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Synthesis and characterization of new substituted macrocyclic complexes and investigation of their antimicrobial activity

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ABSTRACT

New macrocyclic ligand viz., cyclo-(1,2)-dibigunidinylbis{2-hydroxy-ω-substituted -benzoyl)}-acetophenone containing substituted benzyolacetophenone and their complexes with Ni(II), Cu(II) & Fe(III) has been synthesized. The ligand and its complexes were investigated by different spectroscopic methods and also evaluated for their antimicrobial activity .The Characterization of these complexes is achieved by elemental analysis, spectral (IR, ^{1}H NMR, electronic spectra) and thermal analysis. Molar conductance and magnetic moment measurement have also been determined. Microanalytical data clearly shows 1:1 molar ratio of ligand to metal in all complexes. Elemental analysis are in good agreement with the formation of complexes $[M (L) (H_2O)_2]Cl_2$ where M= Ni(II), Cu(II) and Fe(III); [L= L_1- L_V]. On the bases of electronic spectroscopy and magnetic susceptibilities study octahedral geometry assigned for Ni(II) & Fe(III) complexes and distorted octahedral geometry for Cu(II) complexes. The ligand and complexes were evaluated for their antimicrobial activity against different species of bacteria and fungi. Their biopotency has been discussed.

Key words: Macrocyclic substituted biguanides, template synthesis and antimicrobial activity.

INTRODUCTION

A very important area of bioinorganic chemistry is the chemistry of macrocyclic complexes because of their vital application such as therapeutic agents[1], magnetic resonance imaging (MRI) contrast agent[2,3], anticoagulant of serine proteases for the treatment of thrombosis disorder, as luminescent Sensors[4] and artificial restriction enzyme for cleavage of DNA & RNA[5].

The study of macrocyclic substituted biguanides recently attracted the attention of researchers because of it unique importance[6]. The various substituents at different position have shown a marked effect on the antimicrobial activity of ligands in terms of detoxification, difference in site of action and physiological deposition[7]. Coordination with metal ion alters their antimicrobial activity up to a remarkable extent. Copper together with iron and manganese are essential metallic elements and exhibit sufficient antimicrobial activity when associated with certain metal-protein complexes participating in oxygen transport and electronic transfer reactions[8].

The metal ion plays an important role in a vast number of widely differing biological processes [9]. Some of these processes are highly specific in their metal ion requirements while in others one metal ion replaces other. For example iron is an essential structural component of myoglobin, chromium is essential for mammalian metabolism and together with insulin is responsible for maintaining blood sugar level. Biguanides have been used for a variety of needs in medical practice but their clinical importance has been remarked by anti malarial and oral hypoglycemic properties.

In the light of above mentioned discussion , the present manuscript involves preparation, characterization and antimicrobial activities of cyclo (1,2) di bigunidinyl bis {2-hydroxy-ù-(substitutedbenzoyl)}-acetophenone (Fig. A) and its complexes with Ni(II), Cu(II) and Fe(III).

EXPERIMENTAL

Chemicals and methods

Dicyandiamide, Ammonium chloride, copper sulphate pentahydrate, sodium hydroxide, benzoic acid, 2-chlorobenzoic acid, 4-chlorobenzoic acid, 2-nitrobenzoic acid, 4-nitrobenzoic acid, hydrochloric acid, phosphorus oxychloride, o-hydroxyacetophenone, Nickel chloride, $CuCl_2.xH_2O$ and anhydrous ferric chloride were used as obtained from E.MERCK. All the solvents (AR grade) were used as such.

Elemental (C, H,) analysis of dried sample was carried out by standard micro methods in the centre for Instrumental analysis, Punjab University, Chandigarh. Nitrogen and chlorine were estimated by Kjeldahl's and volhard's methods respectively. The metals Cu (II), Ni(II) and Fe(III) were estimated by atomic absorption spectrophotometer model AA-120, using the appropriate hollow cathode lamp. Purity of the compound was checked by T.L.C. on silica gel-G using anhydrous methanol and THF (1:3) as a solvent. Appearance of single spot indicated the presence of one compound and hence their purity. Cu (II) ions were estimated iodometerically and the Fe (III) ions were reduced to Fe (II) and then estimated with standard $K_2Cr_2O_7$ solution using N-phenylanthranilic acid as indicator.

Magnetic susceptibility measurements were made at room temperature using Magnetic susceptibility Gouy balance using $Hg[Co(NCS)_4]$ as the calibrating agent. The diamagnetic corrections were computed using Pascal's constant. The data were corrected for diamagnetic susceptibilities and temperature independent Para-magnetism.

Molar conductivity of freshly prepared 0.001M solution of each complex in dry DMSO were measured at 25 ± 0.5^0C using digital conductivity meter, model ME-976-C having cell with cell constant 0.98. Molecular wt. determination of each complex in dry DMSO was determined cryoscopically.

H^1 NMR spectra were recorded on Bruker Avance II 400MH_Z Spectrometer at RSIC, Punjab University, Chandigarh. The spectra were obtained using DMSO as solvent.

IR spectra were recorded by using a Perkin-Elmer FTIR 31725X. The spectra were recorded by preparing potassium bromide pellets.

Electronic spectra of the complexes were recorded on a 6305 UV spectrophotometer in DMF solution.

Thermo-gravimetric analysis of the complexes was carried out in static air with open sample holder and a small boat, the heating rate was 10^0C/min.

The antifungal and antibacterial activities were determined by the well plate technique. Two fold serial dilution technique is used to find minimum Inhibitory concentration (MIC).

Synthesis of ligands [L_1-L_V]

An ethanolic solution of (20ml) of Biguanide (10 mmol) was mixed with an ethanolic solution (15ml) of 2-hydroxy-ω-(benzoyl / 2-chlorobenzoyl / 4-chlorobenzoyl / 2-nitrobenzoyl / 4-nitrobenzoyl) acetophenone (20 mmol).The resultant solution was refluxed on a water bath for 2 hrs. The ligands separated on initially cooling the reaction mixture at room temperature after that in refrigerator for 3hrs. After filtration the ligand was recrystallized from ethanol. Yield 35-50%.

IR bands(cm^{-1}): [3300-3280, 3260-3175,n(N-H)];[1360-1300,1280-1240,n(C-N)]; [1675-1655, nC=N in (>C=N-H)];[1640-1615 nC=N in (>C=N-C)].

Synthesis of macrocyclic complexes

(a) Non- Template synthesis

All the complexes were prepared by a common method. Ethanolic solution of metal chloride was mixed with hot ethanolic solution of ligand in1:1 molar ratio.

The reaction mixture was refluxed for 4 hours on a water bath. The colored complexes obtained on cooling the reaction mixture. The complexes were filtered and dried over P_2O_5 under vacuum.

(b) Template synthesis

The reaction is carried out in 1:2:2 molar ratios. A solution of metal chloride in ethanol is reacted with biguanide at 5^0C on stirring for 1hr. This is followed by the addition of ethanolic solution of 2-hydroxy-ù-(benzoyl/2-chlorobenzoyl/4-chlorobenzoyl/2-nitrobenzoyl/4-nitrobenzoyl) acetophenone. The mixture was stirred continuously for 5hrs. The resulting solid was recovered after filtration and dried over P_2O_5 under vaccum.

Antimicrobial activity

The antimicrobial activity of all the ligands, the complexes and the adducts was evaluated against the following phytopathogenic fungi and bacteria :

Fungi

A. alternata, F.oxysporum and *A.niger.*

Bacteria

Escherichia coli and *S. aureus.*

Czapek Yeast Extract Agar medium, Potato Dextrose Medium and Potato Sucrose Medium were used for growing the culture of fungi and Nutrient agar medium for that of bacteria.

RESULTS AND DISCUSSION

Physical properties and analytical data shows that all the complexes corresponds to $[M\{L\}(H_2O)_2]Cl_2$ where M= Ni(II), Cu(II) and Fe(III) (Table 1). Melting point of all the complexes was not sharp but decomposes above 290^0C. These complexes were colored and insoluble in most of the common organic solvents except polar solvent such as DMSO and DMF. The molar conductance ($140\text{-}170 W^{-1}cm^{-2}mol^{-1}$) value indicates their ionic nature and mol.wt. data suggested their monomeric behavior.

The ^{1}HNMR spectra of the ligands were recorded in deuterated dimethyl sulphoxide using TMS as an internal standard. The ^{1}H spectra of the

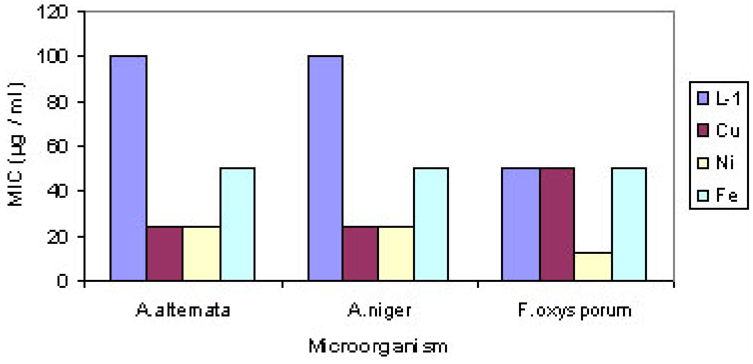


Fig. 1:

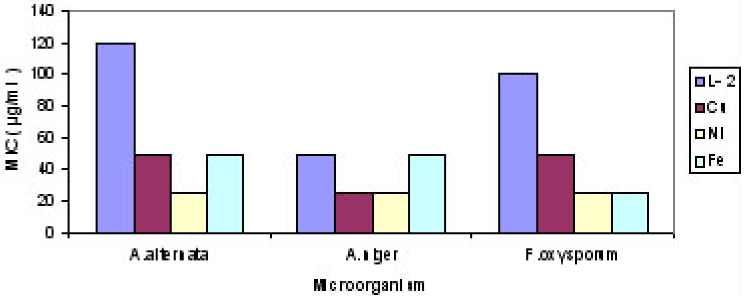
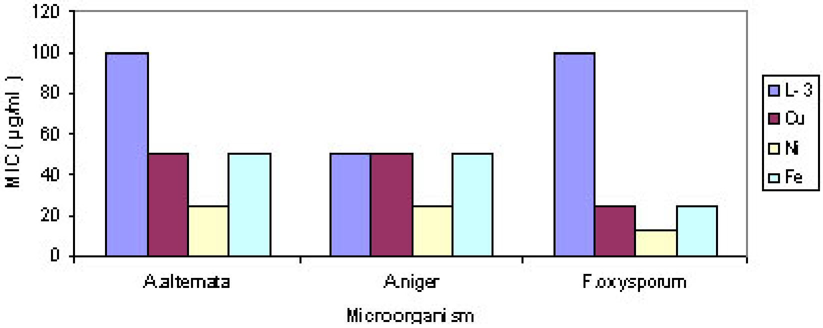


Fig. 2:



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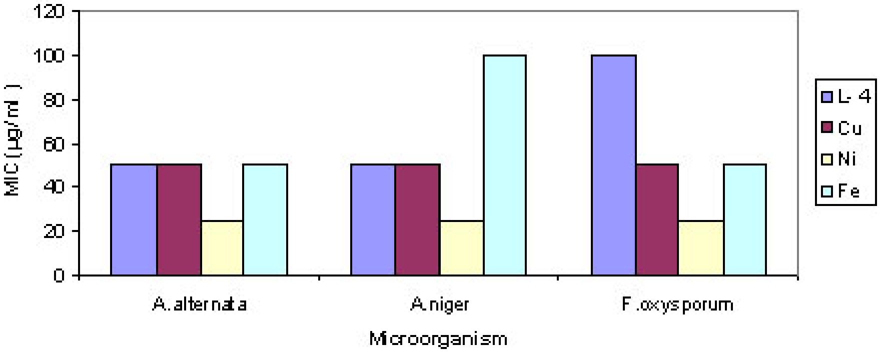

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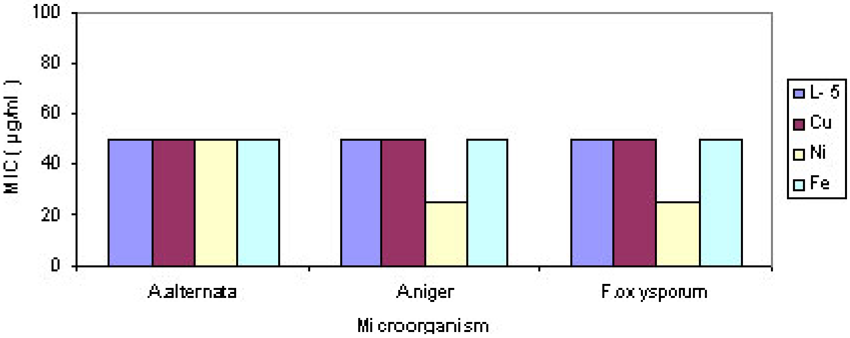

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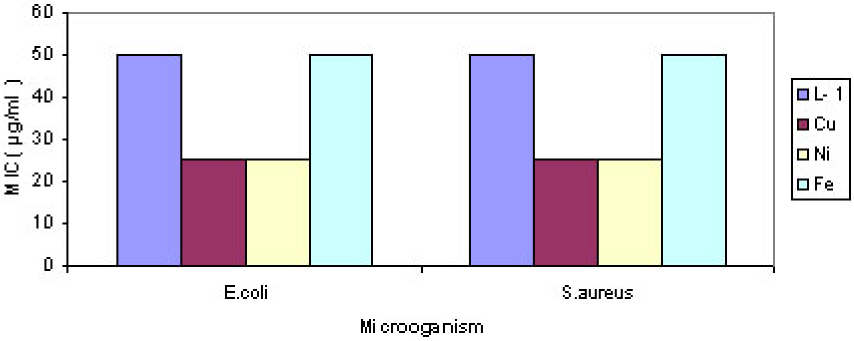

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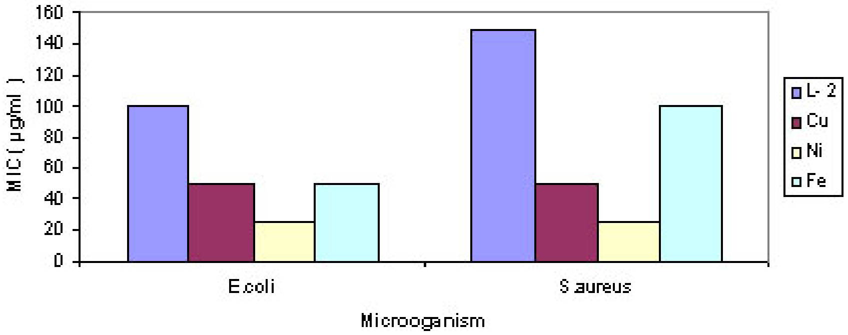
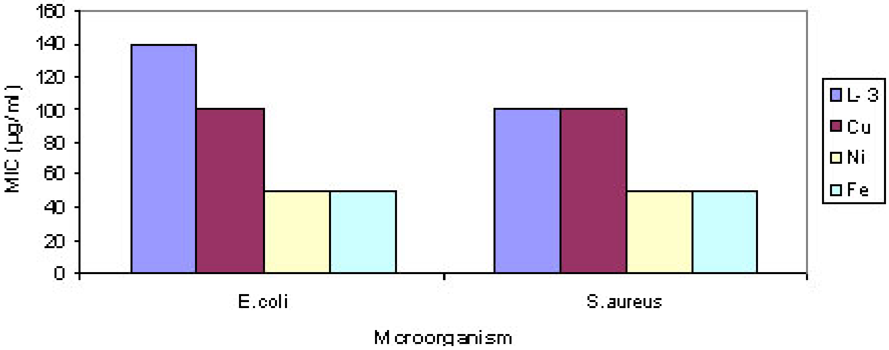


Fig. 7:



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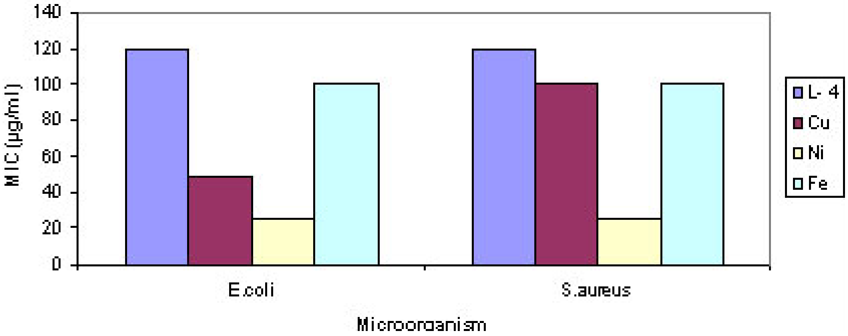

Fig. 9:

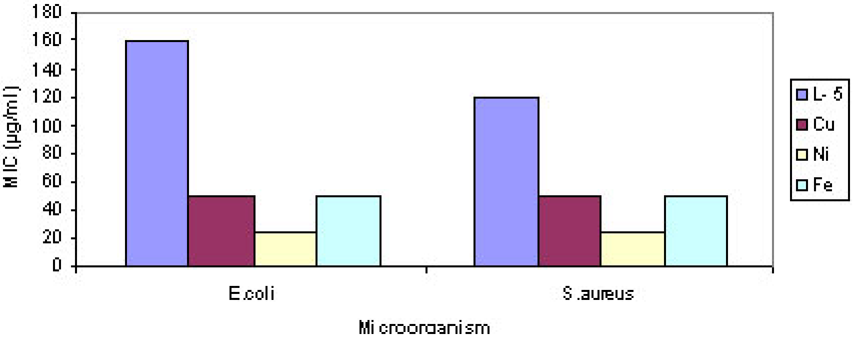

Fig. 10:

ligands showed a singlet at 2.0 ppm which is assign to the protons of >CH_2 group present. A multiplet is observed at 7.2 to 8.1 ppm which was due to the presence of aromatic protons. A singlet at 6.9 ppm clearly confirms the presence of phenolic protons in the ligands. Multiplet observed at 3.4-3.5 ppm was attributed to the protons of –C=NH group present in the ligand, it is suggested here that the carbon atom of this group is present in sp^2 hybridization state therefore the electron density around –NH proton decreases because of electron negativity effect of sp^2 hybridization, thus it shows some what down field chemical shift value. Another singlet present at 3.2ppm in the spectra which was probably due to the presence of >NH protons. The spectra of ligands (cyclo (1, 2) - dibiguanidinyl bis [2-hydroxy -ù- (2-chlorobenzoyl / 4-chlorobenzoyl / 2-nitrobenzoyl / 4-nitrobenzoyl) acetophenone]) has signals for the up fielded and down fielded aromatic protons because due to the presence of electron

Table 1: Physical and analytical data of nickel (II), Copper (II) and Iron (III complexes of Cyclo (1,2)-dibguanidinyl bis-[2-hydroxy-o-(benzoyl)2-chlorobenzoyl/4 -chlorobenzyol/2-nitrobenzoyl/4-nitrobenzoyl)acetophenone (L_r-L_v)

Compound	Molecular Formula	Yield (%)	Analytical data % observed (calculated)					Mole-cular weight
			C	H	N	Cl	M	
$[Ni(L_I)(H_2O)_2]Cl_2$	$C_{34}H_{34}N_{10}O_4Cl_2Ni$	65	52.23	4.31	17.95	9.09	7.28	768.5
			(52.65)	(4.38)	(18.06)	(9.16)	(7.60)	(774.9)
$[Ni(L_{II})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_4Cl_2Ni$	72	48.08	3.59	16.15	16.51	6.08	834.7
			(48.40)	(3.79)	(16.60)	(16.84)	(6.98)	(842.9)
$[Ni(L_{III})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_4Cl_2Ni$	74	47.78	3.71	16.30	16.01	6.27	83.57
			(48.40)	(3.79)	(16.60)	(16.84)	(6.98)	(842.9)
$[Ni(L_{IV})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_8Cl_2Ni$	72	46.96	3.39	19.07	8.10	6.39	863.1
			(47.12)	(3.69)	(19.40)	(8.22)	(6.80)	(865.7)
$[Ni(L_V)(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_8Cl_2Ni$	73	47.08	3.63	19.26	8.14	6.59	861.7
			(47.12)	(3.69)	(19.40)	(8.22)	(6.80)	(865.7)
$[Cu(L_I)(H_2O)_2]Cl_2$	$C_{34}H_{34}N_{10}O_4Cl_2Ni$	68	52.23	4.31	17.95	9.09	7.28	768.5
			(52.27)	(4.36)	(17.97)	(9.10)	(8.15)	(780.5)
$[Cu(L_{II})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_4Cl_2Ni$	70	48.09	3.37	16.17	16.61	7.39	841.7
			(48.17)	(3.77)	(16.52)	(16.75)	(7.48)	(847.5)
$[Cu(L_{III})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_4Cl_2Ni$	75	48.06	3.48	16.10	16.45	6.98	840.7
			(48.17)	(3.77)	(16.52)	(16.75)	(7.48)	(847.5)
$[Cu\ (L_{IV})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_8Cl_2Ni$	71	46.76	3.49	19.17	8.09	7.13	865.5
			(46.86)	(3.67)	(19.29)	(8.15)	(7.29)	(870.5)
$[Cu(L_V)(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_8Cl_2Ni$	71	46.70	3.45	19.19	8.07	7.19	867.1
			(46.86)	(3.67)	(19.29)	(8.15)	(7.29)	(870.5)
$[Fe(L_I)(H_2O)_2]Cl_2$	$C_{34}H_{34}N_{10}O_4Cl_2Ni$	75	52.72	4.12	17.99	9.14	7.08	767.5
			(52.89)	(4.27)	(18.13)	(9.18)	(7.23)	(772.10)
$[Fe(L_{II})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_4Cl_2Ni$	73	48.10	3.17	16.35	16.51	6.00	831.7
			(48.55)	(3.68)	(16.64)	(16.86)	(6.63)	(840.9)
$[Fe(L_{III})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_4Cl_2Ni$	71	48.11	3.47	16.35	16.31	6.10	830.0
			(48.55)	(3.68)	(16.64)	(16.86)	(6.63)	(840.9)
$[Fe(L_{IV})(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_8Cl_2Ni$	72	46.85	3.17	19.00	8.09	6.28	848.1
			(47.36)	(3.59)	(19.48)	(8.22)	(6.47)	(860.0)
$[Fe(L_V)(H_2O)_2]Cl_2$	$C_{34}H_{32}N_{10}O_8Cl_2Ni$	72	46.99	3.07	18.97	8.05	6.07	850.1
			(47.36)	(3.59)	(19.48)	(8.22)	(6.47)	(860.0)

withdrawing groups(2-Cl, 4-Cl, 2-NO_2 and 4-NO_2) the different protons at different position felt different magnetic field. In case of the chloro ligands the ortho proton is down fielded more then in case of nitro ligands this fact is clearly shown in spectra where a signal at 8.2ppm is observed in case of chloro ligands where as same is observed at 7.8ppm in case of nitro ligands.

The IR spectra of free ligands and their metal complexes have been recorded. The comparative study confirms the formation of macrocyclic framework. The IR spectra of free ligands shows the absorption band in the region of 1645-1615 cm^{-1} due to (C=N) stretching mode of >C=N-C group. This absorption band shifted downward to 1625-1590cm^{-1} in the spectra of metal complexes it suggests the involvement of imine nitrogen of this group in the bond formation with metal ion[10. This facts further confirmed by shifting of ν(C-N) to 1318-1307 and 1270-1240cm^{-1} which was observed in the region of 1340-1320 and 1295-1265 cm^{-1} in the spectra of ligands[11]. Nitrogen of N-H group did not participate in coordination. This fact gets supports from the evidence that the strecting vibration due to ν(C=N) mode of (>C=N-H) group and (N-H) group did not change in the spectra of metal complexes.

A new band around 1242-1227 cm^{-1} appeared in the spectra of complexes probably due to chelate ring vibration. In the spectra of complexes a broad band in the region of 3440-3395 cm^{-1} is formed. This band confirmed the presence of coordinated water molecule because this band appeared due to ν(O-H) mode. The spectra of complexes shows the down fall in the >C=N stretching frequency (25-20cm^{-1}) of >C=N-C group and n(C-N) by 30-15cm^{-1} in the spectra of the metal complexes due to the participation of nitrogen atom of >C=N-C group in coordination with metal atom. Participation of oxygen and nitrogen was further confirmed by the presence of new bands at 515-475 and 455-430 cm^{-1} which were attributed to ν(M-O) and ν(M-N) vibrations[12].

Table 2 shows the magnetic data of all complexes. The data shows the distorted octahedral geometry for Cu complexes and octahedral complexes for Ni and Fe complexes. The distorted octahedral geometry for Cu complexes confirmed by their effective magnetic moment values which ranges from 1.81- 1.90 B.M[13] .

The electronic spectra of copper complexes showed asymmetric broad maxima in the range of 13200-14600cm^{-1} which was assigned to $^2E_g \rightarrow {}^2T_{2g}$ electronic transition a characteristic for distorted octahedral geometry[6,14]. The J-T effect play a crucial role and the main cause for the distortion in the symmetry of Cu(II) complexes. In case of iron(III) three bands were found in the region of 12500-13000, 14500-15500, and 18500-24500 cm^{-1} due to $^6A_{1g} \rightarrow {}^4T_{1g}{}^{(G)}$, $^6A_{1g} \rightarrow {}^4T_{2g}{}^{(G)}$ and $^6A_{1g} \rightarrow {}^4A_{1g}{}^{(G)}$ transition which were in good agreement for octahedral geometry[6,10]. The magnetic moments for compounds $[Fe(L_1(H_2O)_2]Cl_2$ to $[Fe(L_V(H_2O)_2]Cl_2$ were 5.50 – 6.0 B.M. which gave indication for high spin d^5 configuration of octahedral geometry. These evidences are in good agreement with octahedral geometry for the Fe(III) complexes. In the present investigation, the nickel (II) complexes of the ligands L_I - L_V displayed three absorption bands in the region 10300-10830, 17610-17890 and 24110-25300 cm^{-1} which were assigned to $^3A_{2g}{}^{(F)} \rightarrow {}^3T_{2g}{}^{(F)}$ (ν_1), $^3A_{2g} \rightarrow {}^3T_{1g}{}^{(F)}$ (ν_2) and $^3A_{2g} \rightarrow {}^3T_{1g}{}^{(P)}$ (ν_3) transitions, respectively. The values were in good agreement with the octahedral geometry[15].

Antimicrobial activity

In vitro growth inhibitory activity against phytopethogenic fungi, viz., *Alternaria alternata, F.oxysporum* and *A.niger* and bacteria viz. *Escherichia coli* and *S.aureus.* were studied for all the ligands and their complexes. Two fold serial dilution technique is applied for the determination of minimum inhibitory concentration(MIC) value. The bar plots (Concentration vs compounds) were drawn to compare the activity pattern of the ligands and their metal complexes (Figs. 1-10). The results reveal that all the ligands exhibited the promising activity against tested microorganisms. The results showed that the antimicrobial activity of ligands increases on complexation with metal ions[16,17]. A comparative study of the ligands and their metal complexes indicate that the newly synthesized complexes have indeed been found to be more active in inhibiting the growth of fungi and bacteria than the precursors themselves[18]. The metal chelates inhibited the growth of microorganisms.

Table 2: Infrared, electron spectral data and magnetic moment of the complexes

Complexes	ν(N-H) of (>C=NH)	ν(C=N) of (>C=NH)	ν(C=N)	ν(C-N)	ν(M-O)	n (M-N)	ν_{max} (cm^{-1})	Magnetic Moment (μ) (B.M.)
$[Ni(L_I)(H_2O)_2]Cl_2$	3315,3185	1685	1615	1320,1265	500	460	10830,17890,25300	3.22
$[Ni(L_{II})(H_2O)_2]Cl_2$	3345,3250	1675	1600	1310,1255	495	470	10800,17610,25200	3.21
$[Ni(L_{III})(H_2O)_2]Cl_2$	3350,3255	1670	1595	1315,1250	490	445	10810,17610,25250	3.23
$[Ni(L_{IV})(H_2O)_2]Cl_2$	3290,3200	1665	1610	1310,1255	490	440	10300,17640,24110	3.16
$[Ni(L_V)(H_2O)_2]Cl_2$	3300,3190	1660	1615	1305,1250	495	445	10310,17650,24115	3.18
$[Cu(L_I)(H_2O)_2]Cl_2$	3315,3185	1685	1615	1320,1250	490	460	14800	1.97
$[Cu(L_{II})(H_2O)_2]Cl_2$	3345,3250	1675	1590	1310,1255	495	440	14250	1.98
$[Cu(L_{III})(H_2O)_2]Cl_2$	3350,3255	1670	1595	1310,1255	500	445	14200	1.99
$[Cu\ (L_{IV})(H_2O)_2]Cl_2$	3290,3200	1665	1610	1310,1255	490	440	13500	1.95
$[Cu(L_V)(H_2O)_2]Cl_2$	3300,3190	1660	1615	1305,1250	495	455	13550	1.96
$[Fe(L_I)(H_2O)_2]Cl_2$	3315,3185	1685	1605	1310,1260	495	460	12510,15510,22400	5.90
$[Fe(L_{II})(H_2O)_2]Cl_2$	3345,3250	1675	1600	1310,1255	500	445	12780,15540,23150	5.92
$[Fe(L_{III})(H_2O)_2]Cl_2$	3350,3255	1670	1590	1315,1250	490	440	12500,15500,23500	5.94
$[Fe(L_{IV})(H_2O)_2]Cl_2$	3290,3200	1665	1615	1315,1265	495	455	13350,15515,24110	5.90
$[Fe(L_V)(H_2O)_2]Cl_2$	3300,3190	1660	1620	1315,1255	500	445	13400,15525,24100	5.91

It is assumed that the production of the enzymes is being affected, as the microorganisms are unable to utilize food for itself or intake of nutrient decreases. The polarity of metal ion decreased after chelation because of partial sharing of its positive charge with the donor group and possible electron delocalization over the whole chelation ring. Because of this the lipophilic character of organism cell-metal complex increased, which subsequently favours its permeation through the lipid layers of the membrane and the normal cell process being impaired.

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