

Synthesis, characterization and antibacterial studies of 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one as bidentate ligand with some transition metals

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ABSTRACT

The new solid complexes of 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one, with Mn(II), Co(II), Ni(II) and Zr(IV) have been synthesized. The isolated metal complexes were characterized by melting point, C, H and N elemental analysis, magnetic studies, conductance measurements, infrared, UV-Vis. and mass spectra. The molar conductance in DMF solution indicates that the complexes are ionic. The thermal analyses (TGA and DTG) have been applied to study the thermal stability and the compositions of the prepared compounds. The results suggested that the ligand acts as a bidentate through the N and Cl atoms in all complexes forming 1:2 (M:L) molar ratio. The antibacterial activity of the ligand and synthesized complexes has been evaluated against three Gram-positive and three Gram-negative bacteria. The complexes showed high activities compared with free ligand.

Keywords: Dichlorobenzoxazin-4-one; metal ions; IR; Thermal analyses; Antibacterial activity.

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1. INTRODUCTION

Recently, the heterocyclic compounds especially containing nitrogen and sulphur donor atoms play an important role in pharmaceutical activities especially anti-inflammatory, anti-cancer and anti-viral activities [1-7]. In addition, they can form different types of coordination compounds due to several electron rich donor centers [8, 9].

A detailed literature research has shown that no work is reported on the 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one (L). Thus our aim of the work has been to study the coordination behavior of some novel 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one metal complexes. The complexes of Mn(II), Co(II), Ni(II) and Zr(IV) with L were synthesized and characterized by elemental analyses, conductivity, magnetic susceptibility measurements at room temperature, IR, UV-Vis., ^1H NMR, mass spectra and thermal analyses as well as some results of bioactivity tests are also included in order to examine the mode of binding and their structure.

2. EXPERIMENTAL

All the chemicals, organic solvents and inorganic reagents used for all synthesis of compounds and analytical work were of analytical reagent grade (BDH or Fluka or Sigma or Merck). The black solid of 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one (L) was prepared according to published method [10-12] by heating of 1mmol (0.206 g) of 3,5-dichloro anthranilic acid and 25 mL of acetic anhydride in round bottom flask for 3 hr in oil bath. The reaction mixture was concentrated and left at room temperature until the precipitate is formed, filtrate it under vacuum and the solid product was recrystallized from suitable solvent (ethanol). The black solid complex $[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$ was prepared by adding 4mmol (0.676 g) of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in 25 mL mixture of an ethanol-water

(1:1) drop wisely to a stirred solution of 8mmol (1.840 g) of ligand (L) in 25 mL of the same mixture in 1:2 molar ratio. The reaction mixture was stirred for 4 h where upon the complex precipitated. The black solid complex collected by filtration and purified by washing with an ethanol-water mixture (1:1) and diethyl ether, then dried under vacuum over calcium chloride anhydride. The black solid complexes $[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$, $[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and $[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$ were prepared in a similar manner described above by using $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$, respectively, in 1:2 molar ratio.

Elemental microanalysis of the four solid complexes and free ligand for C, H and N was carried out on a Perkin-Elmer CHN 2400 at the Micro-analytical Center, Cairo University. The percentage of the metal ions were determined gravimetrically by transforming the solid products into oxide or sulphate and also determined by using atomic absorption method. Spectrometer model PYE-UNICAM SP 1900 fitted with the corresponding lamp was used for this purposed. Infrared spectra of the prepared compounds were recorded as KBr pellets on a Perkin-Elmer FTIR type 1650 spectrophotometer in wave number region $4000\text{--}400\text{ cm}^{-1}$. Thermogravimetric (TGA) and differential thermogravimetric (DTG) analyses were carried out in dynamic nitrogen atmosphere (20 mL min^{-1}) with a heating rate of $10\text{ }^\circ\text{C/min}$ using Shimadzu TGA-50H thermal analyzer within the temperature range from room temperature to $1200\text{ }^\circ\text{C}$. Electronic spectra were obtained in the region of $800\text{--}200\text{ nm}$ using UV-3101PC Shimadzu. Mass spectra were recorded on GCMS-QP-2010 plus shimadzu (ESI-70 ev) in the range from $0\text{--}1090$. Molar conductivities of the prepared solutions of the ligand and four metal complexes in DMSO at $1.0 \times 10^{-3}\text{ M}$ were measured on CONSORT K410. All measurements were carried out at ambient temperature with freshly prepared

solution. Melting points were determined on an electrothermal-9100 apparatus. Magnetic measurements were carried out on a Scerwood scientific magnetic balance using Gouy method and $\text{Hg}[\text{Co}(\text{SCN})_4]$ as a calibrate.

3. ANTIBACTERIAL ACTIVITY

The effect of the tested new compounds 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one and its metal complexes on linear growth of tested gram positive and gram negative bacteria species such as *S. aureus*, *B. subtilis*, *E. faecalis*, *P. aeruginosa*, *E. coli* and *S. typhimurium* was recorded and investigated by a previously reported modified method of Beecher and Wong [13]. The Müller-Hington agar (30.0% Beef extract, 1.75% Casein hydrolysate, 0.15% Starch and 1.7% Agar) was prepared and then cooled to 47 °C and seeded with tested microorganisms. After solidification 5mm diameter holes were punched by a sterile cork-borer. The investigated compounds, i.e., ligand, metal salts and their complexes, were introduced in holes (only 100 μL) after being dissolved in DMSO at 10^{-3} M. These culture plates were then incubated at 37 °C for 20 h. The activity was determined by measuring the diameter of the inhibition zones (in mm). Growth inhibition was calculated with reference to the positive control, i.e., gentamicin and ampicillin.

4. RESULTS AND DISCUSSION

Mn(II), Co(II), Ni(II) and Zr(IV) ions reacted with 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one(L) forming the new solid complexes with molecular formulae $[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$, $[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$, $[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and $[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$. The found and calculated percentages of C, H and N data agree well with each other and these

prove the suggested molecular formulae (Table 1). The molar ratio for all synthesized complexes is L:M=2:1.

The molar conductivity is a method of testing the degree of ionization of the complexes, the molecular ions that a complex liberates in solution (in case of presence anions outside the coordination sphere), the higher will be its molar conductivity and vice versa [14-16]. The molar conductance values of the ligand (L) and their metal complexes in DMSO with standard reference, using 10^{-3} M solutions at room temperature were found to be in the range 10 to $170 \text{ S cm}^{-1} \text{ mol}^{-1}$. The data indicated that all the complexes were electrolyte and the sulphate, chloride and acetate ions found as counter ions (outside the complex sphere). Qualitative reactions [17] also agree well with the molar conductance data which revealed the presence of sulphate, chloride and acetate ions as counter ions. The complex solutions were tested with an aqueous solutions of BaCl_2 , AgNO_3 and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ forming a white precipitate of BaSO_4 , AgCl and red brown color of $\text{Fe}(\text{CH}_3\text{COO})_3$. Also, the Infrared spectra confirm the presence of sulphate and acetate as counter ions (Table 2) [18]. The magnetic moments (as B.M.) of the complexes were measured at room temperature. The complexes of Mn(II), Co(II) and Ni (II) are found in paramagnetism and their magnetic moments are 5.14, 3.90 and 4.64 B.M., respectively, with molecular geometries octahedral. The thermogravimetric and infrared spectroscopic data also confirm the presence of water in the composition of the complexes.

Table 1: Elemental analysis and Physico-analytical data for L and its metal complexes.

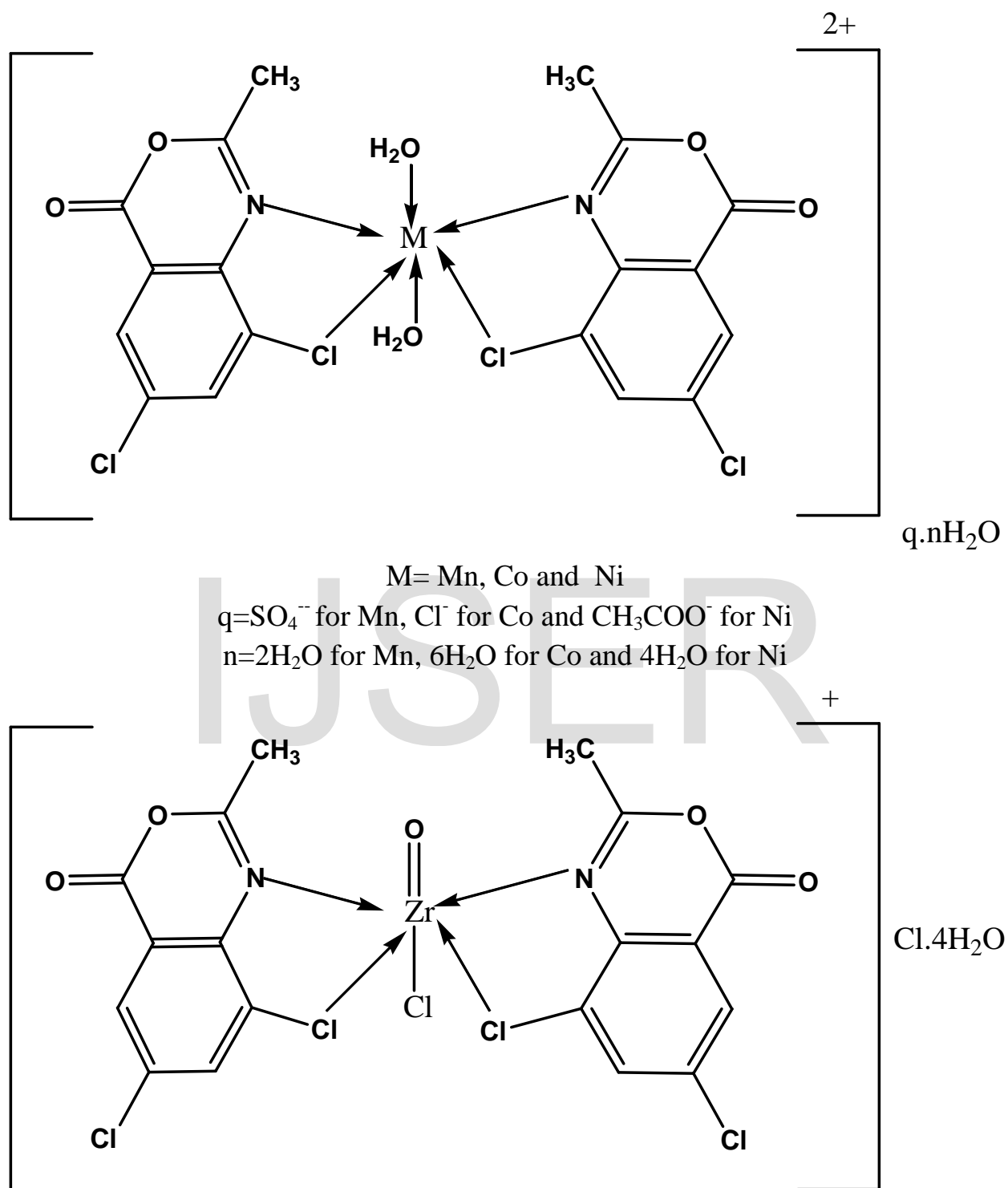
Compounds M.Wt. (M.F.)	Yield %	M.p. °C	Color	Found (Calcd.)(%)						μ_{eff} (B.M)	(S cm ² mol ⁻¹)
				C	H	N	M	Cl	S		
L 230 (C ₉ H ₅ O ₂ NCl ₂)	79.35	200-204	Black	(46.96) 46.93	(2.17) 2.15	(6.09) 6.03	- -	(30.87) 30.83	-	-	10
[Mn(L) ₂ (H ₂ O) ₂]SO ₄ .2H ₂ O 682.94 (C ₁₈ H ₁₈ O ₁₂ N ₂ Cl ₄ SMn)	75.31	290-294	Black	(31.63) 31.60	(2.63) 2.60	(4.10) 4.00	(8.04) 8.00	(20.79) 20.74	(4.68) 4.65	5.14	95
[Co(L) ₂ (H ₂ O) ₂]Cl ₂ .6H ₂ O 733.9 (C ₁₈ H ₂₆ O ₁₂ N ₂ Cl ₆ Co)	76.16	270-273	Black	(29.43) 29.32	(3.54) 3.50	(3.82) 3.80	(8.03) 8.00	(29.02) 29.00	-	3.90	170
[Ni(L) ₂ (H ₂ O) ₂](CH ₃ COO) ₂ .4H ₂ O 744.7 (C ₂₂ H ₂₈ O ₁₄ N ₂ Cl ₄ Ni)	73.96	296-298	Black	(35.45) 35.40	(3.76) 3.74	(3.76) 3.73	(7.88) 7.85	(19.06) 19.00	-	4.64	155
[ZrO(L) ₂ Cl]Cl.4H ₂ O 710.2(C ₁₈ H ₁₈ O ₉ N ₂ Cl ₆ Zr)	74.21	319-322	Black	(30.41) 30.38	(2.53) 2.50	(3.94) 3.90	(12.84) 12.80	(29.99) 29.95	-	-	98

4.1. INFRARED SPECTRA

The infrared spectra of the four complexes are compared with those of the free ligand 6,8-dichloro-2-methyl-3, 1-(4H)-benzoxazin-4-one (L) in order to determine the coordination sites that may be involved in chelation. The presence of the spectral absorption bands in the region $3460\text{--}3420\text{ cm}^{-1}$ indicates the presence of water molecules in the obtained complexes [19, 20]. The (C=O) stretching vibration of free L and its complexes is found at around 1665 cm^{-1} . The shift of (C=O) in ligand and complexes does not change which indicates that L in coordination but there is no formation of a bond between the carbonyl oxygen of L with the central metal ions, and that nitrogen atom is the donor atom. The band observed at 1582 cm^{-1} in the spectrum of the ligand has been assigned to the stretching of (C=N) [21-29]. The shift of the characteristic band of (C=N) to a lower value from 1582 cm^{-1} to around 1561 cm^{-1} indicate the involvement of C=N group in the interaction of ligand with metal ion [30]. Also, the data given in Table 2 shows that (Zr=O) is a strong band at 802 cm^{-1} [31, 32].

The coordination of the metal ions via nitrogen of L and oxygen of water molecules is also confirmed by (M-O) and (M-N) bands at 580, 525 and 500 cm^{-1} for Mn(II), at 586, 559 and 463 cm^{-1} for Co(II), at 584, 555 and 502 cm^{-1} for Ni(II) and at 586, 505 and 470 cm^{-1} for Zr(IV). Therefore, from the infrared spectra, it is concluded that the free L behaves as a neutral bidentate ligand with nitrogen and chlorine atoms as a donor site, involved in the coordination sphere.

The proposed structure of the metal complexes on the basis of the results discussed in this paper may be as follows (Scheme 1):



Scheme 1: The coordination mode of Mn(II), Co(II), Ni(II) and Zr(V), with 6,8-dichloro-2-methyl-3,1-(4H)-benzoxazin-4-one

According to the proposed structures of the complexes under investigation, the complexes possess a two-fold axis and two plane of symmetry and hence they are C_{2v} symmetry. The C_{2v} complexes are expected to display 129, 129, 129 and 117 vibrational fundamentals for Mn(II), Co(II), Ni(II) and Zr(IV) which are all non-degenerate. These are distributed between A_1 , A_2 , B_1 and B_2 motions; all are IR and Raman active, except for the A_2 modes which are only Raman active.

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Table 2: Infrared frequencies (cm^{-1}) and tentative assignments for (A) L, (B) $[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$, (C) $[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$, (D) $[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and (E) $[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$.

Compound	(O-H); H_2O ; COOH	(C=O); COOH	(C=O)	(C=N)	(Zr=O)	(M-N)
L	-	-	1666vs	1582vs	-	-
$[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$	3450m	-	1666vs	1560vs	-	580m, 525m, 500w
$[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$	3420w	-	1666vs	1562vs	-	586m, 559w, 463w
$[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$	3460w	1713s	1666vs	1563vs	-	584m, 555m, 502w
$[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$	3430mbr	-	1663vs	1560s	802s	586w, 505m 470m

vs = very strong, s=strong, w=weak, ms= medium strong, m= medium, mbr= medium broad and =stretching

4.2. UV-VISIBLE SPECTRA

The formation of the metal complexes of the ligand L was also confirmed by the electronic liquid UV-Visible spectra. The electronic absorption spectra for each of the L along with its Mn(II), Co(II), Ni(II) and Zr(IV) complexes in DMSO-d₆ solution and the wavelength interval from 200 to 800 nm range are shown in Figure (1). It shows that free L appears at 235, 244, 266 and 300 nm (Table 3). The first three bands at 235, 244, and 266 nm may be attributed to $\pi \rightarrow \pi^*$ transition and the second band observed at 300 nm is assigned to $n \rightarrow \pi^*$ transitions, these transitions occur in case of unsaturated hydrocarbons which contain ketone groups [31]. The shift of the bands to higher or lower values in Mn(II), Co(II), Ni(II) and Zr(IV), complexes and presence of new bands in the spectra of complexes indicated that the formation of their metal complexes. The four complexes have bands at range 496-615 nm upon the complexation may be due to the transition of the type of ligand to metal charge-transfer and (d-d) transition [24].

The reflectance spectrum of Mn(II) complex showed one band at 597 nm which is assigned to ${}^6A_{1g} \rightarrow {}^4T_{2g}({}^4G)$ transition [33, 34]. The Co(II) complex absorption spectrum show one absorption band at 600 nm which is assigned to ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transition in favor of octahedral geometry [34-36]. The Ni(II) complex absorption spectrum show one absorption band at 615 nm which are assigned to ${}^3A_{2g}(P) \rightarrow {}^3T_{1g}(P)$ transition, in favor of octahedral geometry [37-39].

Table 3: UV-Vis. Spectra of 6,8-dichloro-2-methyl-3, 1-(4H)- benzoxazin-4-one (L) and its metal complexes.

Assignments (nm)	(L)	Complexes			
		Mn(II)	Co(II)	Ni(II)	Zr(IV)
- * transitions	235	217	220	232	216
	244	230	230	264	266
	266	243	247		
		266	266		
n- * transitions	300	310	315	308	312
Ligand-metal charge transfer	-	496	506	503	498
d-d transition	-	597	600	615	-

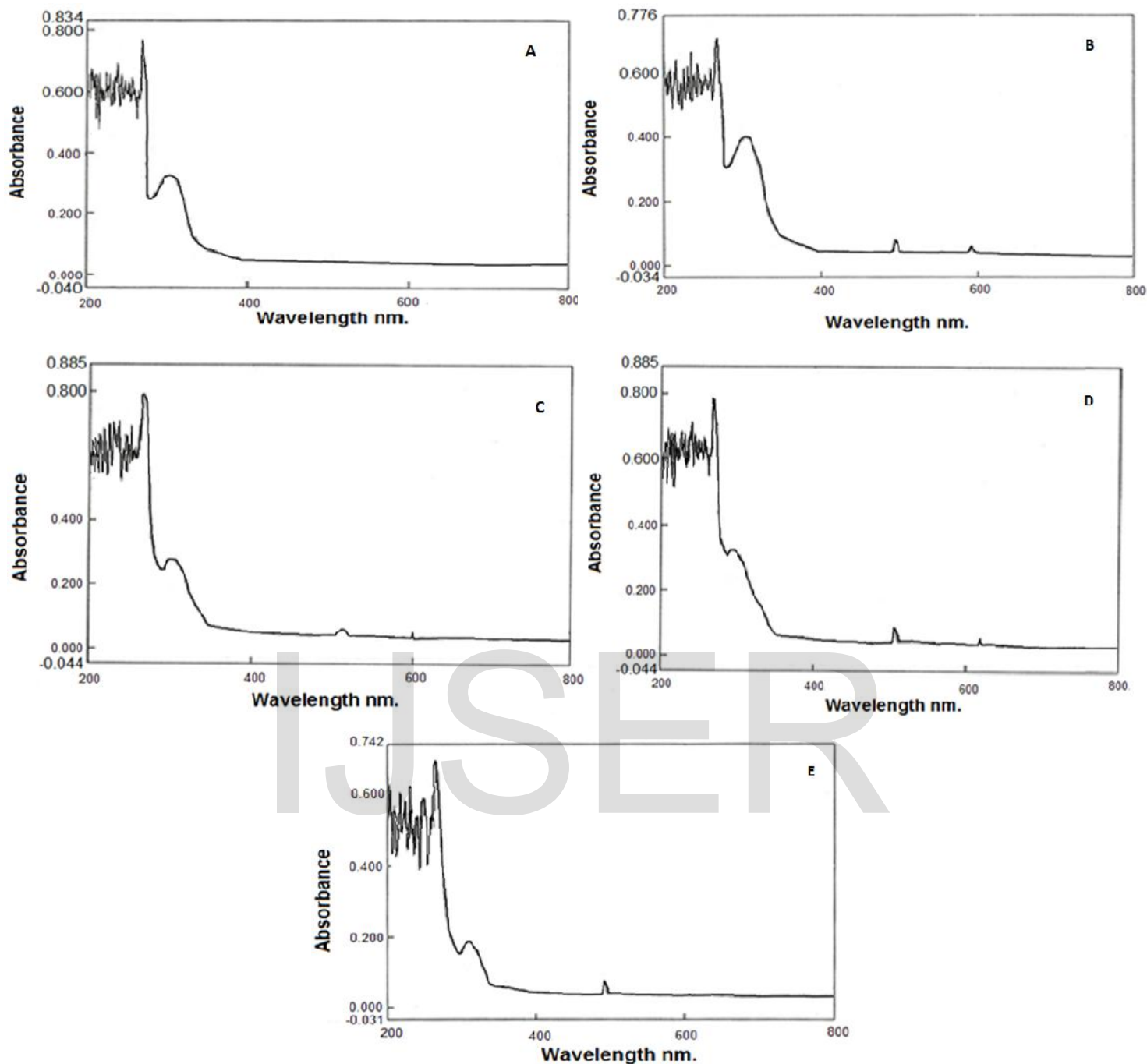
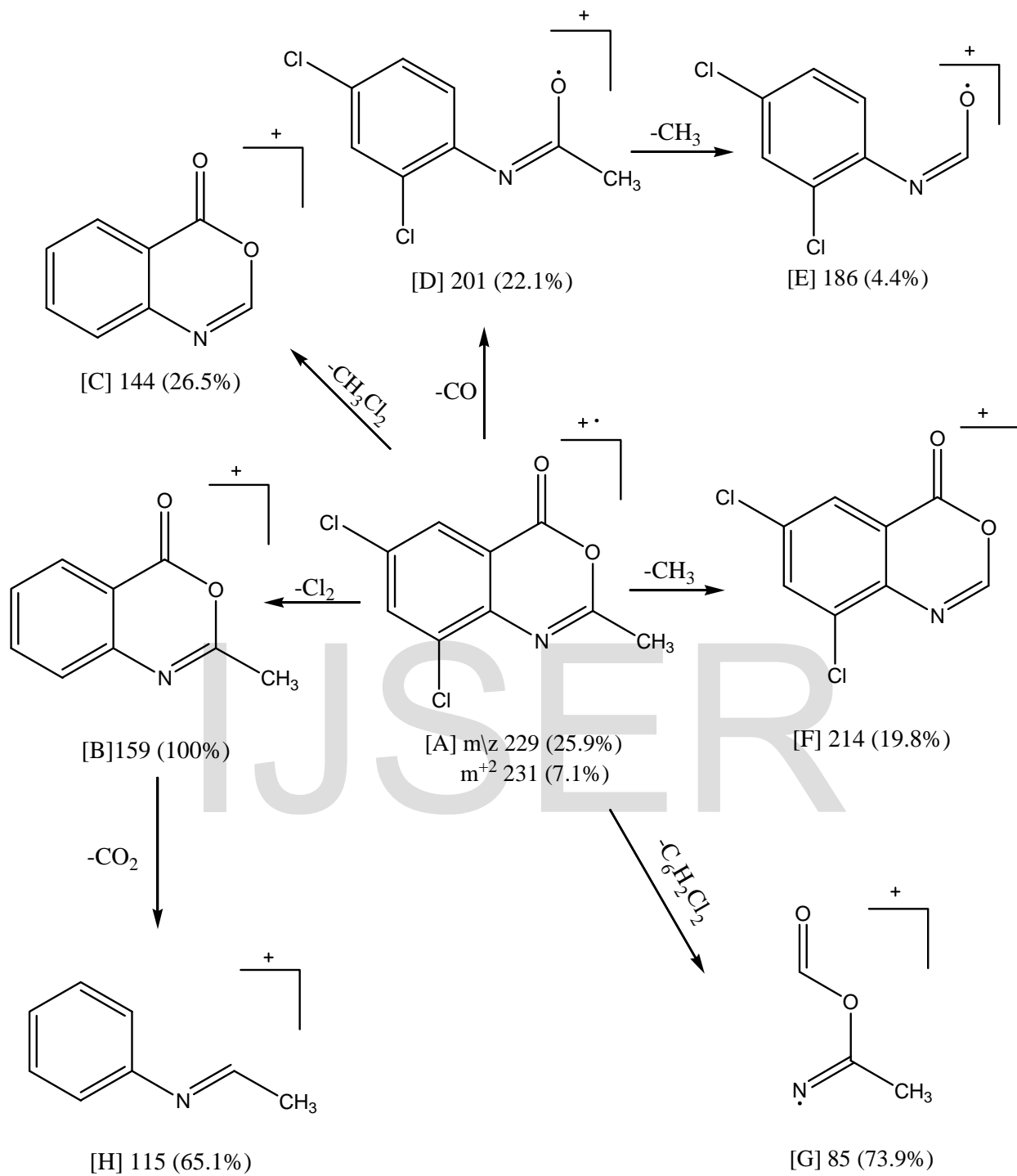


Fig. 1: Electronic reflection Spectra for (A) L; (B) $[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$; (C) $[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$; (D) $[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and (E) $[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$.

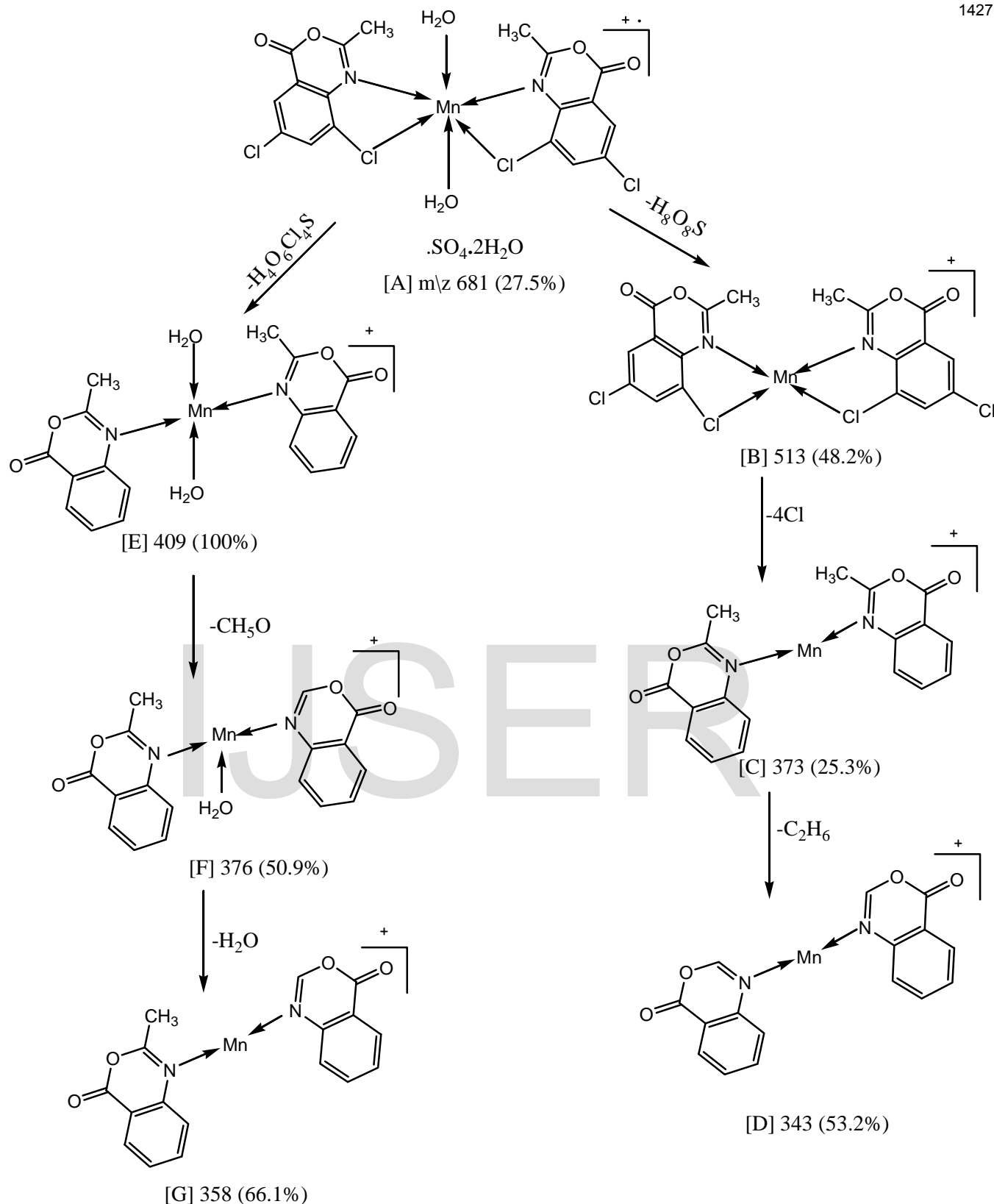
4.3. MASS SPECTRA

Mass spectrometry was found useful as a complementary tool. The structure and stability of coordination complexes under ionization conditions are dependent on various factors like the ligand itself, metal ion, counter ions, solvent, temperature and concentration. Mass spectrum of the ligand (L) $[C_9H_5NO_2Cl_2]$ is in a good agreement with the suggested structure (Fig. 2 and Scheme 2). The ligand showed molecular ion peak (M^+) at $m/z = 229$ (25.9 %), and M^{+2} at $m/z = 231$ (7.1 %) abundance which agree well with the suggested formula. The molecular ion peak [A] gave a fragment [B] by losing Cl_2 which refer to base peak at $m/z = 159$ (100 %), which loses CO_2 to give a fragment [H] at $m/z = 115$ (65.1 %), the molecular ion peak [A] gave a fragment [F] by losing CH_3 with $m/z = 214$ (19.8 %). Also it gave fragment [G] at $m/z = 85$ (73.9 %) by losing $C_6H_4Cl_2$, it also lose CH_3Cl_2 to give a fragment [C] at $m/z = 144$ (26.5 %), the molecular ion peak [A] gave a fragment [D] at $m/z = 201$ (22.1 %), which can lose CH_3 to give fragment [E] at $m/z = 186$ (4.4 %).

The fragmentation patterns of our studies complexes were obtained from the mass spectra (Fig. 2). The mass spectra of Mn(II), Co(II), Ni(II) and Zr(IV) complexes displayed molecular peaks at m/z (%) 681 (27.5 %), 732 (12.7 %), 743 (8.7 %) and 708 (27.8 %), respectively, suggesting that the molecular weights of the assigned product matching with elemental and thermogravimetric analyses. Fragmentation pattern of the complex $[Mn(L)_2(H_2O)_2]SO_4 \cdot 2H_2O$ is given as an example in scheme 3, appeared at $m/z = 681$ (27.5 %) loses H_8O_8S to give fragment [B] at $m/z = 513$ (48.2 %) which loses $4Cl^-$ to give fragment [C] a $m/z = 373$ (25.3 %) which gave a fragment [D] by losing C_2H_6 at $m/z = 343$ (53.2 %) and The molecular ion peak [A] can lose $H_4O_6Cl_4S$ to give a fragment [E] which refers to the base peak at $m/z = 409$ (100 %), which can lose CH_5O to give fragment [F] at $m/z = 376$ (50.9 %) which gave a fragment [G] at $m/z = 358$ (66.1 %).



Scheme2: Fragmentation pattern of L.



Scheme 3: Fragmentation pattern of $[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$.

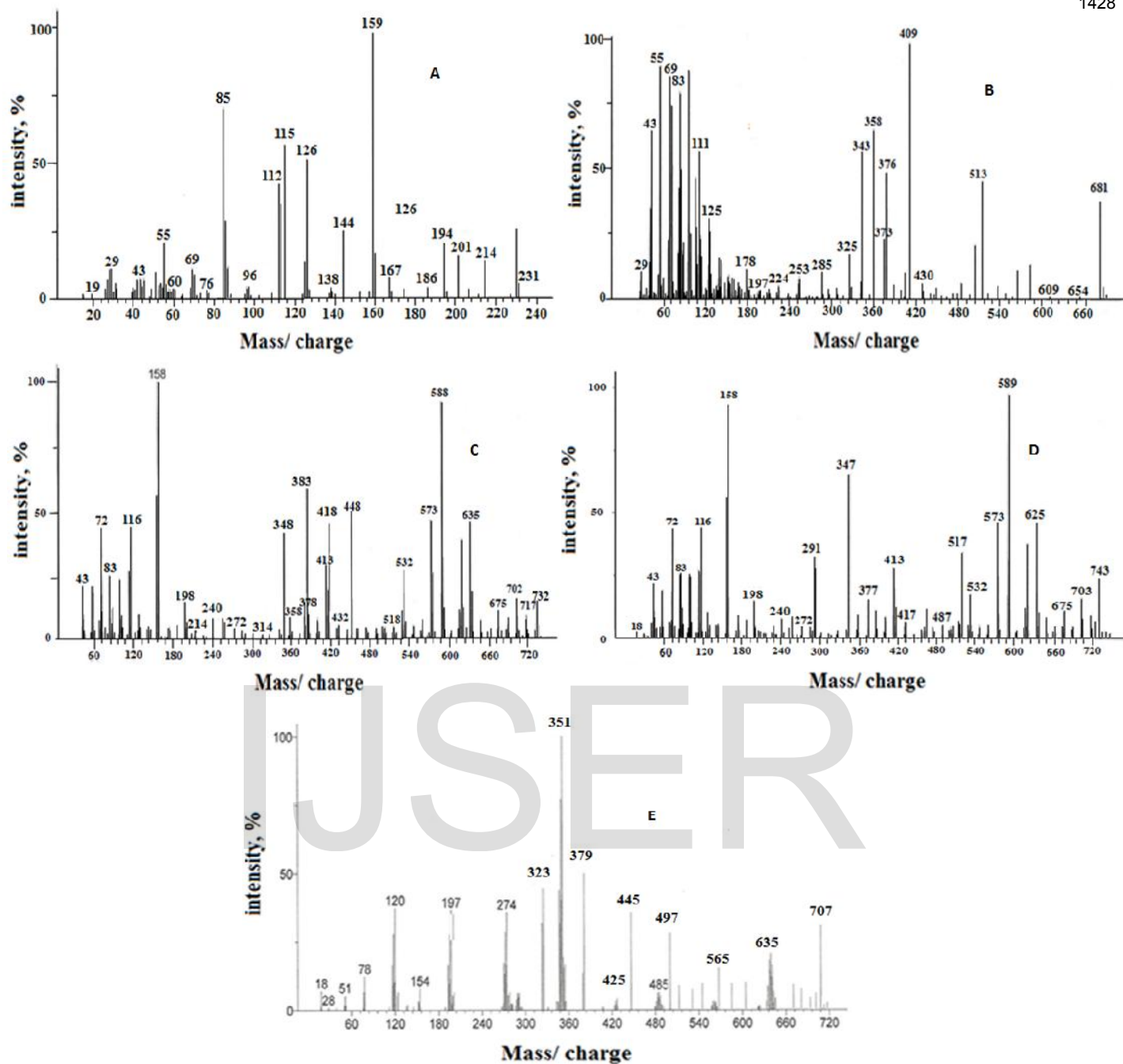


Fig. 2: Mass Spectrum diagrams for (A) L; (B) $[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$; (C) $[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$; (D) $[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and (E) $[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$.

4.4. THERMAL ANALYSES

Thermogravimetric analyses (TGA and DTG) of the ligand L under investigation and the four solid complexes were carried out to get information about the thermal stability of these new compounds. The TGA and DTG curves of the ligand and their complexes are shown in Figure (3). The maximum temperature values along with the weight losses for each compound within the corresponding temperature ranges are listed in Table 4.

Ligand ($C_9H_5NO_2Cl_2$) is thermally stable at the temperature range 25-74 °C. Decomposition of this ligand started at 74 °C and finished at 650 °C with one stage at one maximum 190 °C and is accompanied by a weight loss 89.55%, corresponding exactly to the loss of $2CO+2C_2H_2+HCN+Cl_2$ which is very closely to calculated value 89.57%.

The thermal decomposition of Mn(II) complex within two main degradation steps at temperature range from 44 to 650 °C. The first step of decomposition occurs from 44 to 110 °C is accompanied by a weight loss of 5.19% in agreement with the theoretical values 5.27 % for the loss of two uncoordinated water molecules at maximum temperature 77 °C. The second step of decomposition occurs at two maxima 182 and 503 °C with a weight loss of 72.76% which almost agree with the calculated one 72.63 % this associated with the loss of ligand and two coordinated water molecules forming manganese sulphate as a final product.

The thermal decomposition of $[Co(L)_2(H_2O)_2]Cl_2.6H_2O$ complex proceeds with two degradation steps. The first one occurred at a temperature range 28 to 85 °C with a maximum temperature at 44 °C and the weight loss found at this stage equals to 14.35 % which is in good agreement with the calculated values of 14.72% and the weight loss found at this stage corresponds to loss of six water crystallization molecules, while the second one occurred at temperature range from 86 to 632 °C with two maxima temperature at 128 and 170 °C with a weight loss

of 65.32% in agreement with the theoretical values 65.27% and give $5\text{C}_2\text{H}_2+2\text{HCl}+2\text{Cl}_2+2\text{NO}+\text{H}_2\text{O}+2\text{CO}$. The actual weight loss from these two steps is 79.67%, very closed to the calculated value 79.99%.

The thermal degradation for the $[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ exhibits two degradation steps. The first step of decomposition occurred in the range 51-127 °C, with a maximum temperature at 89 °C, is accompanied by a weight loss of 9.60% corresponding exactly to the loss of four water molecules which is in a good agreement with the calculated values 9.67%. The second step of degradation occurred at two maxima at 190 and 438 °C and is accompanied by a weight loss of 74.01%, which is approximately corresponding to the calculated loss 73.86% of $4\text{HCl}+7\text{C}_2\text{H}_2+\text{H}_2\text{O}+2\text{NO}_2+4\text{CO}$ leaving $\text{NiO}+4\text{C}$ as residue. The actual weight loss from these two steps is 83.61%, close to the calculated value 83.53%.

For Zr(IV) complex the thermal decomposition exhibits two main degradation steps. The first step of decomposition occurred from 38 to 154 °C with one maximum temperature at 63 °C is accompanied by a weight loss of 7.35% in agreement with the theoretical value 10.14% for the loss of four uncoordinated water molecules. The second step of decomposition occurs at three maxima temperature at 189, 256 and 548 °C with a weight loss of 66.81% in agreement with the theoretical value 64.07% and the weight loss found at this stage corresponds to loss of $5\text{C}_2\text{H}_2+3\text{Cl}_2+\text{N}_2+3\text{CO}$.

Table 4: The maximum temperature T_{\max} ($^{\circ}\text{C}$) and weight loss values of the decomposition stages for L and its complexes with Mn(II), Co(II), Ni(II) and Zr(IV).

Compounds	Decomposition	T_{\max} ($^{\circ}\text{C}$)	Weight loss (%)		Lost species
			Calc.	Found	
L ($\text{C}_9\text{H}_5\text{Cl}_2\text{NO}_2$)	First step	190	89.57	89.55	$2\text{CO}+2\text{C}_2\text{H}_2+\text{HCN}+\text{Cl}_2$
	Total loss		89.57	89.55	
	Residue		10.43	10.45	2C
$[\text{Mn}((\text{L})_2(\text{H}_2\text{O})_2)]\text{SO}_4 \cdot 2\text{H}_2\text{O}$ ($\text{C}_{18}\text{H}_{18}\text{O}_{12}\text{N}_2\text{Cl}_4\text{SMn}$)	First step	77	5.27	5.19	$2\text{H}_2\text{O}$
	Second step	182,503	72.63	72.76	$7\text{C}_2\text{H}_2+2\text{NO}+2\text{Cl}_2+4\text{CO}$
	Total loss		77.90	77.95	
	Residue		22.10	22.05	MnSO_4
$[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ ($\text{C}_{18}\text{H}_{26}\text{O}_{12}\text{N}_2\text{Cl}_6\text{Co}$)	First step	44	14.72	14.35	$6\text{H}_2\text{O}$
	Second step	128,170	65.27	65.32	$5\text{C}_2\text{H}_2+2\text{HCl}+2\text{Cl}_2+2\text{NO}+$ $\text{H}_2\text{O}+2\text{CO}$
	Total loss		79.99	79.67	
	Residue		20.01	20.30	$\text{CoO}+6\text{C}$
$[\text{Ni}((\text{L})_2(\text{H}_2\text{O})_2)](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ ($\text{C}_{22}\text{H}_{28}\text{O}_{14}\text{N}_2\text{Cl}_4\text{Ni}$)	First step	89	9.67	9.60	$4\text{H}_2\text{O}$
	Second step	190,316, 438	73.86	74.01	$4\text{HCl}+7\text{C}_2\text{H}_2+\text{H}_2\text{O}+2\text{NO}_2$ $+4\text{CO}$
	Total loss		83.53	83.61	
	Residue		16.47	16.39	$\text{NiO}+4\text{C}$
$[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$ ($\text{C}_{18}\text{H}_{18}\text{O}_9\text{N}_2\text{Cl}_6\text{Zr}$)	First step	63	10.14	9.85	$4\text{H}_2\text{O}$
	Second step	189,256, 548	64.07	64.31	$5\text{C}_2\text{H}_2+3\text{Cl}_2+\text{N}_2+3\text{CO}$
	Total loss		74.21	74.16	
	Residue		25.79	25.84	ZrO_2+5C

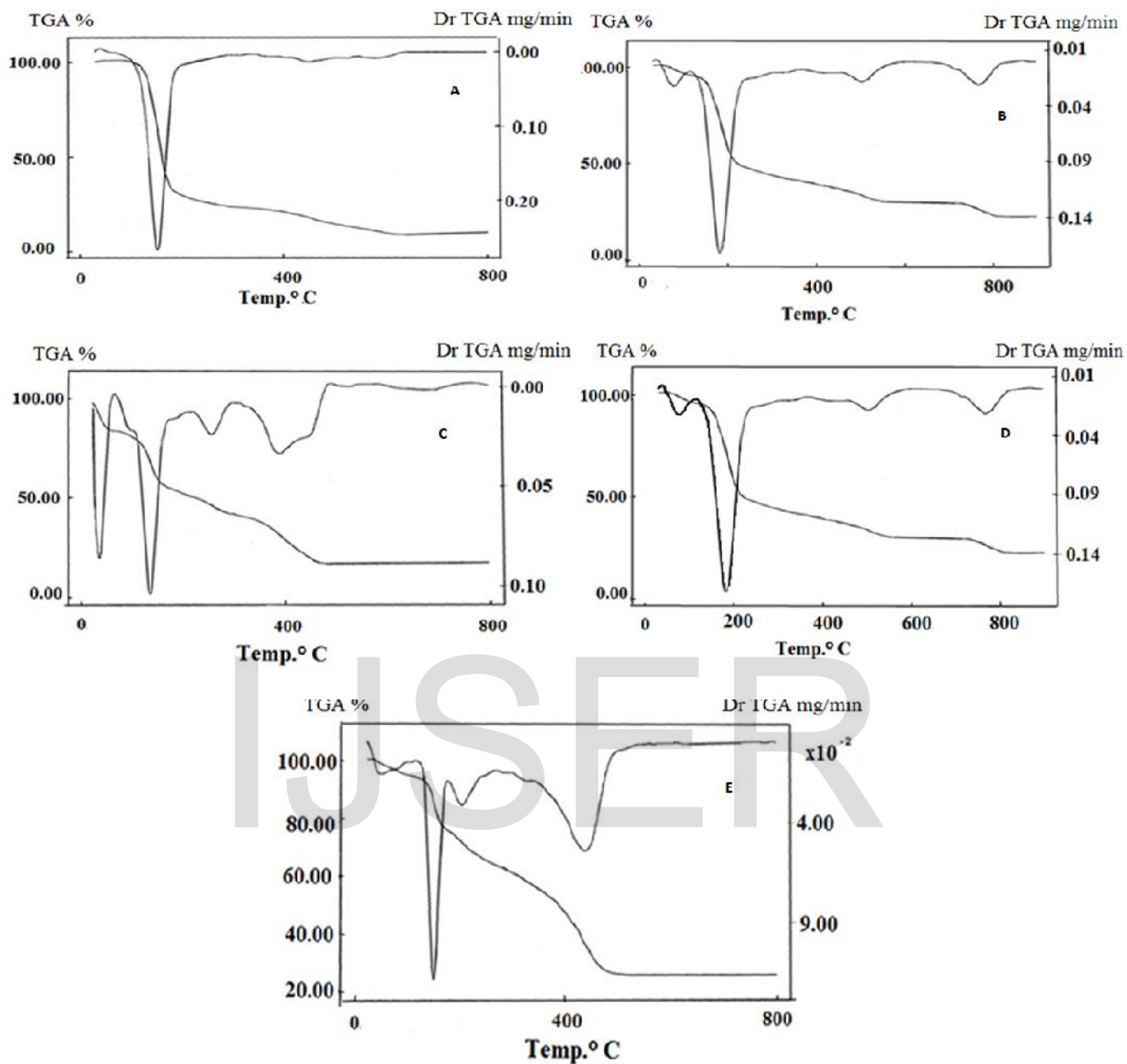


Fig. 3: TGA and DTG diagram for (A) L; (B) $[\text{Mn}(\text{L})_2(\text{H}_2\text{O})_2]\text{SO}_4 \cdot 2\text{H}_2\text{O}$; (C) $[\text{Co}(\text{L})_2(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$; (D) $[\text{Ni}(\text{L})_2(\text{H}_2\text{O})_2](\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and (E) $[\text{ZrO}(\text{L})_2\text{Cl}]\text{Cl} \cdot 4\text{H}_2\text{O}$.

4.5. ANTIMICROBIAL ACTIVITY:

The ligand as well as their metal complexes was evaluated for their some bacterial strains, such as *Staphylococcus aureus*, *Bacillus subtilus* and *Enterococcus faecalis* as gram positive bacteria and *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurimas* gram negative bacteria. As assessed by color, the complexes remain intact during biological testing Figure (4) and Table 5. A comparative study of ligand and their metal complexes showed that the metal complexes exhibit higher antibacterial activity for Gram-positive and Gram-negative bacteria. Where, for Mn(II) complex, it showed a moderate significant effect on *Staphylococcus aureus*, *Bacillus subtilus* and *Enterococcus faecalis* and a highly significant on *Escherichia coli* but has no significant effect on *Pseudomonas aeruginosa* and *Salmonella typhimurim*. For Co(II) complex, it has a highly significant on *Staphylococcus aureus*, *Bacillus subtilus* and *Escherichia coli* and a moderate effect on *Pseudomonas aeruginosa* but no significant effect on *Salmonella typhimurim* and *Enterococcus faecalis*. For Ni(II) complex, it has a very highly significant on *Staphylococcus aureus*, *Bacillus subtilus*, *Salmonella typhimurim* and *Escherichia coli*, and a highly significant on *Enterococcus faecalis* but no significant effect on *Pseudomonas aeruginosa*. For Zr(IV) complex, it has a moderate significant effect on *Staphylococcus aureus*, *Bacillus subtilus*, *Salmonella typhimurim* and *Escherichia coli*, while it has a highly significant on *Pseudomonas aeruginosa* and *Enterococcus faecalis*. All metal salts showed no antibacterial activity.

The results are promising compared with the previous studies [40-45]. Such increased activity of metal chelate can be explained on the basis of the oxidation state of the metal ion, overtone concept and chelation theory. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials in which liposolubility is an

important factor that controls the antimicrobial activity. On chelation the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of the complexes [42]. This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. In general for metal complexes showing antimicrobial activity, the following five principal factors should be considered: i) the chelate effect; ii) the nature of the ligands; iii) the total charge of the complex; iv) the nature of the ion neutralizing the ionic complex; v) the nuclearity of the metal center in the complex. All of the five above mentioned factors are present to our compounds except the second factor.

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Table 5: The inhibition diameter zone values (mm) for L and its metal complexes:

Compounds		Microbial species (Tested microorganisms)					
		Gram Positive Bacteria			Gram Negative Bacteria		
		S.aureus	B. subtilis	E.faecalis	P.aeruginosa	S.typhimurium	E.Coli
L		11.4 ±0.58	13.6 ±0.28	10.2 ±0.33	16.1 ±0.22	14.0 ±0.58	12.2 ±0.28
L/Mn (II)		19.3 ⁺¹ ±0.44	20.1 ⁺¹ ±0.58	18.9 ⁺¹ ±0.44	18.3 ^{NS} ±0.32	16.6 ^{NS} ±0.44	23.8 ⁺² ±0.58
L/Co (II)		24.9 ⁺² ±0.58	26.6 ⁺² ±0.44	12.1 ^{NS} ±0.44	23.4 ⁺¹ ±0.28	15.3 ^{NS} ±0.58	29.3 ⁺² ±0.28
L/Ni (II)		33.7 ⁺³ ±0.58	38.4 ⁺³ ±0.44	28.9 ⁺² ±0.44	ND	38.3 ⁺³ ±0.28	32.4 ⁺³ ±0.58
L/Zr (IV)		18.8 ⁺¹ ±0.28	19.7 ⁺¹ ±0.58	34.2 ⁺² ±0.23	29.2 ⁺² ±0.23	20.4 ⁺¹ ±0.58	19.1 ⁺¹ ±0.28
MnSO ₄ .H ₂ O		0	0	0	0	0	0
CoCl ₂ .6H ₂ O		0	0	0	0	0	0
Ni(CH ₃ COO) ₂		0	0	0	0	0	0
ZrOCl ₂ .8H ₂ O		0	0	0	0	0	0
Standard	Ampicillin	27.4 ±0.18	32.4 ±0.10	20.4 ±0.11	-	-	-
	Gentamicin	-	-	-	17.3 ±0.15	28.8 ±0.24	22.3 ±0.18

ND: Non-detectable i.e., the inhibition zones exceeds the plate diameter

Statistical significance P^(NS) P not significant, P > 0.05; P⁽⁺¹⁾ P significant, P < 0.05; P⁽⁺²⁾ P highly significant, P < 0.01; P⁽⁺³⁾ P very highly significant, P < 0.001; student's t-test.

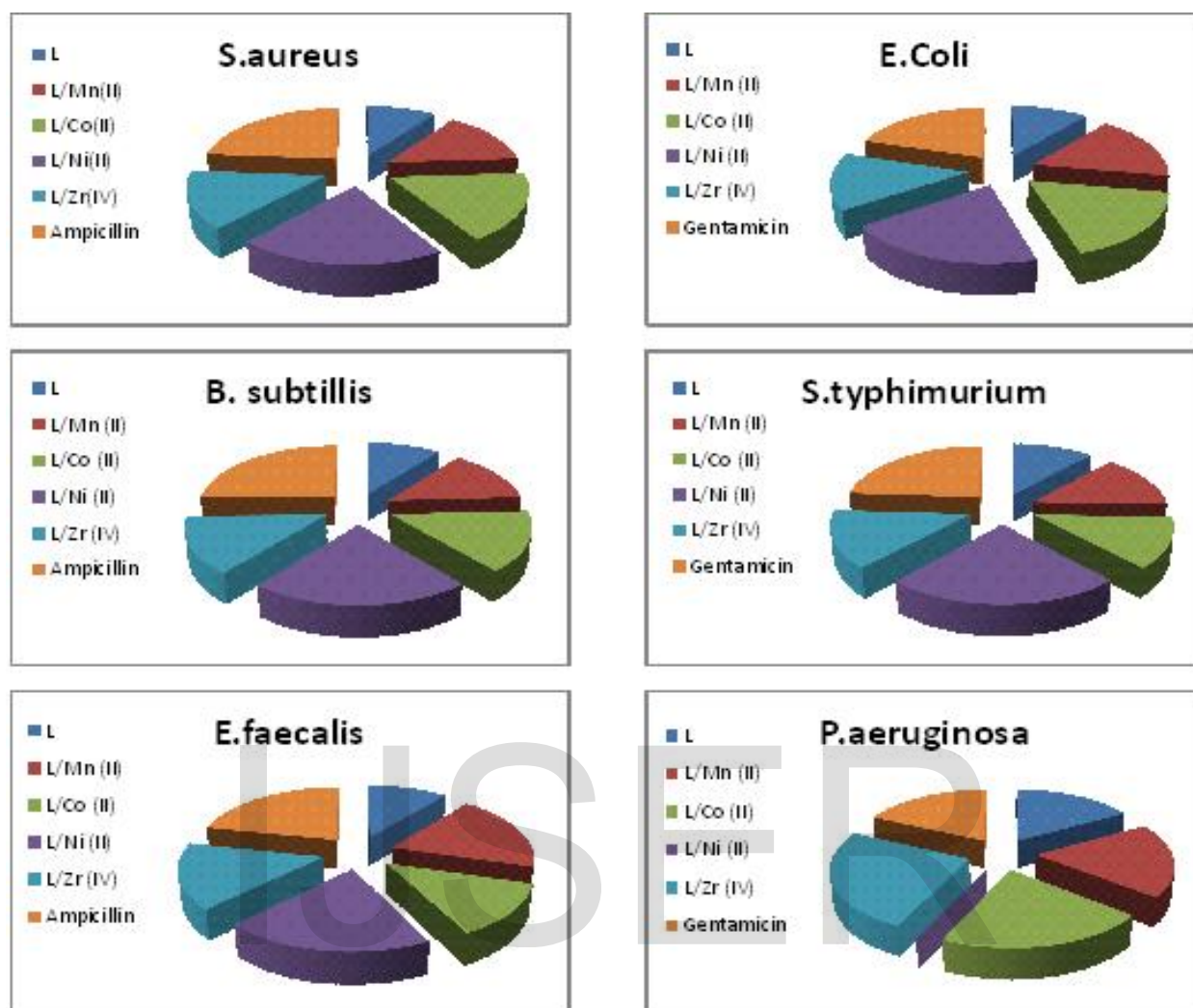


Fig.4: Statistical representation for biological activity of L and its metal complexes.

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