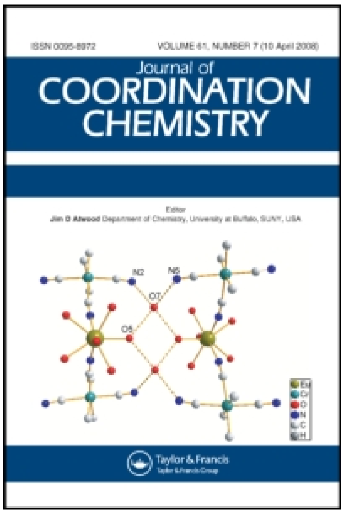

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Antimicrobial studies of *N*-*N*′-dicarboxydiethyloxamide and its Co(II), Ni(II), Cu(II) and Zn(II) complexes

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Neutral complexes of Cu(II), Ni(II), Co(II), and Zn(II) have been synthesized from the oxamide-based ligand derived from leucine and diethyloxalate. The structural features have been deduced from their microanalytical, IR, UV/Vis, mass, ^{1}H and ^{13}C NMR spectral data. The Co(II) and Ni(II) chelates have octahedral geometries and the Cu(II) chelate is a square-pyramidal geometry. The non-electrolytic and monomeric nature of the complexes is shown by their magnetic susceptibility and low conductance data. The biological activities of the ligand and its metal chelates against gram-positive and negative bacteria and fungi are also reported. All the compounds are antimicrobially active and show higher activity than the free ligand.

Keywords: Oxamide; Transition metal(II) complexes; Spectral and antimicrobial study

1. Introduction

Design and synthesis of new amino acid derived metal complexes [1] are of continuing interest because of their relevance in biological processes [2, 3]. Transition metal complexes afford new pathways to unusual geometries, with unique features such as birefringence, polarizability, paramagnetism [4], and color. New approaches using metal complexes in photopolymerization [5] and ionically-conducting nanocomposite materials [6] have been examined. Synthesis and the study of metal complexes containing amide groups [7] with coordination capabilities towards many metal ions [8], form versatile compounds which can be employed in biochemistry [9], materials chemistry and physical chemistry.

A large number of oxamide ligands derived by simply condensing aliphatic, aromatic or heterocyclic amines with diethyl oxalate or malonate compounds are discussed by Ojima *et al*. [10]. Such compounds result in mononuclear, dinuclear or polynuclear metal complexes [11, 12]. We have developed a new class of oxamides incorporating carboxylic acids into the side arm [13] to better bind metal ions. The present work concerns the synthesis, characterization and biological activity of the ligand named as LeuDEO including its metal complexes with Ni(II), Cu(II), and Zn(II) metal ions. Characterization

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is based on elemental analysis, IR, ^{1}H and ^{13}C NMR, UV/vis, mass spectra and magnetic studies. The ligand and all the metal complexes have been screened against four bacteria (2 gram positive and 2 gram negative) and three fungi strains for antimicrobial activity.

2. Experimental

2.1. *General remarks*

All chemicals used were of reagent grade. Solvents were dried and distilled before use according to standard procedures. Leucine and diethyl oxalate were purchased from CDH India Ltd. while metal chlorides were received from Qualigens India Ltd. Redistilled H_2O was used wherever it was needed.

FTIR spectra were recorded on a Perkin Elmer FTIR 621 spectrometer (4000–400 cm^{-1}) using potassium bromide pellets. Electronic spectra were recorded at room temperature on a Perkin-Elmer Lambda EZ-201 UV/Vis spectrophotometer in DMSO. ^{1}H and ^{13}C NMR spectra of the ligand and its complex were recorded in DMSO-d$_6$ on a Bruker Specrospin DPX-300 MHz NMR instrument at room temperature using TMS as internal standard. Molar conductivity of 10^{-3} M solution of the solid complexes in DMSO were measured on a Corning Conductivity Meter NY 14,831 model 441. Magnetic susceptibility of the complexes were measured by Guoy's method on a Faraday balance using Hg[Co(SCN)$_4$] as calibrant at room temperature. The effective magnetic moments were calculated from the expression $\mu_{\text{eff}} = 2.828(\chi_M T)^{1/2}$ BM, where χ_M is the molar susceptibility corrected using Pascal's constant for the diamagnetism of all atoms. Microanalysis of carbon, nitrogen and hydrogen were carried out on a Perkin Elmer model-2400 elemental analyzer. Melting points were determined by open capillary system and are uncorrected.

2.2. *Synthesis*

2.2.1. Ligand, LeuDEO (*N-N′*-dicarboxydiethyloxamide). Synthesis of the ligand was adopted in the literature [10]. L-Leucine (20 mmol, 2.62 g) was dissolved in 30 mL of water stirring at room temperature and neutralized by using an equivalent of NaOH. The mixture was refluxed for 10 min and then an ethanolic solution of diethyle oxalate (10 mmol, 1.36 mL) was added dropwise to the reaction flask. The solution was stirred for 30 min at room temperature and then refluxed at 60°C for 5 h. The solution was cooled in an ice bath, then HCl was added (pH-1), resulting in white precipitate which was filtered, washed with ethanol and diethyl ether and finally dried over $CaCl_2$. Yield – 52%. ^{1}H NMR (D_2O, TMS, δ ppm). 11.20 (s, 2H, –COOH), 8.1 (s, 2H, –NH), 4.46 (t, 2H, –C*H*–NH–), 1.64–1.83 (m, 6H, form methylene and methane protons), 1.01 (d, 12 H, $-CH_3$); ^{13}C NMR (D_2O, TMS, δ ppm). 175.2 (–COOH), 163 (–C=O, amide), 52 (–NH–CH–), 40.3 (–NH–CH–*C*H$_2$–), 22.9 (CH_3), 22.5 (–*C*H–$(CH_3)_2$).

2.2.2. Complex, Cu(LeuDEO) (*N-N′*-dicarboxydiethyloxamide copper complex). A solution of LeuDEO (2 mmol, 0.63 g) in distilled water was prepared and added dropwise into an ethanolic solution of $CuCl_2 \cdot 2H_2O$ (2 mmol, 0.34 g) under stirring and refluxed for 2 h. The solution was kept overnight, resulting in turquoise precipitate which was filtered, washed with cold ethanol and finally with diethyl ether. Yield – 71%.

All other metal complexes were prepared by the method used for Cu(LeuDEO).

2.3. *Antimicrobial screening*

Paper disc diffusion [14] was used to study the sensitivity of pathogenic bacteria to the ligand and metal complexes. According to the method [15], freshly prepared 0.1 mL inoculums of the test organisms were spread uniformly on the surface of the agar medium in each Petri plate. Each compound was prepared in three concentrations i.e. 100, 200 and 300 ppm in DMSO. Sterilized paper discs (Whatman filter paper no. 4) of 5 mm diameter impregnated with test agent were placed upon the surface of inoculated plate. The plates were incubated for 24 h for bacteria and 48 h for fungi at $35 \pm 2^{\circ}C$. During the incubation process, the test agent diffuses from the disc into the agar. Plates are observed for zone of inhibition (a clear area) around the disc, indicating that the test agent inhibited growth of organism. Tetracycline was used as standard drug and solvent DMSO was used as blank.

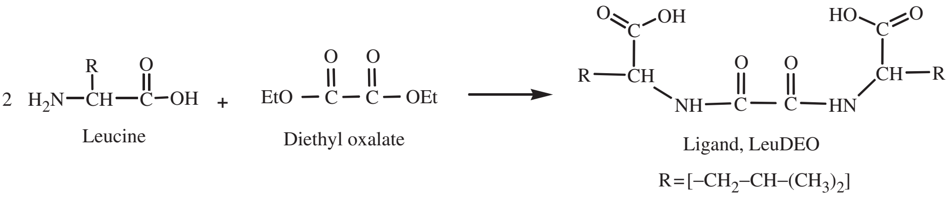
3. Results and discussion

The synthetic route of LeuDEO is described in scheme 1. Attempts to reproduce the ligand synthesis by varying the experimental conditions were completely unsuccessful. Hot solutions of the ligand were used during complexation, where the formation of M(LeuDEO), M=Cu(II) and Zn(II) and M(LeuDEO)$(H_2O)_2$, M=Ni(II) and Co(II), metal complexes (figure 1) was rapid and gave good yields with the ligand tetradentate.

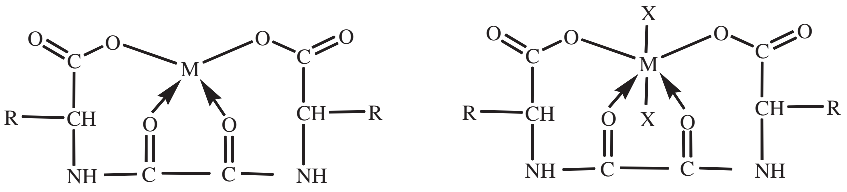
All the complexes are colored and stable on prolonged exposure in air, readily soluble in coordinating solvents such as DMSO and DMF, but insoluble in other organic solvents. The analytical data, table 1, indicate that the complexes are mononuclear. The molar conductance [16] in DMSO falls in the expected range 9-21 $\Omega^{-1}\,cm^2\,mol^{-1}$ for non-electrolytes, indicating the absence of chloride ions.

3.1. *NMR – spectral study*

^1H and ^{13}C NMR spectra of the ligand and the diamagnetic Zn(II) complex, [Zn(LeuDEO)], were recorded in DMSO-d_6; ^1H and ^{13}C NMR (figures 2 and 3) are detailed in the Experimental section.



Scheme 1. Synthesis of LeuDEO.



where M = Cu(II), Zn(II)
R = [$(H_3C)_2$–CH–CH_2–]

where M = Co(II), Ni(II)
X = H2O

Figure 1. Suggested structure of complexes.

Spectra of the ligand show a singlet at 8.1 ppm, due to –NH which was unaffected in the metal complex, strongly suggesting noninvolvement in coordination. Methine (–CH) and methylene (–CH–) protons merge to a multiplet in the 1.64–1.83 ppm region. Methine protons adjacent to –NH (–*CH*–NH–) are a triplet at 4.46 ppm. Downfield shift of this methine is due to the adjacent amide. A singlet at 11.20 ppm is due to –OH group of the carboxylic acid [17]. This peak was absent in the Zn(II) complex, indicating deprotonation on complexation and coordination through the alcoholic oxygen. Terminal methyl group showed characteristic absorption at 1.01 ppm. All other absorptions in the complexes were at their usual positions, slightly downfield in comparison to the free parent ligand, confirming coordination.

Similarly, the ^{13}C NMR spectra of the ligand and Zn(II) complex support the proposed structure. In the ligand, the amide carbon (*C*=O) is at 163 ppm, but significantly downfield at 148 ppm in the complex. Signal of carbon adjacent to NH group (–CH–NH–) appeared at 52 ppm. Signal at 175.2 ppm was assigned to carbon of the carboxylic acid group, significantly downfield in the complex spectrum.

3.2. *IR – spectral study*

The IR bands of the ligand and its complexes are summarized in table 2. In order to study the binding of LeuDEO to transition metal ions in the new complexes, the IR spectrum of the free ligand was compared with the spectra of metal complexes. The $-NH_2$ band of leucine disappears as a consequence of the reaction of L-Leucine with diethyloxalate and the appearance of a prominent band at 1656 cm^{-1} assignable to υ(C=O) [18] infers the formation of LeuDEO; in metal complexes this band is observed at lower frequency (22–35 cm^{-1}), confirming coordination to metal [19]. In the ligand, a single medium intensity band observed at 3360 cm^{-1} is assigned to amide N–H stretching frequency. The IR spectrum of the free ligand displays a broad band at 3460–3230 cm^{-1} corresponding to O–H of carboxylic acid, which is further supported by a medium intensity absorption at 2650 cm^{-1}. In metal complexes, IR spectra showed the absence of the band at $\sim$3460 cm^{-1}, indicating deprotonation of the hydroxyl group. It may be concluded that the ligand is dibasic tetradentate in all the complexes.

In the far infrared region all the complexes exhibit bands around 530–510 cm^{-1} for υ(M–O) [20]. Other bands were at their usual positions and in complexes found to shift towards lower wave number (10–30 cm^{-1}), suggesting the coordination of

Table 1. Analytical and physical data of the compounds.

Compound	Empirical Formula	Formula weight	Yield (%)	Color	M.p. (°C)	Molar Cond. (Ω^{-1}cm^{2}mol^{-1})	Found (calcd.) (%)			
							C	H	N	M
LeuDEO	$C_{14}H_{24}N_2O_6$	316.35	52	White	>300	–	53.11 (53.15)	7.59 (7.65)	8.89 (8.86)	–
Co(LeuDEO)$(H_2O)_2$	$C_{14}H_{26}N_2O_8Co$	409.30	56	Pink	250d	18	41.08 (41.10)	6.40 (6.43)	6.85 (6.86)	14.40 (14.43)
Ni(LeuDEO)$(H_2O)_2$	$C_{14}H_{26}N_2O_8Ni$	409.06	52	Light green	245d	21	41.11 (41.13)	6.41 (6.39)	6.85 (6.87)	14.35 (14.38)
Cu(LeuDEO)	$C_{14}H_{22}N_2O_6Cu$	377.88	71	Turquoise	210d	14	44.50 (44.45)	5.87 (5.89)	7.41 (7.42)	16.82 (16.83)
Zn(LeuDEO)	$C_{14}H_{22}N_2O_6Zn$	380.03	48	White	230d	9	44.28 (44.27)	5.84 (5.86)	7.38 (7.36)	17.22 (17.25)

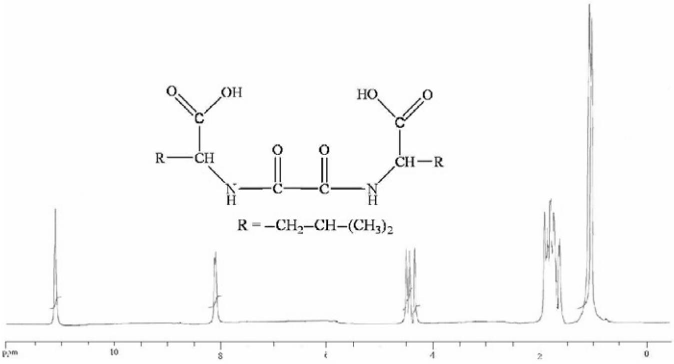


Figure 2. ^{1}H NMR spectrum of LeuDEO.

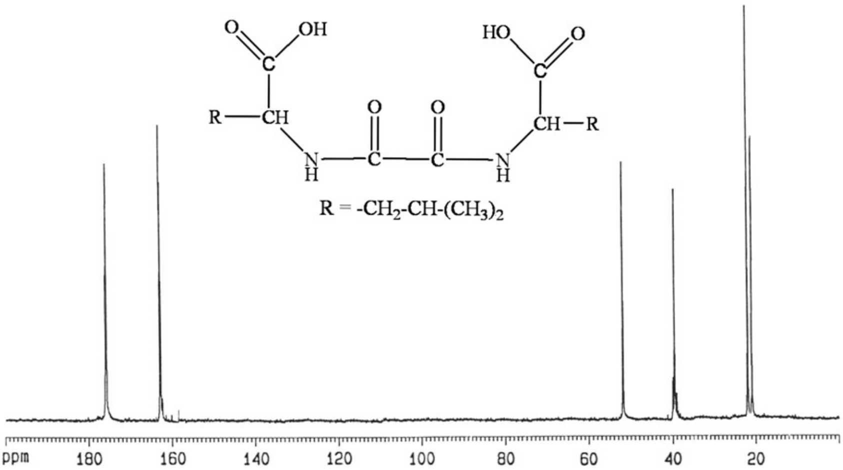


Figure 3. ^{13}C NMR spectrum of LeuDEO.

ligand to metal. These observations are in accord with the proposed structures of M(II) complexes with LeuDEO, where the central metal ion is four or six coordinate.

3.3. *ES-mass spectral study*

In the present study, ES-mass spectra of LeuDEO and Cu(LeuDEO) have been recorded on a Waters LCT Premier XE mass spectrometer and the spectra have been found in favor of proposed molecular formula of the compounds. LeuDEO shows its molecular ion peak (M^{+}) at $m/z = 316.05$ (f.w. = 316.35) and Cu(LeuDEO) exhibits its molecular ion peak (M^{+}) at $m/z = 377.46$ (f.w. 377.88). Spectrum of the complex confirms its monomeric nature.

3.4. *Electronic spectra and magnetic moments*

The UV-Vis spectral bands of the complexes in DMSO and magnetic moments measured at room temperature are given in table 3. The copper(II) complex displays a broad band at 12,400 $\mathrm{cm^{-1}}$ and a well-defined shoulder around 23,120 $\mathrm{cm^{-1}}$,

Table 2. Significant IR spectral data (cm^{-1}) of LeuDEO and its metal complexes.

Compound	ν(NH)	ν (C=O) (oxalate gp.)	ν_s(COO)	ν_{as}(COO)	δ(CH_2)	ν(C–C)	ν(M–O)
LeuDEO	3360	1656	1412	1608	1320	1060	–
Co(LeuDEO)(H_2O)$_2$	3355	1630	1402	1612	1315	1058	530
Ni(LeuDEO)(H_2O)$_2$	3353	1621	1402	1595	1318	1060	515
Cu(LeuDEO)	3355	1634	1400	1598	1320	1060	520
Zn(LeuDEO)	3350	1625	1405	1600	1318	1055	510

Table 3. Electronic spectra, magnetic moment, and ligand field parameters of the complexes.

Complex	Magnetic Momentum (BM)	Absorption (cm^{-1})	Assignment	Dq (cm^{-1})	B (cm^{-1})	β	β%	Geometry
Co(LeuDEO)(H_2O)$_2$	4.58	9210	$^4T_{2g}(F) \leftarrow {}^4T_{1g}(F)$	1218	902	0.80	20	
		17,870	$^4A_{2g}(F) \leftarrow {}^4T_{1g}(F)$					Octahedral
		21,670	$^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$					
Ni(LeuDEO)(H_2O)$_2$	3.30	10,210	$^3T_{2g}(F) \leftarrow {}^3A_{2g}(F)$	712	852	0.82	18	
		14,201	$^3T_{1g}(F) \leftarrow {}^3A_{2g}(F)$					Octahedral
		23,170	$^2T_{1g}(P) \leftarrow {}^3A_{2g}(F)$					
Cu(LeuDEO)	1.82	12,400	$^2A_{1g} \leftarrow {}^2B_{1g}$					Square planar
		23,120	$^2B_{2g} \leftarrow {}^2B_{1g}$					
Zn(LeuDEO)	Diamagnetic							Tetrahedral

attributable to $^2A_{1g} \leftarrow {}^2B_{1g}$ and $^2B_{2g} \leftarrow {}^2B_{1g}$ transitions, respectively, strongly indicating square-planar geometry around copper [21, 22]. The broadness of the band may be taken as an indication of distortion. Magnetic moment of 1.82 BM furthur supports the proposed geometry. For the cobalt(II) complex three d-d bands at 9,210, 17,870, and 21,670 cm^{-1} are assignable to transitions from the $^4T_{1g}(F)$ state to three spin-allowed excited states $^4T_{2g}(F)$, $^4A_{2g}(F)$, and $^4T_{1g}(P)$. The magnetic moment of 4.58 BM and the transitions indicate an octahedral Co(II).

The electronic spectrum of the Ni(II) complex shows three principle d–d bands at 10,210, 14,201 and 23,170 cm^{-1}, attributed to $^3T_{2g}(F) \leftarrow {}^3A_{2g}(F)$, $^3T_{1g}(F) \leftarrow {}^3A_{2g}(F)$ and $^3T_{1g}(P) \leftarrow {}^3A_{2g}(F)$ transitions. The electronic spectral bands and the observed magnetic moment of 3.30 BM are in agreement with the expected octahedral environment around Ni(II). Zinc complex is diamagnetic.

3.5. *Biological activity results*

Antimicrobial activity of LeuDEO and its metal complexes versus some bacteria and fungi have been studied and the results are expressed graphically in figure 4. The bacteria, *Bacillus anthrasis* and *Staphylococcus aureus* (gram positive bacteria) and *Pseudomonas aurugenosa* and *Escherichia coli* (gram negative bacteria), while the fungi *Candida albicans*, *Rhizoctonia bataicola* and *Panicillium italicum* were used for antimicrobial evaluation.

The antimicrobial assay studies reveal that inhibition zone values of the metal complexes are higher than that of the free ligand. The ligand exhibits the best activity against *B. anthracis* (gram +ve), *E. coli* (gram −ve) and *P. italicum* (the fungi).

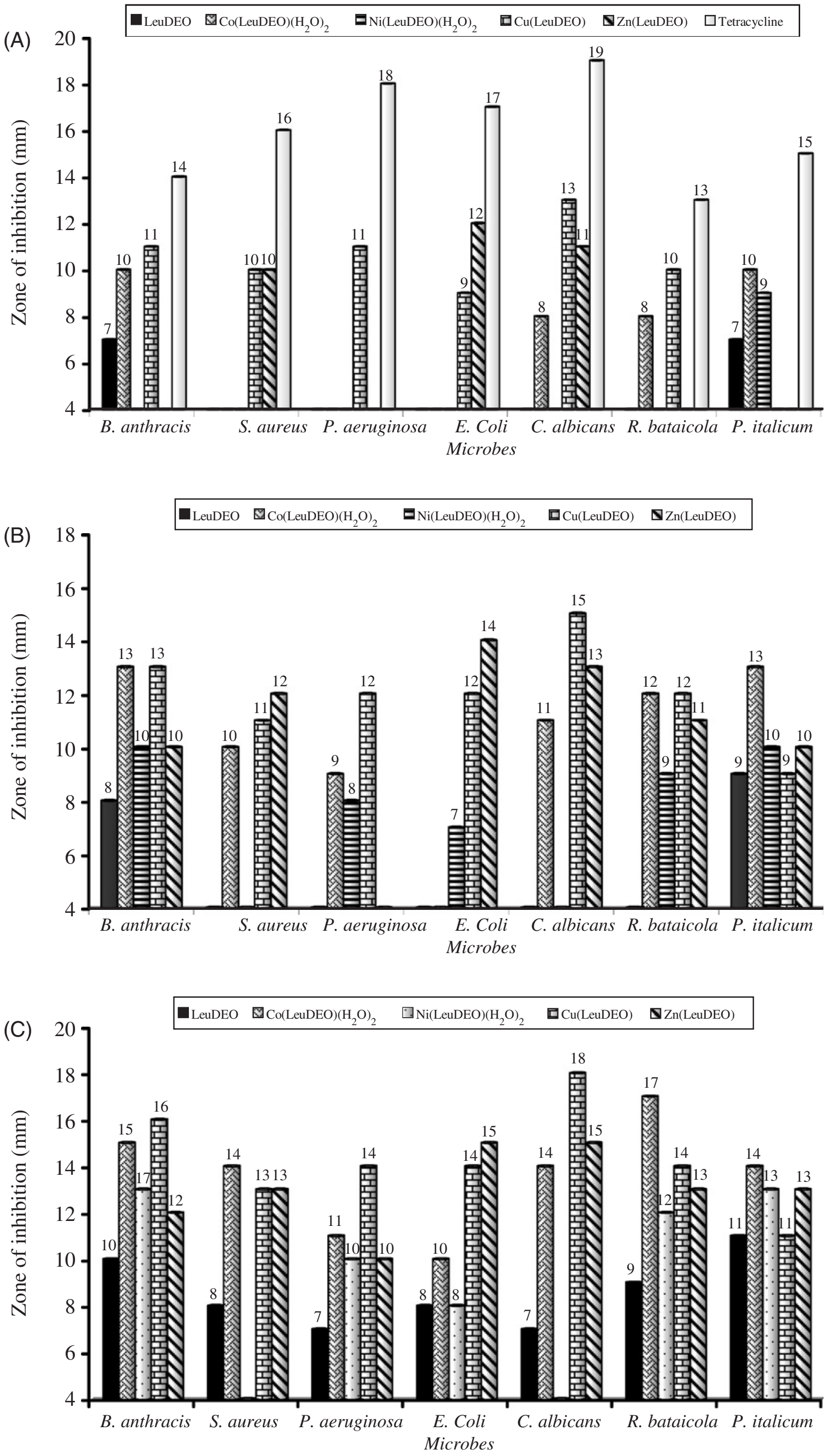


Figure 4. A, B and C–Antimicrobial data for LeuDEO and its metal complexes at 100, 200 and 300 ppm concentrations, respectively.

The complex Cu(LeuDEO) has the highest activity against the *C. albicans* among all the microbes. Co(LeuDEO)(H_2O)$_2$ has very good results when it is examined in 300 ppm concentration against the microbes and is effective against the fungi, particularly *R. bataicola*. Ni(LeuDEO)(H_2O)$_2$ showed poor inhibitory activity against most of the microbes. Cu(LeuDEO) displays moderate inhibitory activity against gram +ve and –ve bacteria along with fungi.

A ligand containing nitrogen and oxygen donors may inhibit the growth of pathogens through various ways, as cell membrane inhibitors, which disorganize the structure or inhibit the function of bacterial membranes [23] as DNA/RNA inhibitors or bind to DNA/RNA so that their messages can't be read.

The metal complexes show increased activity over the ligand, which may be explained on the basis of Overtone's concept and chelation theory [24, 25]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors passage of only lipid-soluble materials such that liposolubility is an important factor in antimicrobial activity. On chelation, the polarity of the metal ion will be reduced due to overlap of the 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. This increased lipophilicity enhances penetration of the complexes into lipid membranes for blocking the metal binding sites in the enzymes of microorganisms.

4. Conclusions

In this report, *N-N'*-dicarboxydiethyloxamide and its transition metal complexes were synthesized by condensation and their structural characteristics were explored via C, H and N analysis, IR, NMR, UV/vis, mass spectroscopy, *etc*. The spectroscopic tools provided ample evidence that the basic side chain of leucine in the ligand is involved in coordination of divalent metal cations.

Antimicrobial activity of LeuDEO and its metal complexes (*in vitro*) against some bacteria and fungi has been analyzed. The results showed better inhibitory activity of the complexes than the ligand. A metal cation bound amino acid (leucine basic side chain) complex M(LeuDEO) may be a good model system to represent functional understanding of a simple (metallo) enzyme-substrate complex (ES).

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