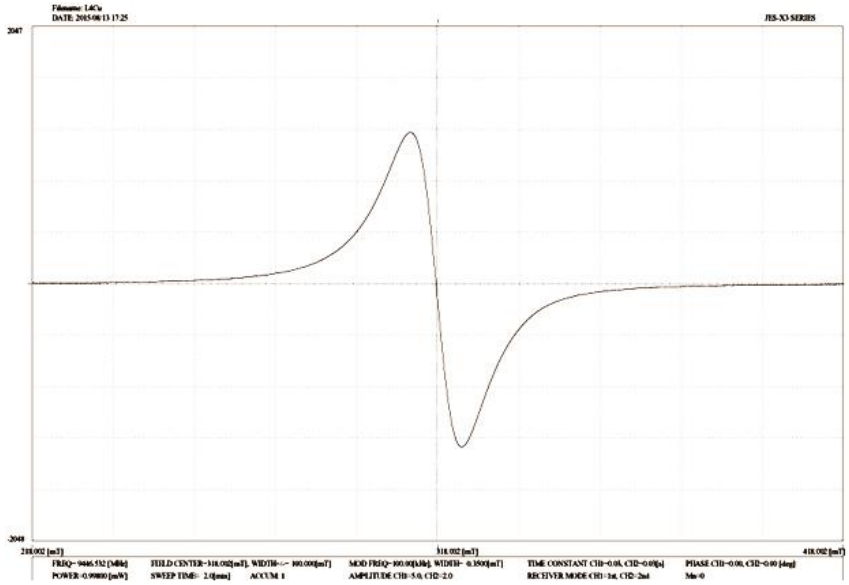


Fig. 21 EPR spectrum of L – Cu complex

Mass Spectra

The mass spectra of the ligand and its transition metal complexes were recorded at ambient temperature. The observed molecular ion peaks in the mass spectra of the ligand and its metal complexes have been used to confirm the proposed formula mass (Scheme 3.4). Electron impact mass spectral data are presented in table 5.

Mass spectrum of the ligand shows peak at *m/z* 512.660 is corresponding to molecular ion M. The ligand has fragmented up to 57 mass number. The mass spectra of complexes L4-Cd, L4-Ni, L4-Cu, L4-Zn, L4-Co and L4-Mn show a prominent peak at 718.635, 664.909, 648.025, 646.175, 668.942 and 664.117 respectively, which are consistent with the molecular mass of the respective metal complexes. The spectral data prove that the ratio between metal and ligand is 1:1 as described in scheme 3.4.

Table 5 Mass spectral data of ligand and its metal complexes

Ligand/complex	**Calculated (*m/z*)**	**Found (*m/z*)**	**Peak assignment**
L4	512.166	512.660	[M]
L4-Cd	718.636	718.635	[M]
L4-Ni	664.916	664.909	[M]
L4-Cu	646.175	646.175	[M]
L4-Zn	648.025	648.025	[M]
L4-Co	668.218	668.942	[M]
L4-Mn	664.118	664.117	[M]

Antibacterial activity

The free lignad and its L4-Cu, L4-Ni and L4-Cd complexes were examined for their *in vitro* antibacterial activity against five human pathogenic bacteria (*E. coli, S. aureus, E. faecalis, P. aurogenes and K. pneumonia*) by well diffusion method using streptomycin as reference. Bacteria can achieve resistance to antibiotics through biochemical and morphological modification [41]. The reference drug streptomycin is also tested for its antibacterial activity at the same concentration under the conditions similar to that of the test compounds concentration. By measuring the size of inhibition diameter, the susceptibility of bacterial strain towards the compounds is judged. The inhibition zone of compounds against the bacterial strains are given in table 6. The minimum inhibition concentration (MIC) values of the compounds and reference against the respective bacterial strains were ranging between 20-150 μg /ml and 6-12.5 μg /ml, respectively. The obtained results suggest that the metal complexes are more active than ligand and less active than the reference against all the bacteria tested.

Among the compounds tested, good activity was observed in L4-Cu and L4 – Cd complexes towards all the five bacterial strains. However, L4-Ni, (MIC: 20-60μg/ml) show moderate activity against bacterial strains. The tested complexes L4-Cu, L4-Cd, L4-Ni, possess high inhibitory power over the bacterial strain. *E. faecalis*, herein the zones of inhibitions are 29, 25 and 20 mm respectively. Moreover L4 -Ni possesses good inhibition activity against *K. Pneumonia*. More activity exhibited in metal complexes than the ligand is due to its chelation, which reduces the polarity of metal ion in complexes and thus favoring permeation of complexes through lipoid layer of microorganisms [42]. The variations of activity of the compounds are shown in figure 22.

Table 6 Antimicrobial activity of ligand and its metal complexes

Zone of inhibition (mm)					
Strains	**L4**	**L4 -Ni**	**L4 - Cu**	**L4 – Cd**	**Reference**
E.coli	15	15	15	16	30
S.aureus	-	11	16	16	30
E.feacalis	20	20	25	25	30
P.aurogenes	-	9	18	20	25
K.pneumonia	8	20	15	19	28
C.albicans	16	16	16	16	25
A.niger	17	17	19	20	30

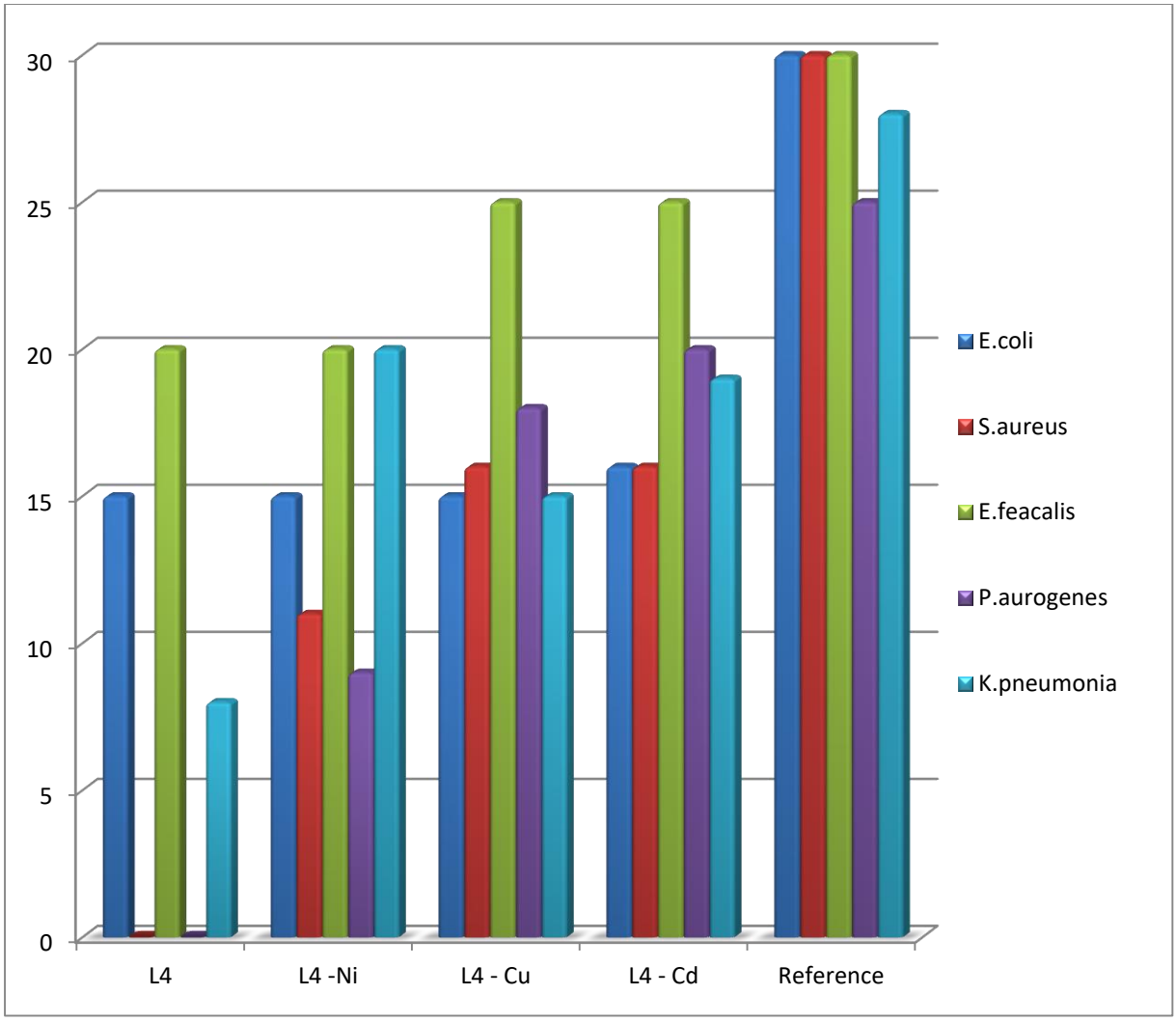


Fig. 22. Antibacterial activity of ligand and its metal complexes

3.4.10 Antifungal activity

The same compounds, used for antibacterial analysis were also used to test their antifungal activity against *candida albicans,* and *A. niger* by well diffusion method. The growth inhibition zone of compounds against microorganisms is summarized in table 6. The MIC values of the compounds and reference *amphotericin B* (AMB) against the respective fungal strains were ranging between 20-100µg / ml 0.03-16µg / ml, respectively. The experimental results were compared with the reference at the same concentration of the test compounds. According to the zone of inhibition, all the three complexes show good inhibition activity towards the fungal strain *candida albicans,* and *A. niger* were noticed. Among the three complexes tested, in L-Cd, higher zone of inhibition against *A. niger*. In general, moderate activity was observed in all the four compounds (figure 23).

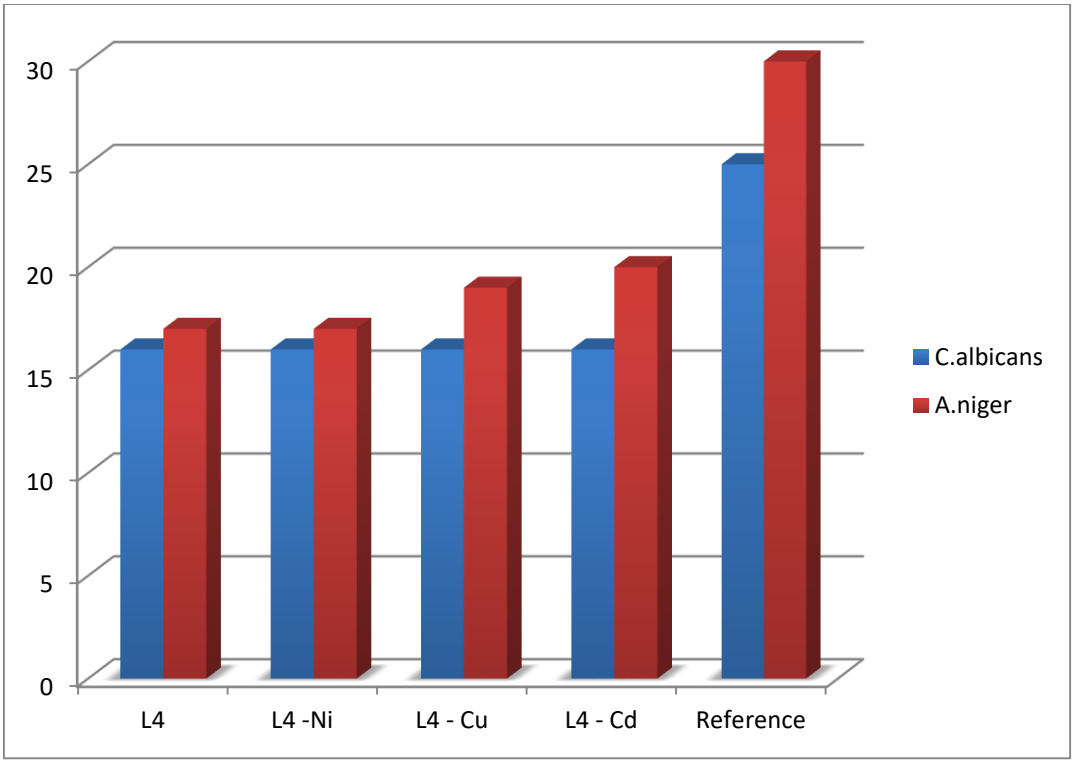


Fig. 23 Antifungal activity of ligand and its metal complexces

Anticancer activity

In order to assess the *in-vitro* anticancer effects of newly synthesized ligand (L4) and its metal complexes (L4–Cd, L4–Cu, L4–Mn and L4–Zn), malignant cell line (human cervical HeLa cell) growth using sulforhodamine B protein (SRB) assay protocols [43] was followed. Since the solubility of the tested compounds in aqueous medium was poor, they were dissolved in DMSO. By using SRB protocols, each drug was tested at four dose levels (10, 20 40, 80µg/ml). Appropriate positive control (reference) Adriamycin (ADR) was used in each experiment and each experiment was repeated thrice. The results, in terms of GI_{50} TGI and LC_{50} are summarised in table 7 and 8.

Using ADR as reference molecule, the obtained data revealed that the 50% inhibition concentration values (GI_{50}) are moderate to good activity ranging from 29 to 56.1 µg/ml. In general all compounds are moderately inhibited HeLa with GI_{50} value range of 29 to 56.1µg/ml. The inhibitory effects of the compounds are in order L –Mn> L-Zn > L-Cu > L-Cd. Corresponding images and growth curves are shown in figures 24 and 25 respectively. However, concentration of drug that produces total inhibition of cells (TGI) shows 55 µg/ml for compound L4-Mn exhibited moderate potent with four different dose levels, maximum inhibitory activity was found at 80 µg/ml. Percentage control growth results shows L4-Mn are line with reference ADR at 80 µg/ml.

Table 7 Parameters calculated from cell line

Compounds	Human Cervical Cancer Cell Line HeLa															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
L4	107.9	106.1	41.6	0.2	123.2	103.9	43.0	5.6	108.4	101.7	40.5	15.5	113.2	103.9	41.7	7.1
L4-Cd	114.5	108.2	75.6	5.0	127.1	116.1	86.9	8.8	101.6	106.1	81.1	15.3	114.4	110.2	81.2	9.7
L4-Mn	96.5	62.7	22.4	-51.2	98.1	72.3	32.2	-45.9	80.7	57.0	21.0	-41.3	91.8	64.0	25.2	-46.2
L4-Zn	98.2	88.9	34.3	11.4	102.5	89.5	40.9	14.2	88.0	75.5	33.8	5.3	96.2	84.7	36.4	10.3
L4-Cu	107.8	106.1	53.7	24.0	109.8	110.5	54.6	22.2	110.6	104.0	45.1	21.1	109.4	106.9	51.2	22.4
ADR	-47.9	-61.0	-68.7	-63.1	-49.6	-70.0	-72.5	-63.4	-66.2	-69.8	-75.0	-69.1	-54.6	-66.9	-72.1	-65.2

Table 8 Parameters calculated from cell line

	Drug Concentrations (µg/ml) calculated from graph		
HeLa	**LC_{50}**	**TGI**	**GI_{50}***
L4	>80	79.7	48.0
L4-Cd	>80	>80	56.1
L4-Mn	>80	55.0	29.0
L4-Zn	>80	>80	43.0
L4-Cu	>80	>80	54.5
ADR	NE	<10	<10

LC_{50} – Concentration of drug causing 50% cell kill

GI_{50} - Concentration of drug causing 50% inhibition of cell growth

TGI - Concentration of drug causing total inhibition of cell growth

FGI_{50} - Values of < 10µmg is considered to demonstrate potent activity in of pure case compounds

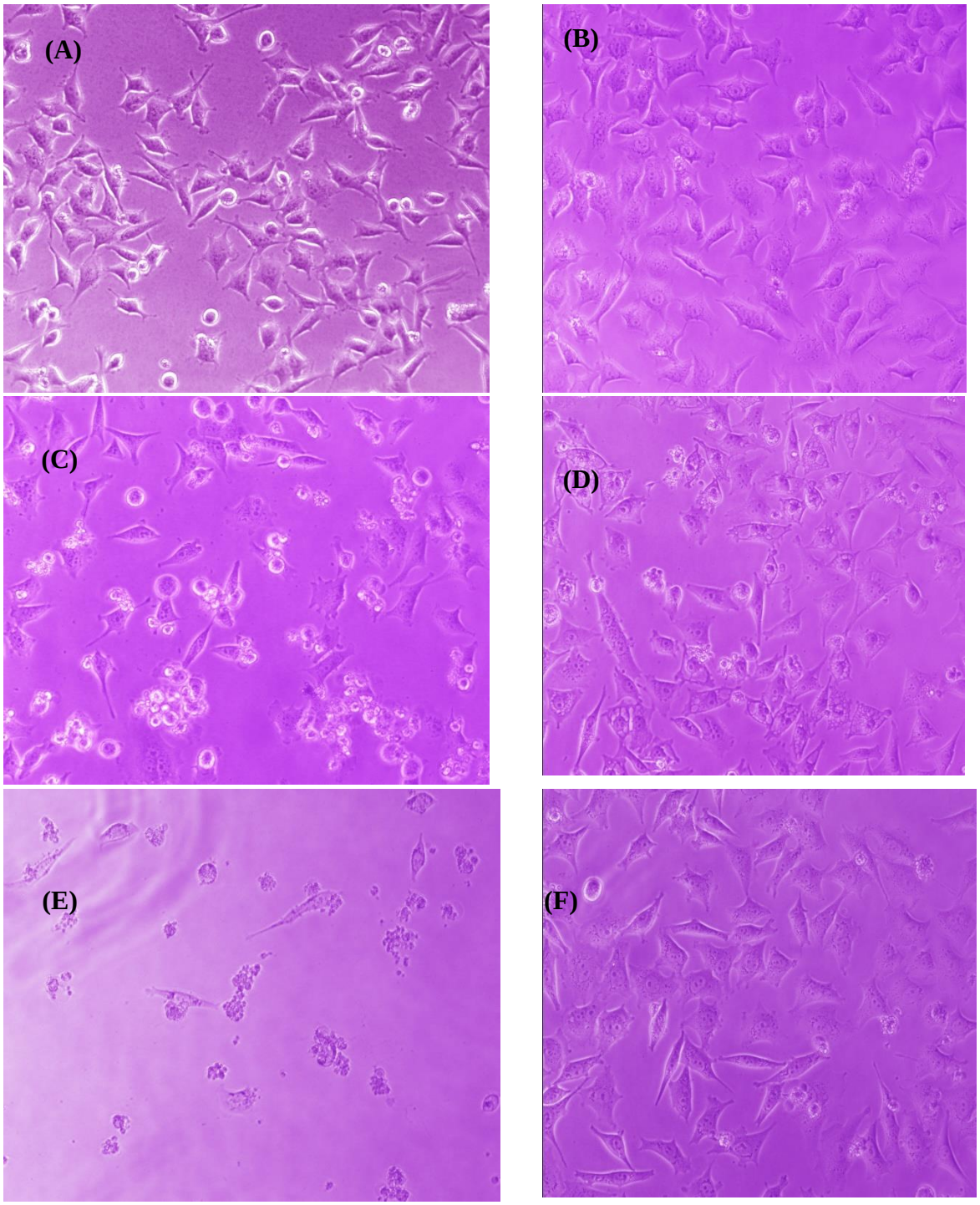


Fig. 24 Anticancer activity: Images of ligand and its metal complexes (a) Reference (b) Ligand (c) L4 – Cd (d) L4 – Cu (e) L4–Mn (f) L4 - Zn

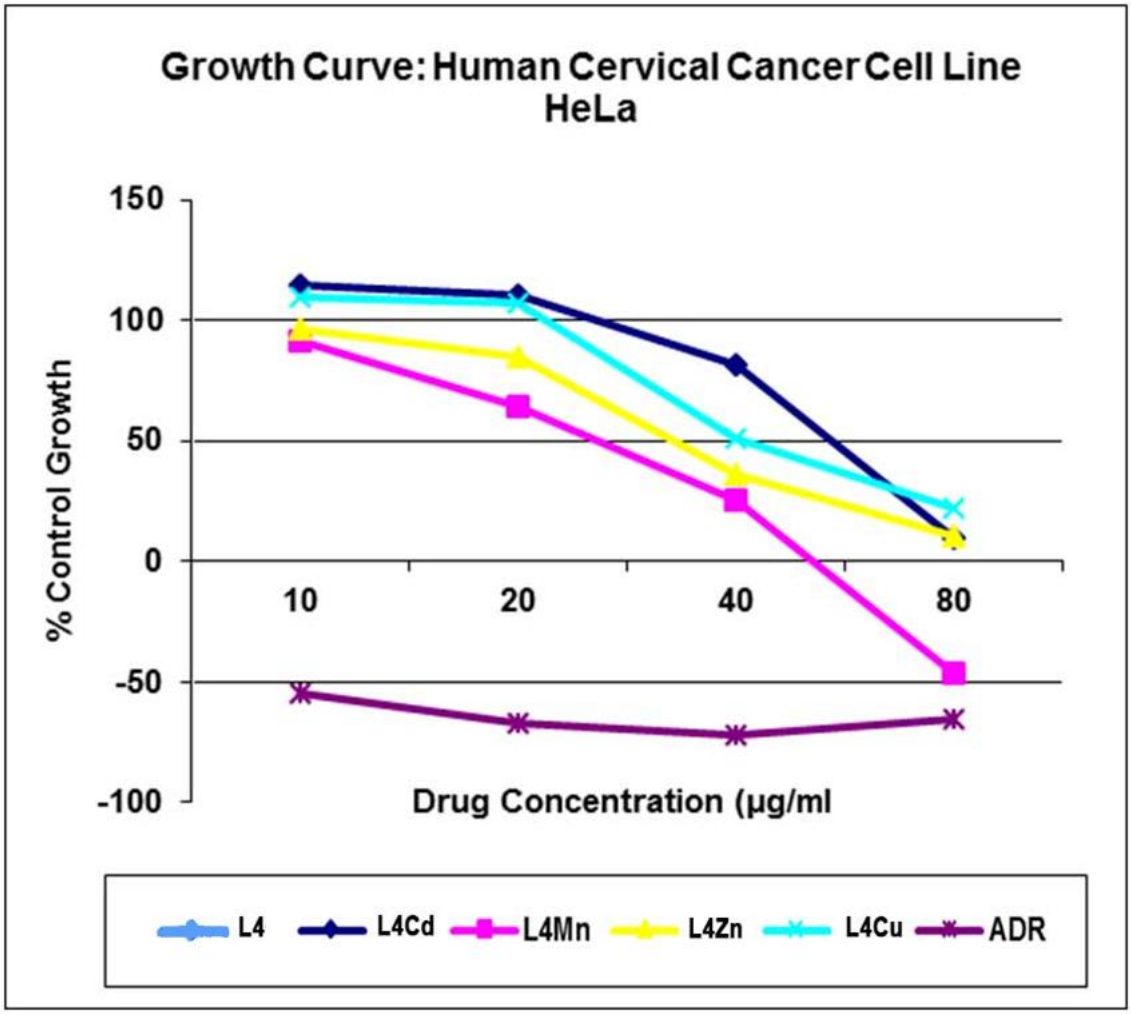


Fig. 25 Anticancer activity: Growth curve.

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