Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

IN VITRO ANTIMICROBIAL ACTIVITY AND PLANT GROWTH ACTIVITY STUDY OF SCHIFF BASE LIGAND (E)-2,4-DIROMO-6-{[(2-(2-METHOXYPHENOXY) ETHYL] IMINOMETHYL} PHENOL AND THEIR COMPLEXES WITH TRANSITION METALS

Vaibhav A. Mane*[1], Sheetal V. Palande[2], Deelip K. Swamy[3]

[1]*Department of Chemistry, N.E.S. Science College, Nanded, Maharashtra, India*
[2]*Department of Chemistry Viva college, Virar, Mumbai, Maharashtra, India*
[3]*Department of chemistry, Pratibha Niketan Mahavidyalaya, Nanded, Maharashtra, India*
**Corresponding author: vaibhav.22mane@gmail.com*

ABSTRACT

A newly synthesized Schiff base was obtained by the reaction of amine (2-(2-methoxyphenoxy)ethylamine) with carbonyl compound (3,4-dibromosalicylaldehyde) and their complexes with transition metals are prepared. The obtained Schiff base and metal complexes are characterized by spectral methods. The Schiff base ligands and their complexes of transition metals are tested for their plant growth regulating activity with seeds of three plants *viz.* Wheat, Mung and Mat been. *In vitro* antimicrobial activity against two bacteria *E.coli, S. aureus* are also tested for Schiff base ligand and its metal complexes.

Keywords: Schiff bases, Metal complexes, Plant growth activity, Antimicrobial

1. INTRODUCTION

In the design of carbon-nitrogen bonds, Schiff bases are major pathways. In the fields like pharmaceutical chemistry, agriculture and industrial fields, Schiff base ligands and their metal complexes have enormous applications [1]. They have the ability to act as catalysts as well as corrosive agents. In coordination chemistry, schiff base ligands are the important class of compounds possessing the wide range of applications [2]. The incorporation of transition metal ions into the Schiff base ligands extends its applications in the field of dye industry, food industry and in the field of biology.

Numerous Schiff base complexes exhibit excellent catalytic activity [3] for various types of reactions such as oxidation, reduction, hydrolysis and biocidal activity as homogenous and heterogenous catalysis. Schiff base ligands with imine group have been widely used in pharmaceutical field because of their physiological activity. Therefore Schiff bases and their metal complexes were widely investigated for their antifungal [4], antibacterial [5], antitumor, antifertility and enzymatic activities [6].

The Schiff bases were tested for their plant growth activity and antibacterial activity in the current research. The present study deals with Schiff base ligand synthesis and its transition metal complexes by condensing with Ni(II),Cu(II),Co(II), Mn(II) and Zn(II) metal salts. Over the years, various metal containing compounds have been proven to possess significant biological applications, especially in anticancer and antimalarial therapy. The syntheses of metal based antibacterial drugs are supposed to be beneficial. However for the synthesis of novel metal based products, block metals have been used. For example, Cu(II) complexes have been reported to be effective anticancer agents. Germination is an economical and simple method for improving the nutritive value of plants studies has been reported [7]. Plant growth regulating activity of complexes of transition metal ions are reported by different co-workers [8]. Various unnatural chelating agents used in biological systems have been reported and the activity of metal chelate is increased compared to ligand [9].

2. MATERIAL AND METHODS

The chemicals and reagents were purchased from Sigma Aldrich and S D Fine chemical companies. The chemicals used are 2-(2-methoxyphenoxy) ethylamine (Sigma Aldrich, AR grade) and 3,5-dibromo-salicylaldehyde (Sigma Aldrich, AR grade), Ethyl alcohol (S D Fine, AR grade), Cobalt(II) chloride dihydrate (S D Fine), Nickel(II) chloride hexahydrate (Sigma Aldrich), Copper(II) chloride dehydrate (Sigma Aldrich), Zinc (II)

chloride (Sigma Aldrich), Manganese(II) chloride tetrahydrate (Sigma Aldrich).
UV-visible spectra of compounds were carried out on Agilent technologies carry-100 UV-vis spectrometer. Using Brucker AMX 400-MHz, NMR spectral measurements were performed. Elemental micro-analysis (C, H, N,S and O) were carried out on Thermo finning ,Italy (Flash EA 1112 series). In JEOL, Japan JES-FA200 ESR spectrometry, ESR spectra of Cu complexes were performed in LT solution form with X-band.

2.1. General procedure

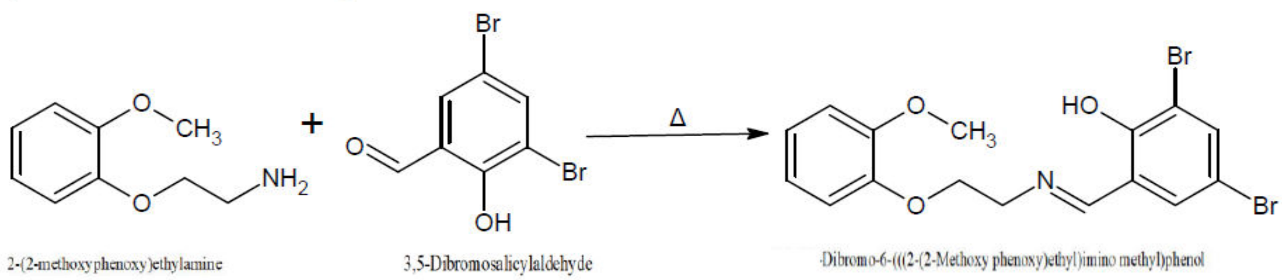
Schiff base ligand (E)-2,4-Dibromo-6-(((2-(2-Methoxyphenoxy)ethyl) iminomethyl) phenol (DBMPEIMP) and their Cu(II), Ni(II), and Zn(II), Co(II), Mn(II) metal complexes were synthesized through the following route.

2.2. Synthesis of Ligand

The reaction mixture of alcoholic (10 ml) solution of 2-(2-methoxyphenoxy) ethylamine (0.01 mol) and 3, 5-dibromosalicylaldehyde (0.01mol) was stirred under reflux for about 2 hours in water bath. A bright yellow colored solid compound was separated; the product obtained was filtered and crystallized. from aqueous ethanol. It was then filtered, recrystallized from ethanol and dried in air (Scheme 1) (Yield-86 %, M.P-140°C).

2.3. Synthesis of Metal Complexes

The Schiff base ligand (E)-2,4-Dibromo-6-(((2-(2-Methoxy phenoxy) ethyl) imino methyl) phenol) (DBMPEIMP) and metal salts ($MX_2.xH_2O$), dissolved in ethanol, were heated in water bath in the ratio of 2:1. The resulting mixture was refluxed for approximately 1hour, precipitating the complex. Through filtration they were removed, washed with cold ethanol and air dried (Yield-68%).



Scheme 1: Preparation of ligand: (E)-2,4-Dibromo-6-(((2-(2-Methoxy phenoxy)ethyl)imino methyl)phenol

2.4. Plant growth activity

Plant growth activity study of Schiff base (E)-2,4-Dibromo-6-(((2-(2-Methoxy phenoxy) ethyl) imino-methyl)phenol and its transition metal complexes was done using the standard bottlers method.
The Schiff base ligand DBMPEIMP and their complexes of transition metals are tested for their plant growth regulating activity with seeds of three plants *viz.* Maize, Wheat, and Mat been. The effect of these compounds on germination parameters, percentage survival, root-shoot ratio and vigour index has been studied. Twenty percent DMSO solution was prepared using double distilled water, in which seeds of equal size of the three plants Mung (*Vigna radiate*),Wheat (*Tritium aestivum*) and Mat been (*Vigna aconitifolia*) were soaked for about 6 hours, the ligand solution (5ppm) and metal complex solution (5ppm) were prepared with double distilled water [10]. The soaked seeds were further washed thoroughly and planted in a petri dish containing moistened blotters with 20 seeds per plate [11]. The plates were observed for germination for 10 days, then the parameters *viz*. percent survival, root length, shoot length, root/shoot ratio and vigour index were measured.

2.5. Antibacterial activity:

The antibacterial activity was measured by agar cup method. Nutrient agar (HI media) was prepared and sterilized at 15Psi for 15 minutes in the autoclave. *In vitro* antibacterial activity study against gram-positive and gram-negative bacteria *Escherichia coli* (ATCC ®25922™), *Staphylococcus aureus* (ATCC® 25922™) were carried out [21] to check their antibacterial potency. Bacterial study revealed that ligands showed good antibacterial potency over standard broad spectrum antibiotic Chloramphenicol and Zn(II) metal complexes were found to be highly active against all the isolates as compared to free ligands and Chloramphenicol. Zone of inhibition (MIC) in mm of the ligands and metal complexes given in Table 11.

3. RESULTS AND DISCUSSION

The formation of complex was indicated by color change and melting point. Physical characteristics of Schiff base and metal complexes are given in Table 1.

The elemental analysis study of the synthesized compounds is in accordance with compounds analytical formula. The basic study of the complexes of Schiff base corresponds to 1:2, metal: ligand stoichiometry. The analysis also shows the extra hydrogen and oxygen atoms signifying the presence of water molecules [12]. Therefore the complex may be represented by the general formula $ML_2.(H_2O)_2$Where M=Ni, Co, Cu, Mn, Zn. The data is represented in Table 2.

3.1. NMR and IR Spectra:

In NMR spectra formation of ligand was confirmed by presence of CH=N peak at 8.5 δ and OH at 4.3 δ.

IR spectral study of ligands along with their metal complexes were done in the range of 400-4000 cm^{-1} to determine the characteristic vibration bands for the different functional groups present in the molecule. The infra-red values for major peaks are assigned in present analysis. The ligand IR spectrum gave a large band at 1642.09 cm^{-1} and 2901.27 cm^{-1} which are due to C=N (azomethine) and -OH stretch respectively [13]. Complexes showed a lower bands of wave numbers for C=N indicating coordinated H_2O moiety in the complexes [14]. For the metal complexes phenolic m (O-H) stretching disappeared due to deprotonation of hydroxy hydrogen and binding of oxygen atom with metal ion [15]. Whereas (M-OH) bond frequency in the metal complexes of Ni(II), Cu(II), and Zn(II) shifted to higher value than their respective ligands as shown in Table 4. Aliphatic m(C-H) vibrations were observed in the range of 2991-2912 cm^{-1} for all the metal complexes. Metal-oxygen bonds are observed in the range of 560-500 cm^{-1}, whereas metal nitrogen bonds were observed in the range of 440-400cm^{-1} [16].

Complex of SB1-Ni showed IR bands at 1620 $cm^{-1}$and 3784 cm^{-1} corresponding to C=N and H_2O, IR values of 609 cm^{-1} and 452 cm^{-1} were assigned to M-O and M-N respectively. Similarly complex of SB1-Zn complex showed bands at υ(C=N) 1627.81 cm^{-1},υ (H_2O)2923.88cm^{-1}, υ(M-O) 609 $cm^{-1}$and υ(M-N) 478 cm^{-1}. Co complex at υ(C=N) 1612.38 cm^{-1}, υ(H_2O) 3440.84cm^{-1},υ(M-O) 609.12 cm^{-1} and υ(M-N) 501.112 cm^{-1}. Mn complex υ(C=N) 1539.88 cm^{-1},υ(H_2O) 3446.17cm^{-1}, υ(M-O) 454.154 $cm^{-1}$and υ(M-N) 496.58 cm^{-1}.

Table 1: Physical characteristics

Sr. No.	Compound	Color	Yield (%)	M.P(°C)
1	Ligand(SB1)DBMPEIMP	Bright yellow	76	120
2	[DBMPEIMP-Ni]	Pale green	82	227
3	[DBMPEIMP-Co]	Blackish green	67	246
4	[DBMPEIMP-Cu]	Orange	78	217
5	[DBMPEIMP-Zn]	Brownish green	64	210
6	[DBMPEIMP-Mn]	Yellow	72	189

Table 2: Elemental analysis of the Schiff base DBMPEIMP and its metal complexes

Compounds	Molecular Formula	Mass	Elemental analysis Observed(Calculated)					
			%C	%H	%N	%O	% Br	%M
Ligand DBMPEIMP-SB1	$C_{16}H_{15}NBr_2O_3$	429.11	44.70 (44.74)	3.50 (3.49)	3.25 (3.26)	11.10 (11.18)	37.20 (37.23)	------
[DBMPEIMP-Ni]	$C_{32}H_{32}N_2Br_4O_8Ni$	950.91	40.35 (40.38)	3.33 (3.36)	2.90 (2.94)	13.40 (13.46)	16.85 (16.80)	6.10 (6.17)
[DBMPEIMP-Co]	$C_{32}H_{32}N_2Br_4O_8Co$	950.53	40.36 (40.39)	3.30 (3.36)	2.92 (2.94)	13.36 (13.39)	33.60 (33.62)	6.15 (6.19)
[DBMPEIMP-Cu]	$C_{32}H_{32}N_2Br_4O_8Cu$	955.14	40.21 (40.20)	3.34 (3.35)	2.91 (2.93)	13.41 (13.40)	33.40 (33.46)	6.60 (6.65)
[DBMPEIMP-Zn]	$C_{32}H_{32}N_2Br_4O_8Zn$	956.98	40.15 (40.12)	3.30 (3.34)	2.89 (2.92)	13.30 (13.37)	33.35 (33.39)	6.80 (6.83)
[DBMPEIMP-Zn]	$C_{32}H_{32}N_2Br_4O_8Mn$	946.53	40.50 (40.56)	3.35 (3.38)	2.90 (2.95)	13.49 (13.52)	33.72 (33.76)	5.75 (5.80)

Table 3: NMR of Schiff base ligand DBMPEIMP-SB1

Compound	HC=N	OH	CH_3	CH	Phenyl Protons
DBMPEIMP-SB1	8.5	4.3	3.2	2.5	6-7

3.2. Thermo Gravimentric Analysis:

In an inert atmosphere at the temperature range of 20°C -1000°C, thermogravimetric study of ligands and their metal complexes was observed to explain the thermal stability of complexes. TGA study of complexes showed the complexes start to degrade in the temperature range of 110°C-200°C is due to elimination of coordinated water molecules [17] and continues to decompose at 310-330°C. Gradual decrease in mass is also seen in the thermogram up to 300°C due to loss of volatile matter and a plateau observed above 350°C, which correlates to the formation of stable metal oxide remains as final residue at higher temperature.

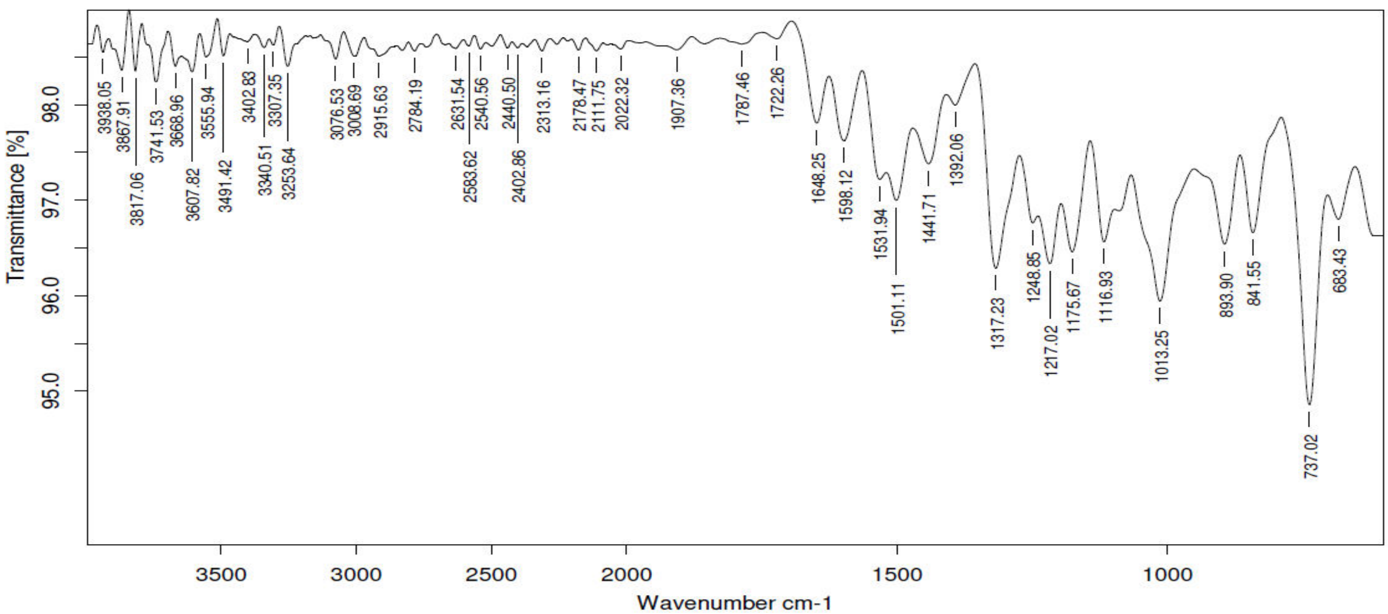


Fig. 1: IR spectra of VAI-3,5-Dibromo Schiff base

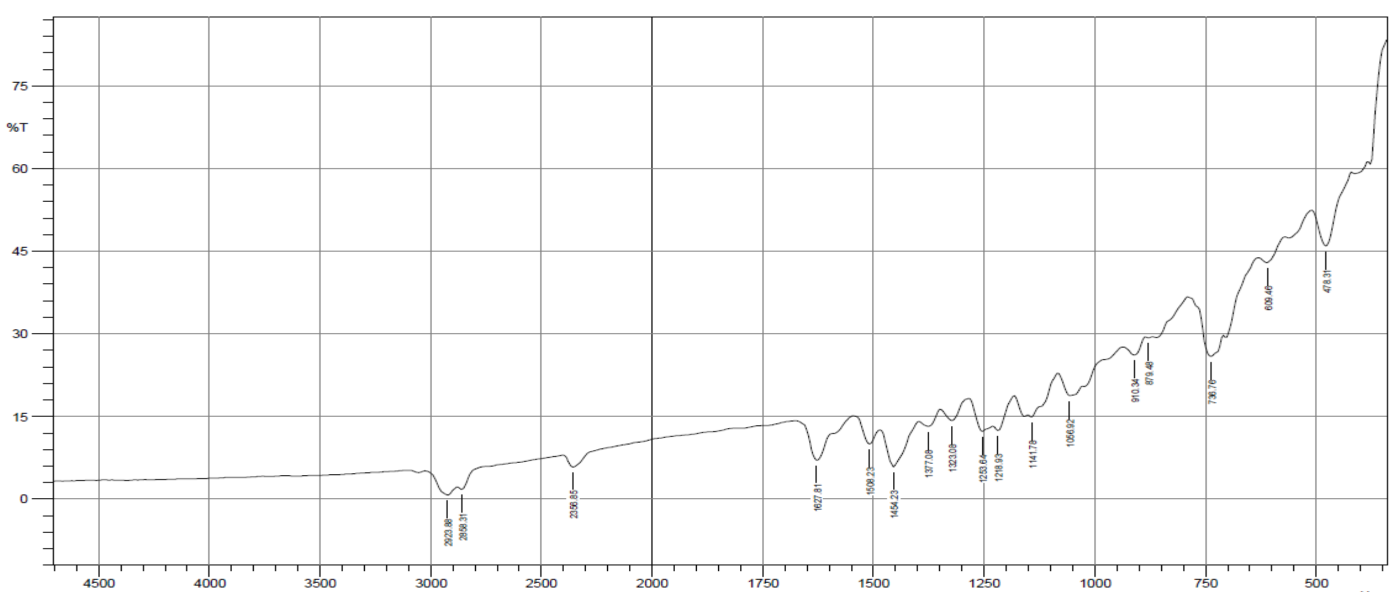


Fig. 2: IR spectra of VAI-3, 5-Dibromo-Zn

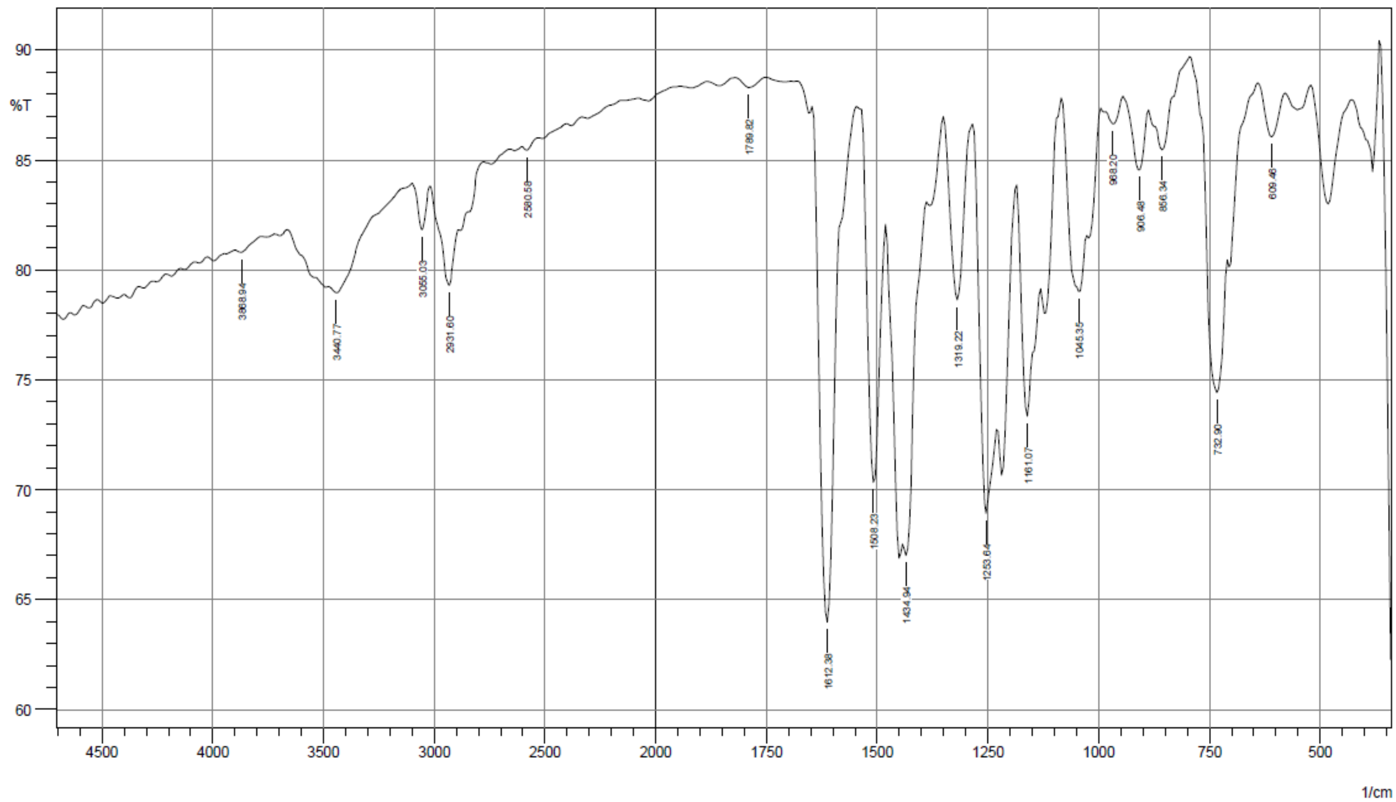


Fig. 3: IR spectra of VAI-3, 5-Dibromo-Co

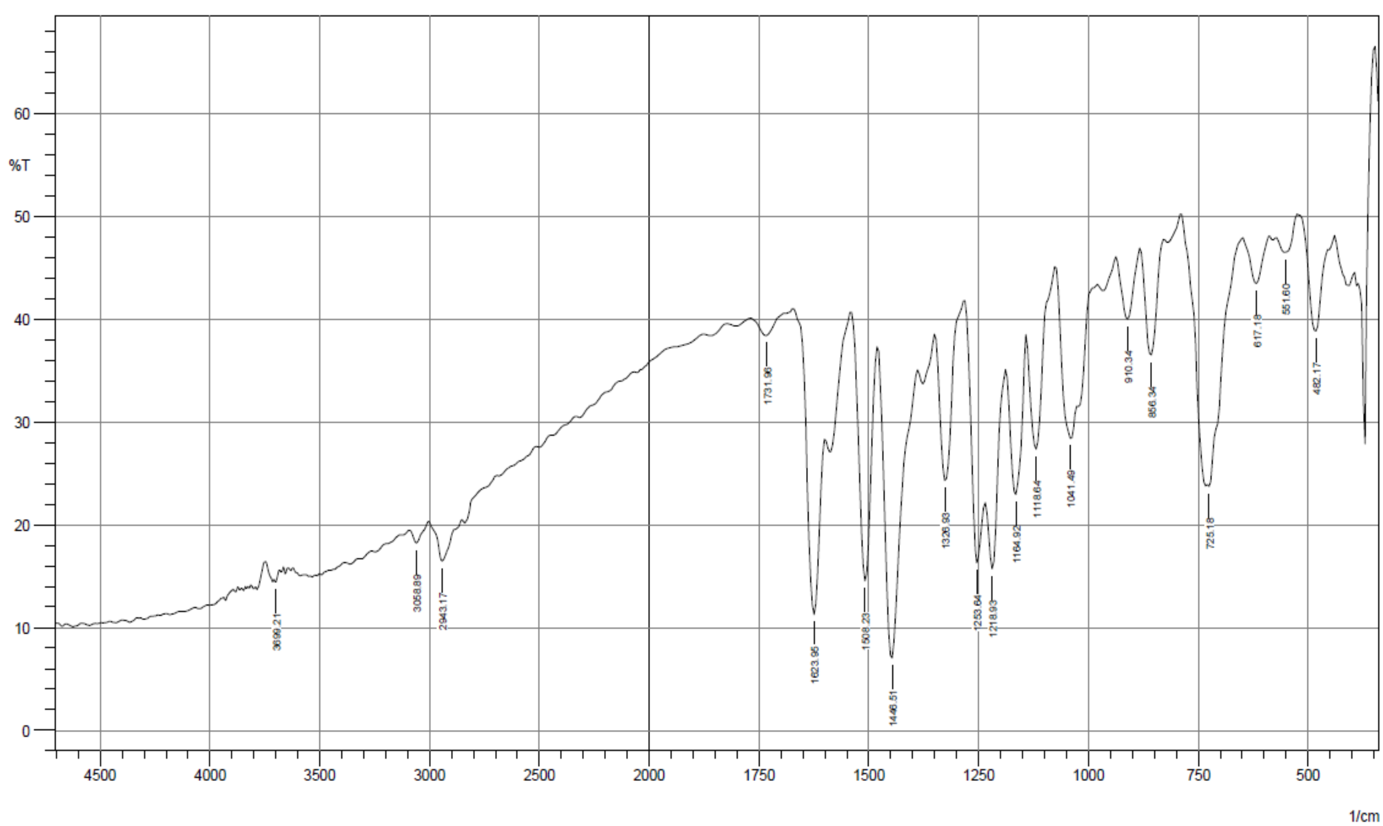


Fig. 4: IR spectra of VAI-3,5-Dibromo-Cu

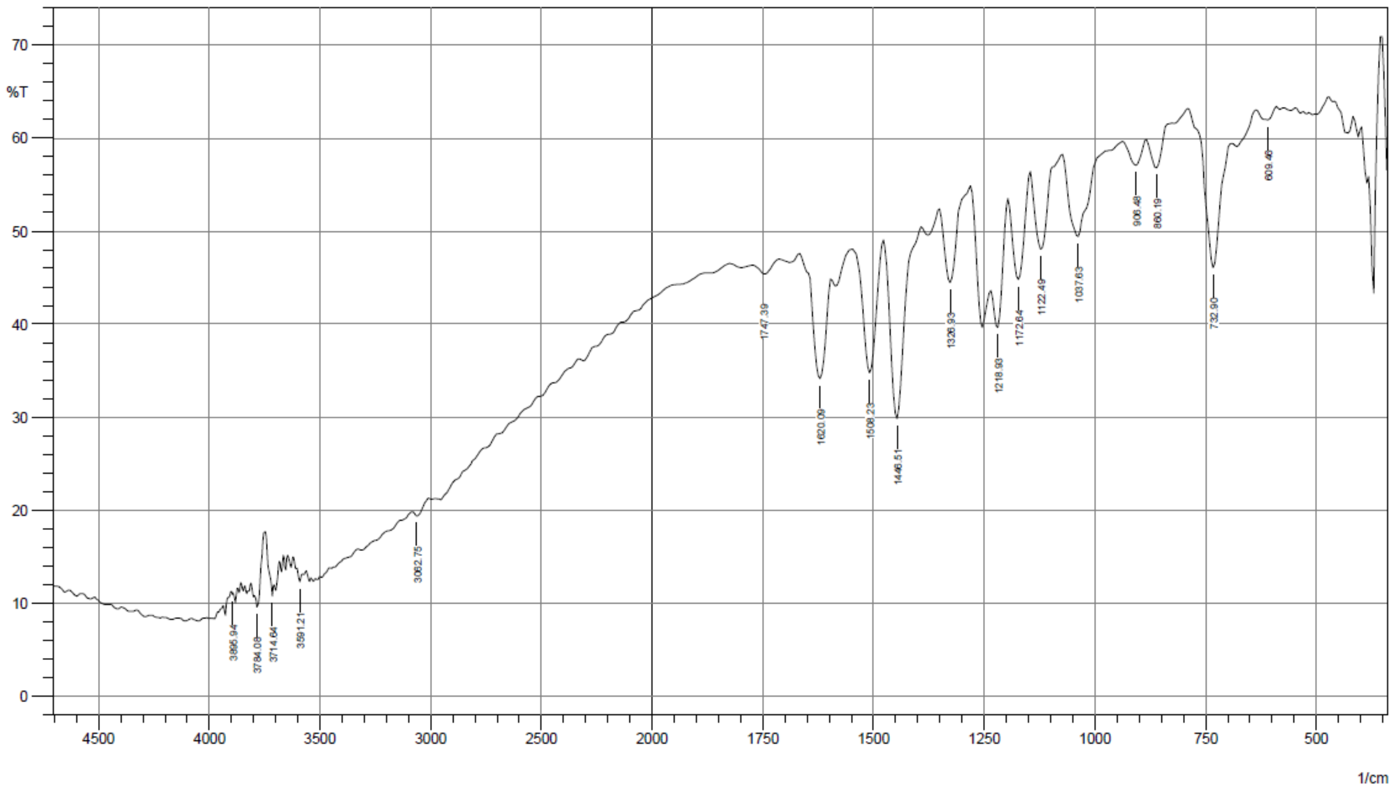


Fig. 5: IR spectra of DBMPEIMP-Mn

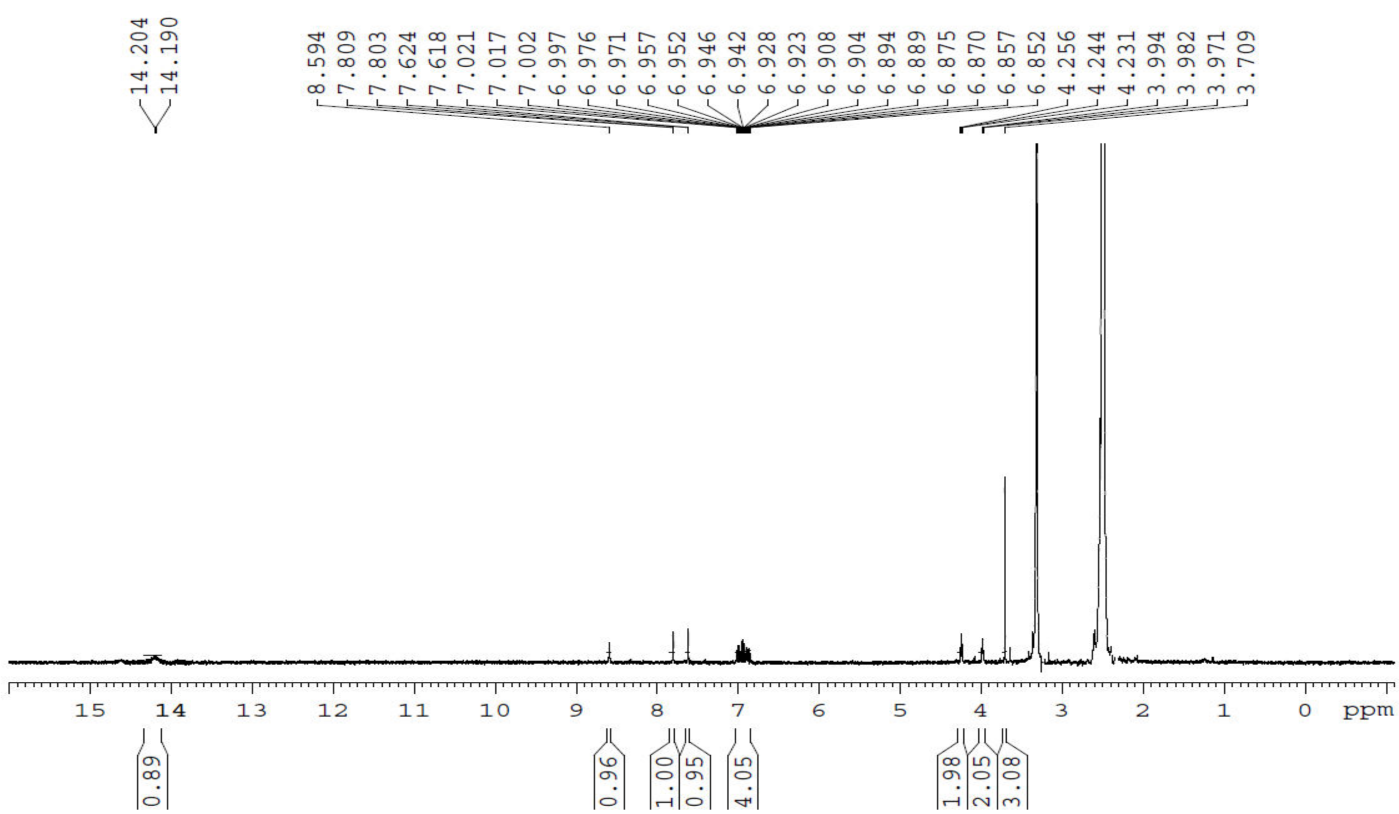


Fig. 6: NMR spectra of Schiff base DBMPEIMP

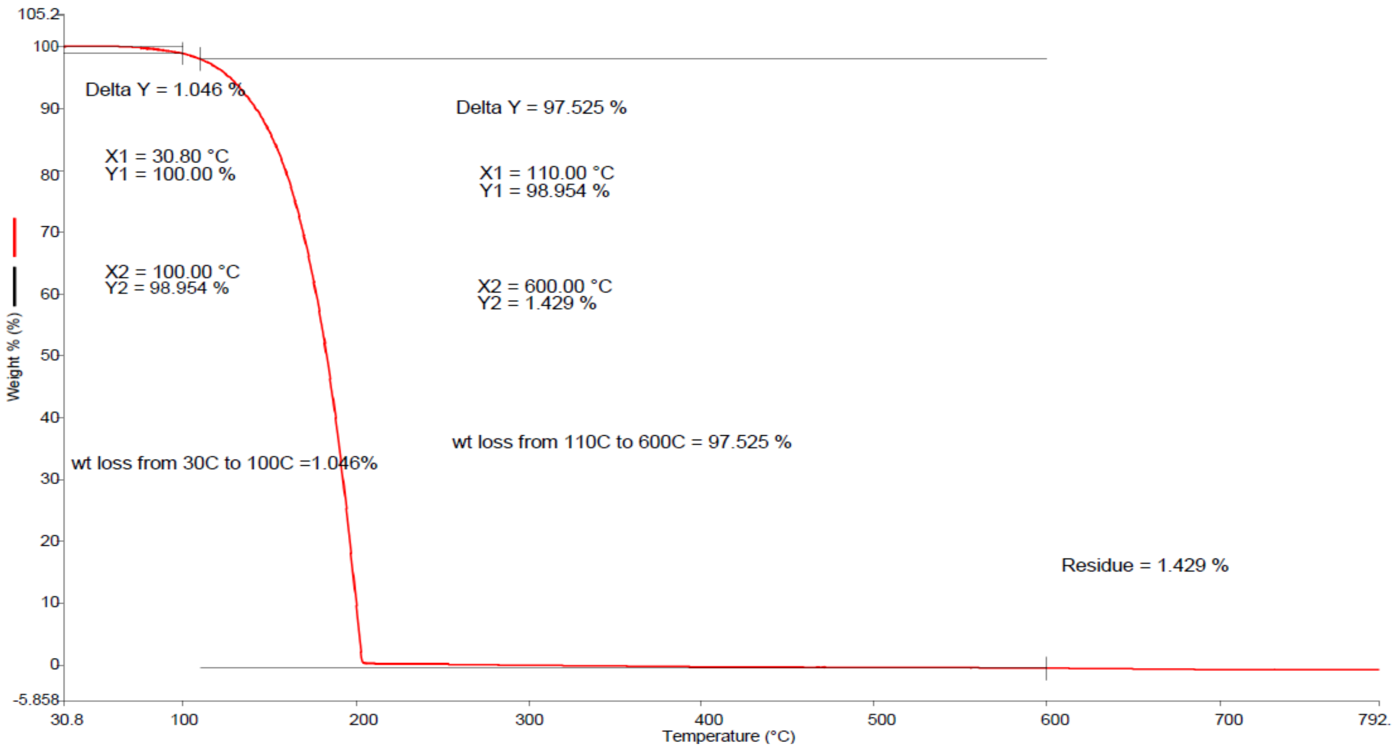


Fig. 7: Schiff base DBMPEIMP -TGA

Table 4: FT-IR Bands for Schiff Base ligand and its metal complexes

Sr. No.	Compound	υ(OH) cm^{-1}	υ(OH) Water cm^{-1}	υ(C=N) cm^{-1}	υ(M-O) cm^{-1}	υ(M-N) cm^{-1}
1	DBMPEIMP-SB1	2901.27	--------	1642.09	--------	-------
2.	DBMPEIMP-Cu	------	3699	1623.95	551	482
3.	DBMPEIMP- Ni	-------	3784	1620	609	452
4.	DBMPEIMP- Zn	--------	2923.88	1627.81	609	478
5.	DBMPEIMP- Co	--------	3440.84	1612.38	609.12	501
6.	DBMPEIMP- Mn	-------	3446.17	1539.88	496.58	454.15

Table 5: Thermogravimetric Data of Schiff base and its metal complexes

Complexes	Temperature Range(°C)	Calculated (%)	Observed (%)	Mass loss
DBMPEIMP-Cu	110 - 200	5.60	6.0	Mass loss due to H_2Omolecules
	250 - 320	14.04	17	Mass loss due to volatile matter
	Above 350	11.63	10.0	Mass of the metal oxide
DBMPEIMP-Ni	110 - 200	8.46	6.0	Mass loss due to H_2O molecules
	250 - 320	16.18	17	Mass loss due to volatile matter
	Above 350	13.12	10.0	Mass of the metal oxide
DBMPEIMP-Co	110 - 200	4.06	6.0	Mass loss due to H_2Omolecules
	250 - 320	14.04	17	Mass loss due to volatile matter
	Above 350	11.45	10.0	Mass of the metal oxide
DBMPEIMP-Zn	110 - 200	6.65	6.0	Mass loss due to H_2Omolecules
	250 - 320	15.62	17	Mass loss due to volatile matter
	Above 350	14.16	10.0	Mass of the metal oxide
DBMPEIMP-Mn	110 - 200	5.62	6.23	Mass loss due to H_2Omolecules
	250 - 320	17.56	16.90	Mass loss due to volatile matter
	Above 350	13.95	10.25	Mass of the metal oxide

3.3. Electric Absorption Spectra

All the UV-Visible spectra of synthesized ligand and their metal complexes were recorded in DMSO solvent. In the electronic spectra, the ligand exhibited energy peaks at 23285 $cm^{-1}$and 29850 cm^{-1}.

The Co(II) complexes exhibited two energy peak at 24479 cm^{-1}, 26041cm^{-1} and 27700 cm^{-1}, which can be assigned to the transitions $4T1_g(F) \rightarrow 4T2_g(F)$, $4T1_g(F) \rightarrow 4T2_g(F)$ and $4T1_g(F) \rightarrow 4T2_g(P)$ for a high spin octahedral geometry respectively [1]. The electronic spectra of the Ni(II) complexes showed d-d transition at 28571 cm^{-1}, 24390 $cm^{-1}$and 22883cm^{-1}. These are assigned to $3A2_g(F) \rightarrow 3T2_g(F)$, $3A2_g(F) \rightarrow 3T1_g(F)$ and $3A2_g(F) \rightarrow 3T2_g(P)$ transitions, respectively. These are consistent with a well-defined octahedral geometry [18].The Zn(II) complexes exhibited only a high intensity band at 26385 cm^{-1} and 29850 cm^{-1}, which is assigned to ligand-metal charge transfer. In case of the Cu(II) complexes, a broad band at 23496 cm^{-1}, 30303 $cm^{-1}$and 27027 cm^{-1} was observed that is assigned to the $2E_g \rightarrow 2T2_g$ transition, which confirms its octahedral geometry.

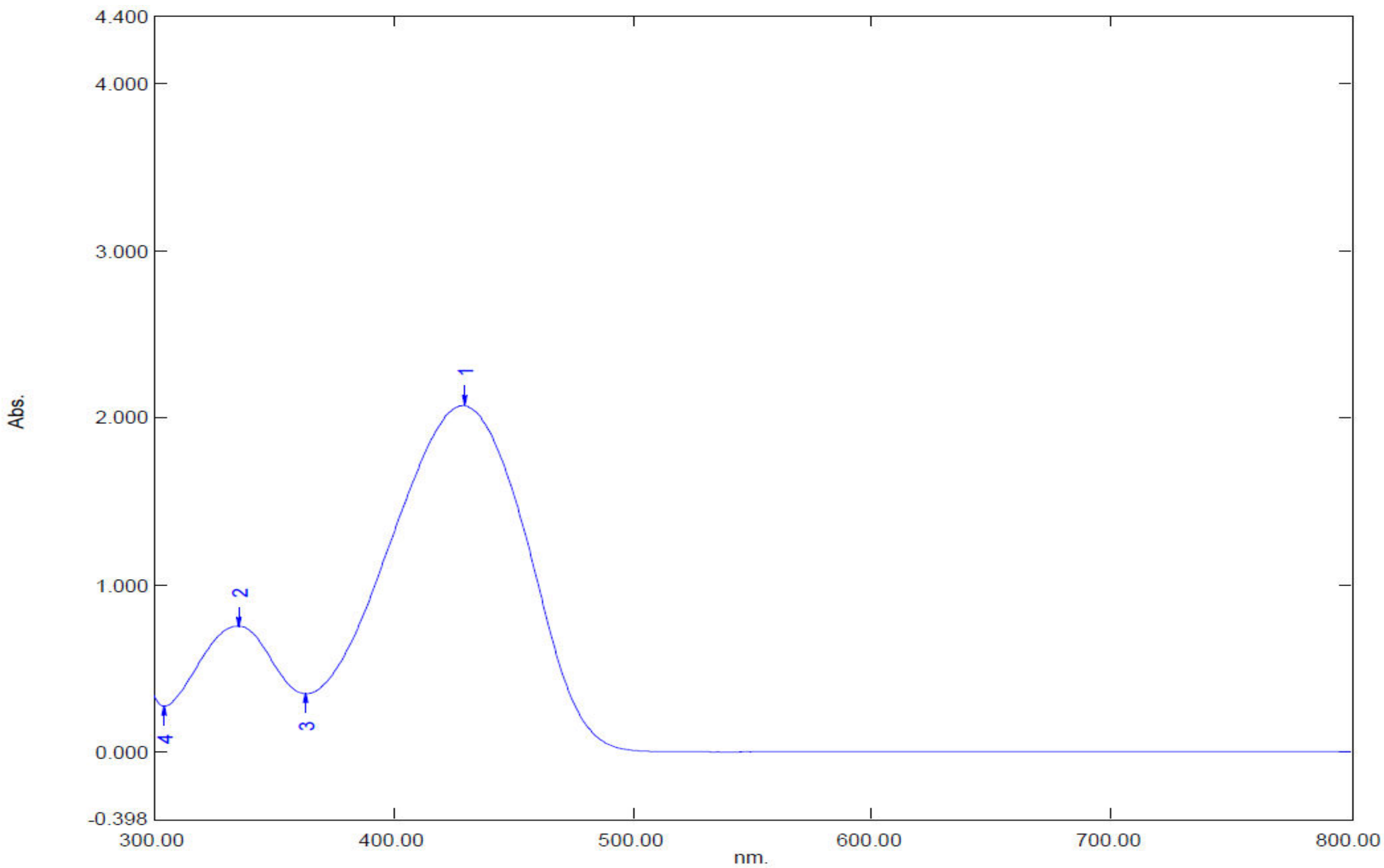


Fig. 8: UV spectra of DBMPEIMP-SB1

3.4. Magnetic Susceptibility Measurements

The effective magnetic moment values for the complexes were determined to explain the geometry of metal complexes. The magnetic moment value 4.24 BM (Bohrs magneton) for Co (II) complex suggests an octahedral environment. The magnetic moment value 3.13BM of Ni (II) complexes suggests an octahedral geometry.

The magnetic moment value of the Cu (II) complexes of 1.63 BM suggests distorted octahedral geometry. Mn (II) complexes with the value of 5.64 BM indicate octahedral geometry [19]. The Zn (II) complexes were found to be diamagnetic, as expected for d^{10} configuration. From the discussion of the results of various physico-chemical studies presented above, it may be concluded that the most probable geometry for the transition metal complexes with general formula $ML_2.2H_2O$ is octahedral and the bonding in the complexes can be represented in Fig. 9.

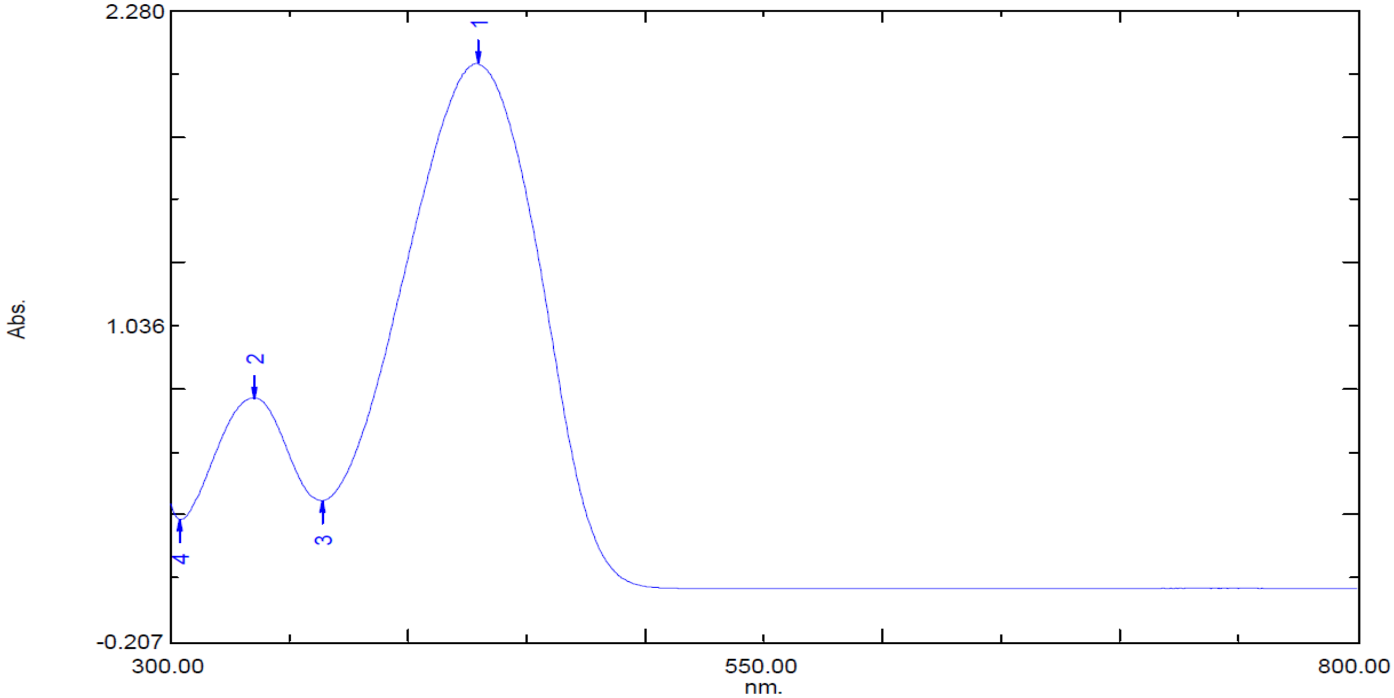


Fig. 9: UV spectra of DBMPEIMP- Ni

Table 6: Magnetic moment values for Schiff base ligand DBMPEIMP metal complexes

Complexes	Experimental values of Magnetic moment (BM)
DBMPEIMP-Cu	1.62
DBMPEIMP- Ni	2.95
DBMPEIMP- Zn	0.00
DBMPEIMP- Co	3.82
DBMPEIMP- Mn	5.23

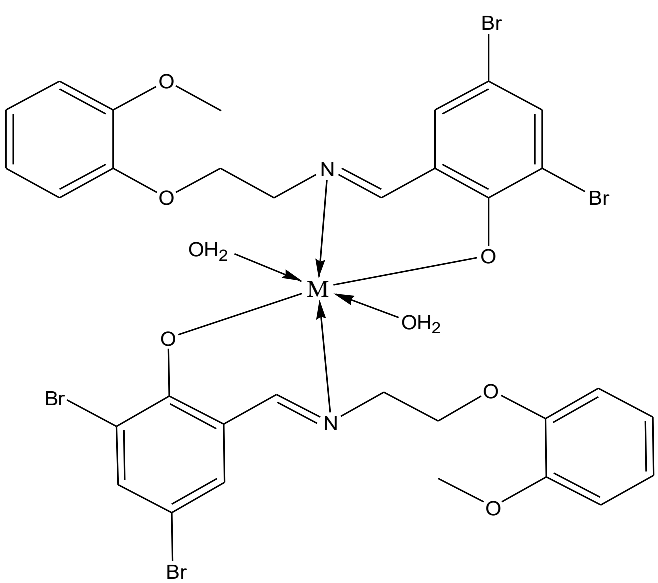


Fig. 10: Structure of complex (M=Ni, Cu, Co, Mn, Zn)

3.5. Molar conductivity

Molar conductivities of all metal complexes were taken in DMSO due to partial solubility in common organic solvents to find out electrolytic nature of the complexes. Solutions of 10^{-1} M concentration were prepared and molar conductivity of the solutions were measured at room temperature to perform conductivity experiments [20]. Specific conductance and molar conductance was calculated using the equation:

Specific conductance (k) = Cell constant × conductance

Molar conductance (ρ) = k × 1000/C

Cell constant of the solution was found to be 0.078 at a room temperature of 30°C. The molar conductance for all the newly synthesized complexes were in the range 23 mhos cm^2 mol^{-1} to 94 mhos cm^2 mol^{-1}, indicating very low conductance[19]. These values indicate that the complexes are non-electrolytic in nature. The conductance values of the metal complexes were given in table below.

All the synthesized compounds were subjected to plant growth activity studies. The results are given in the tables below

From the above observation, it was thus concluded that the synthesized complexes have plant inhibitory activity rather than plant growth activity. In a decreasing order, the activity can be described as follows:

Water>Ligand>Metal complexes

3.6. Antibacterial activity

The synthesized Schiff base ligand and their metal complexes were screened for *in vitro* antibacterial activity against two bacterial strains. It was found that all the compounds possess good antibacterial activity but Ni (II) complexes showed little activity than standard. All the above results were compared with standard antibiotics tetracycline and erythromycin. Tetracycline shows a zone of inhibition of 19 mm (intermediate range) and 16 mm (intermediate range) for *E.coli* and *S. aureus* respectively similarly Erythromycin shows a zone of inhibition of 15 mm (intermediate range) and 18 mm (intermediate range) for *E.coli* and *S. Aureus,* respectively. The antimicrobial activity of the synthesized compounds was evacuated after the incubation period by the measurement of the diameter of zone of inhibition.

Therefore from the above study it is concluded that synthesized compounds shows weak antimicrobial activity than standard, can be summarized as follows: standard > Ligands > complexes.

Table 7: Molar conductance values for metal complexes

Complex	Conductance (mhos)	Specific conductance (mhos cm^{-1})	Molar conductance (mhos cm^2 mol^{-1})
DBMPEIMP-Cu	0.04	3.12×10^{-3}	31.20
DBMPEIMP- Ni	0.12	9.3×10^{-3}	93.60
DBMPEIMP- Zn	0.09	7.02×10^{-3}	70.20
DBMPEIMP- Co	0.07	5.4×10^{-3}	54.60
DBMPEIMP-Mn	0.03	2.34×10^{-3}	23.40

Table 8: Effects of DBMPEIMP-SB1 and its complexes on growth parameters for *Vigna radiata* (Mung) plant

Parameters	Effect of		Effect of complexes				
	Water	**Ligand**	**Ni**	**Cu**	**Mn**	**Co**	**Zn**
Total number of seeds	20	20	20	20	20	20	20
Number of seeds germinated	18	13	14	12	11	13	10
% Germination after 7 days	90	65	70	60	55	65	50
% Survival after 10 days	100	93.33	90.63	88.66	80.61	90.33	89.63
Root length(cm)	3.0	2.1	1.0	1.4	1.3	1.1	1.3
Shoot length(cm)	4.2	3.4	2.5	2.3	2.1	2.4	2.3
Vigor index	612	412.5	210	240.5	187	227.5	198
Root-shoot ratio	0.74	0.63	0.38	0.53	0.80	0.31	0.50

Table 9: Effects of DBMPEIMP-SB1 and its complexes on growth parameters for *Tritium aestivum* (wheat) plant

Parameters	Effect of		Effect of complexes				
	Water	**Ligand**	**Ni**	**Cu**	**Mn**	**Co**	**Zn**
Total number of seeds	20	20	20	20	20	20	20
Number of seeds germinated	18	15	13	11	16	14	13
% Germination after 7 days	90	75	65	55	80	70	65
% Survival after 10 days	100	93.33	91.66	84.61	90.90	84.61	81.81
Root length(cm)	3.1	2.1	0.9	1.6	1.3	1.5	1.6
Shoot length(cm)	4.2	3.4	2.1	2.5	2.6	2.6	2.8
Vigor index	612	412.5	210	240.5	187	227.5	198
Root-shoot ratio	0.74	0.61	0.42	0.64	0.50	0.57	0.57

Table 10: Effects of DBMPEIMP-SB1 and its complexes on growth parameters for Mat been (*Vigna aconitifolia*) plant

Parameters	Effect of		Effect of complexes				
	Water	Ligand	Ni	Cu	Mn	Co	Zn
Total number of seeds	20	20	20	20	20	20	20
Number of seeds germinated	18	13	15	13	12	11	13
% Germination after 7 days	90	65	75	65	60	55	65
% Survival after 10 days	100	93.33	91.66	84.61	90.90	84.61	81.81
Root length(cm)	3.0	2.1	1.3	1.7	1.4	1.2	1.4
Shoot length(cm)	4.2	3.4	2.5	2.1	2.3	2.6	2.7
Vigor index	612	412.5	210	240.5	187	227.5	198
Root-shoot ratio	0.74	0.61	0.52	0.80	0.60	0.46	0.51

Table 11: Antibacterial activity of compounds for organisms *E. coli* and *S. Aureus*

Sample	*E.coli*		Sample	*S. Aureus*	
	Concentration	Zone of Inhibition (mm)		Concentration	Zone of Inhibition (mm)
DBMPEIMP- Ni	20	10	DBMPEP- Ni	20	----
	40	13		40	10
	60	---		60	11
	80	11		80	13
	100	12		100	09
	control	0		Control	0
DBMPEIMP- Cu	20	---	DBMPEIMP- Cu	20	13
	40	12		40	10
	60	10		60	--
	80	09		80	13
	100	13		100	0
	control	0		control	
DBMPEIMP- Zn	20	12	DBMPEIMP- Zn	20	14
	40	13		40	12
	60	--		60	14
	80	14		80	13
	100	10		100	12
	Control	0		control	0

4. CONCLUSION

The Schiff base ligands HL and its Co (II), Cu (II), Ni (II), Mn(II) and Zn(II) metal complexes were synthesized and characterized by various analytical techniques. The biological activity of some metal complexes was higher than that of the free ligand. The Ni (II) complexes had the highest activity index.

In the present investigation plant growth activity suggest that the root shoot ratio has very low values for the complexes as compared to ligand and water.

5. ACKNOWLEDGEMENTS

The authors are thankful to authorities of Science college Nanded and Microanalytical laboratory, Department of Chemistry, University Of Mumbai for providing characterization facilities and IIT Mumbai SAIF for ESR facility.

Conflicts of interest

Authors state that there is no conflict of interest.

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