

Synthesis, Characterization and Antimicrobial Activity Studies of Mixed-1,10-phenanthroline- $Mn^{(II)}$, $Co^{(II)}$, $Cu^{(II)}$, $Ni^{(II)}$ and $Hg^{(II)}$ Complexes with Schiff Base [2,2'-(1Z,1'Z)-(biphenyl-4,4'-diylbis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)diphenol]

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Abstract: The $M(II)$ complexes $[M_2(phen)_2(L)(H_2O)_2Cl_2]$ in (2:1:2 ($M:L:phen$) molar ratio, (where $M(II) = Mn(II), Co(II), Cu(II), Ni(II)$ and $Hg(II)$, $phen = 1,10$ -phenanthroline; $L = 2,2'$ -(1Z,1'Z)-(biphenyl-4,4'-diylbis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)diphenol) were synthesized. The mixed complexes have been prepared and characterized using 1H and ^{13}C NMR, UV/Visible, FT-IR spectra methods and elemental microanalysis, as well as magnetic susceptibility and conductivity measurements. The metal complexes were tested *in vitro* against three types of pathogenic bacteria microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* to assess their antimicrobial properties. From this study shows that all the mixed complexes have octahedral geometry. In addition, it has high activity against test bacteria.

Keywords: mixed complexes, benzidine, 1, 10-phenanthroline, antibacterial

1. Introduction

For many years it was great interest to study of binuclear Schiff base ligands through its ability to link with transition metal complexes [1]. This is due to two reasons, the first reason is the relation between magnetic exchange effects and structures in hetero- and homo- binuclear metal complexes [2], and the second reason is the use of such complexes to imitate parts of bimetallic bio sites in several enzyme and proteins [3]. Outwardly, the generality suitable structure depends on nature of the central transition metal atoms which have been linked with different Lewis acids whether they are soft or hard [4].

It has been known that metal ions have been subject of interest and embroiled in biological processes of life [5]. These metal ions have the mode of action that is often complex but they are involved in bonding to the heteroatoms of the heterocyclic residues of biological molecules such as nucleic acids [6], protein [7], enzymes and others [6]. In coordination chemistry a bidentate ligand as 1,10-Phenanthroline which is a organic compound has heterocycles symbolizes one of the great repeatedly used in inorganic chemistry as chelate ligands, it forms powerful complexes with most metal ions [8]. This is a molecule which has moderate size would be of most amount for studies on complexes, 1,10-phenanthroline which has the intra-ligand vibrations may be obviously particular and unattached from the remnant of the vibrational spectrum [9]. In the present paper, reports about the preparation and characterization of new $Co^{(II)}$, $Ni^{(II)}$, $Mn^{(II)}$, $Cu^{(II)}$ and $Hg^{(II)}$ complexes with Schiff base ligand [2,2'-(1Z,1'Z)-(biphenyl-4,4'-diylbis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)diphenol] and 1,10-phenanthroline.

2. Experimental

Material

All chemicals used in this work are of analytical reagent grade and used as received from supply.

Methods

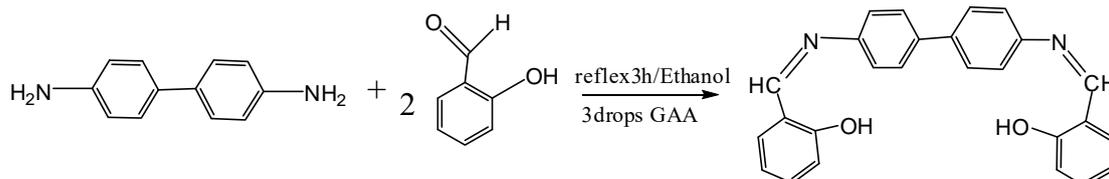
Micro analytical data, 1H , ^{13}C -NMR spectra of the compounds were registered at Bruker spectropin ultra shield magnets (300 MHz) measurement utilizing tetra methyl silane (TMS) as an internal standard and DMSO- d_6 as a solvent in Sharif University of technology in Iran. Products were examined by FT-IR spectra were recorded on SHIMADZU FTIR-8400 Fourier Transform Infrared spectrophotometer as KBr disc. The chloride content for complexes was determined using potentiometric titration method on (686-Titro Processor-665 Dosim A- Metrohm /Swiss). Magnetic susceptibility instruments were got at room temperature on the solid state applying Faraday's Method using Bruker BM6 instrument at 298°K. Elemental microanalysis (C.H.N %) of the synthesized compounds was carried out in the central service laboratory, College of Education for pure science, Ibn Al-Haitham using a C.H.N Analyzer on Perkin Elmer 2400 series II. Melting point was determined by utilizing (start melting point Apparatus) type Digimelt (MSRS). The suggested molecular structures of the compounds were drawing by utilizing (chem. Office prog. 3DX) (2006).

Synthesis of Ligand [H_2L]:

The ligand [H_2L] has prepared according to the procedure published in literature [10], scheme (1). A hot solution of (0.28 mL, 2 mmol.) of salicylaldehyde and (0.184 g, 1mmol.) of benzidine dissolved in 10 mL and dissolved in 15 mL of absolute ethanol to this ethanolic solution, 3drops of glacial

acetic acid were added as a condensing agent and refluxed for 4 hrs. upon cooling a precipitate formed, was filtered off and recrystallized from a mixture of acetone : methanol :

distilled water (5:5:2) giving yellow precipitate, yield (70 %), m.p. (over 260 °C) dec.

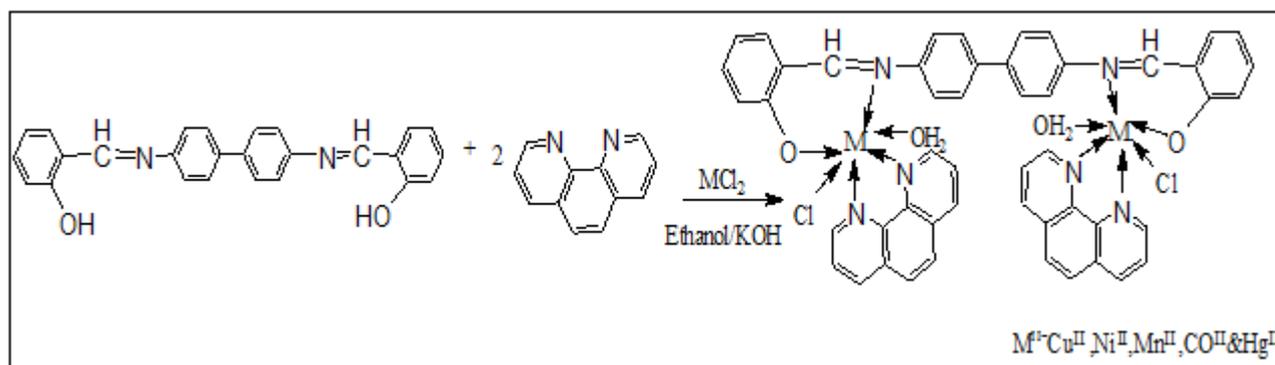


Scheme 1: Synthesis route of Ligand [H₂L]

Synthesis of mixed-ligand binuclear complexes [M₂(phen)₂(L)(H₂O)₂Cl₂]

To a solution of metal chloride (II) (MCl₂; M^(II) = Co^(II), Ni^(II), Mn^(II), Cu^(II) and Hg^(II)) (2 mmol) dissolved in 10 ml ethanol, ethanolic solutions of both 1,10-phenanthroline (2 mmol, 0.36 g) and KOH solution of H₂L (1 mmol, 0.393 g) were added simultaneously under stirring using stoichiometric amount (2:1:2) of Metal: ligand: (phen) molar

ratio. The stirring of reaction mixture was continued at room temperature for 24 h, producing to the isolation of solid product which was filtered, washed with hot ethanol and finally by using anhydrous calcium chloride dried in vacuum, Scheme(2)shows synthesis route of mixed -ligand binuclear complexes .



Scheme 2: Synthesis route of mixed -ligand binuclear complexes

NMR spectrum for ligand (H₂L)

¹H-NMR spectrum of the ligand (H₂L) in D₂O Table (2), Fig. (5) shows singlet signal at 3.4 ppm due to HC=N protons, multiplet signals at (7.2-7.9 ppm) due to aromatic protons and singlet signal at 9.6 ppm due to protons of O-H phenolic groups [11].

¹³C-NMR spectrum of the free ligand Table (3), Fig. (6) shows multiplet signals at (122-126ppm) due to aromatic carbons, the peak at 133 ppm. result to the carbons of imine C=N groups and the peak at 170 ppm result to the carbons of phenolic COH [12].

Electronic spectra, magnetic moments and conductivity measurements

The electronic spectrum of Schiff base ligand (H₂L), Figure (3) exhibits two high intense absorption peaks at (256 nm) (39062 cm⁻¹) and (334nm) (29940cm⁻¹) which assigned to (π → π*) and (n → π*) transitions respectively. The electronic spectrum of 1,10-phenanthroline, Figure (4) displays high intense absorption peaks at (202 nm) (49504 cm⁻¹) and (228 nm) (43859 cm⁻¹) which assigned to (π → π*) and peak at (264 nm) (37878 cm⁻¹) which assigned to (n→π*) transition respectively, the data recorded in Table (5)[13].Theses transitions were also found in the spectra of the complexes, but they were shifted towards lower in range (30769-28571) cm⁻¹ [14].

All the mixed complexes have different colors, it depends on the ligands and metal salts, then this is a significant indication of electronic transitions and coordination, therefore they are displayed different characteristic absorption peaks in their position and intensity [15]. The electronic spectrum of [Co₂(phen)₂(L)(H₂O)₂Cl₂] showed one intense peak at (283 nm) (35335 cm⁻¹) which was assigned to the ligand field. And another intense peak at (356 nm) (28089 cm⁻¹) is due to charge transfer transition. And the peaks at visible region at (626 nm) (15974 cm⁻¹), (675 nm) (14814 cm⁻¹) and (917 nm) (1090 cm⁻¹). Theses peaks are assigned to (⁴T_{1g(F)} → ⁴A_{2g(P)}, ⁴T_{1g(F)} → ⁴A_{2g(P)} and (⁴T_{1g(F)} → ⁴T_{2g(F)}) (d-d) transitions respectively, confirming an octahedral structure around (Co^{II}) ion complex[16].

The electronic spectrum of [Ni₂(phen)₂(L)(H₂O)₂Cl₂] displayed one intense peak at (259 nm) (38610cm⁻¹) was assigned to the ligand field. And another intense peak at (361 nm) (27700 cm⁻¹) is due to charge transfer transition. And the peaks at visible region at (410nm) (24390 cm⁻¹) and (681 nm) (14684cm⁻¹). Theses peaks are assigned to (³A_{2g(F)} → ³T_{1g(P)}) and (³A_{2g(F)} → ³T_{1g(F)}) (d-d) transitions confirming an octahedral structure around (Ni^{II}) ion complex[17].

The electronic spectrum of [Mn₂(phen)₂(L)(H₂O)₂Cl₂] showed one intense peak at(261 nm) (38314 cm⁻¹) which was assigned to the ligand field. And another intense peaks at (338 nm) (29585 cm⁻¹) and (370 nm) (27027 cm⁻¹) are due to

charge transfer transitions. Also the peaks at visible region at (409 nm) (24449 cm^{-1}) and (747nm) (22371 cm^{-1}). These peaks are assigned to (${}^6A_{1g} \rightarrow {}^4T_{2g(D)}$) and (${}^6A_{1g} \rightarrow {}^4T_{2g(G)}$)(d-d) transitions confirming an octahedral structure around (Mn^{II}) ion complex[18].

The electronic spectrum of $[Cu_2(phen)_2(L)(H_2O)_2Cl_2]$ displayed one intense peak at (282 nm) (35460 cm^{-1}) which was assigned to the ligand field. And another intense peaks at (343 nm) (29154 cm^{-1}) and (375 nm) (26666 cm^{-1}) are due to charge transfer transitions. And the peak in visible region at (787nm) (12706 cm^{-1}) is due to assigned to (${}^2B_{1g} \rightarrow {}^2A_{2g}$)(d-d) transition confirming a distorted octahedral structure around (Cu^{II}) ion complex[19].

The UV-Vis spectrum of $[Hg_2(phen)_2(L)(H_2O)_2Cl_2]$ showed absorption peak at (290 nm) (34482 cm^{-1}) was assigned to the ligand field. And another intense peak at (349 nm) (28653 cm^{-1}) and at (349 nm) (28653 cm^{-1}) and (368 nm) (27173 cm^{-1}) are due to charge transfer transitions. The transition happened, this is a good result for an octahedral structure around (Hg^{II}) ion complex [20]. Where a comparison between the data recorded of the mixed ligand and $Mn^{(II)}$, $Co^{(II)}$, $Cu^{(II)}$, $Ni^{(II)}$ and $Hg^{(II)}$ metal ions are given Table (5).

The molar conductivities in ethanol solvent indicate that all metal complexes are non-electrolytes Table (5). These data together with magnetic moment indicate an octahedral geometry around the $Co^{(II)}$, $Mn^{(II)}$, $Cu^{(II)}$, $Ni^{(II)}$ and $Hg^{(II)}$ metal atoms studied[21].

The Chloride content

The chloride content measurements for all complexes (Table-1) assess on approximated data for theoretical data.

The IR spectra

Table (2) shows tentative assignments of the IR peaks for the free ligands and mixed-ligand complexes. The (H_2L) may coordinate with metal ions through tetradentate chelating [22]. The infrared spectrum of the free Schiff base ligand shows a broad band at (3446 cm^{-1}), which indicates to carboxyl-OH group [23]. The $\nu(O-H)$ band is absent in the IR spectrum of the complexes indicated that the carboxyl OH protons were lost upon complexation [24]. The bond in the Schiff base ligand spectrum was observed at (1612 cm^{-1}) due to the $\nu(C=N)$ band of the Schiff base ligand that was shifted slightly to lower frequency in range ($1602-1593\text{ cm}^{-1}$) due to amine linkage that was shifted towards lower frequency in all the complexes. Also, in the spectrum of the 1,10-phenanthroline appeared the band (1620) due to $\nu(C=N)$ in range at ($1617-1614$), indicating that the 1,10-phenanthroline was coordinated to the metal atoms through azomethine nitrogen. The absence of stretching and bending vibrations of free carbonyl group in salicylaldehyde at (1745 cm^{-1}) indicates the absence of this group in these complexes. The IR spectra of the Schiff base ligand showed the absorption band at (1613 cm^{-1}) due to phenolic $\nu(C-O)$ groups. The phenolic (C-O) vibrations on complexation with $Co^{(II)}$, $Cu^{(II)}$, $Ni^{(II)}$, $Mn^{(II)}$ and $Hg^{(II)}$ ions have also shown a shift to higher frequency indicating proof of the coordination to O was provided by the appearance of the bands (M-O) in the ($452-428\text{ cm}^{-1}$) region in the spectra of the complexes.

Further there was the coordination of these ions through phenolic oxygen of the Schiff base[24]. In addition proof of the coordination to N was provided by the appearance of the bands (M-N) in the ($644-638\text{ cm}^{-1}$) region in the spectra of the complexes.

In vitro antibacterial activity:

The *in vitro* antibacterial activity of ligand [H_2L] and its complexes type, $[M_2(phen)_2(L)(H_2O)_2Cl_2]$ [$M = Co(II)$, $Mn(II)$, $Cu(II)$, $Hg(II)$ and $Ni(II)$], were tested using the bacterial cultures of: (*Staphylococcus aureus*), (*Escherichia coli*), (*Bacillus subtilis*) and (*Pseudomonas aeruginosa*), resistant by method such as the disc diffusion. Then it was determined by the minimum inhibitory concentration (MIC) of the ligand and complexes. The disk poured in ethanol was used as the control. Test compounds, which can restrain the apparent growth after 24 h incubation at $37\text{ }^\circ\text{C}$ [25]. The susceptibility was given the basis of diameter of zone of inhibition against Gram(+) and Gram(-) strains of bacteria. The zones of inhibition (mm) of ligand and its complexes against Gram-positive and Gram-negative strains of bacteria are shown in Table 6. The higher antimicrobial activity of the metal complexes as compared to Schiff base ligand may be explained in terms of chelation which makes metal complexes to act as more powerful and potent antimicrobial agents, thus inhibiting the growth of the microorganisms [26]. Furthermore, coordination diminishes the polarity of the metal ion fundamentally on account of the partial participation of its charge (+) with the donor groups within the chelate ring system. The purpose of this work which was the distinct difference in the antimicrobial property of compound further justifies. This study is important due to the ability that the compounds might be more efficient against bacteria for which a thorough examination regarding toxicity, the structure effective relationship and in their biological effects which would be beneficial in designing more potent antibacterial agents for therapeutic use if required.

3. Conclusion

A Schiff base ligand, H_2L derived from the condensation of salicylaldehyde and benzidine was synthesized and characterized. The mixed ligand complexes with $Co(II)$, $Ni(II)$, $Cu(II)$ and $Zn(II)$ ions, Schiff base ligand and 1,10-phenanthroline were prepared by the template synthesis due to poor yield of ligand. The bonding of the ligand in the mixed complexes and the overall geometry has been deduced on the basis of various spectroscopic techniques. The comparative *in vitro* antimicrobial results suggested that the metal complex shows a significant antimicrobial activity as compared to ligand, H_2L and its $Co^{(II)}$, $Mn^{(II)}$, $Cu^{(II)}$, $Hg^{(II)}$ and $Ni^{(II)}$ complexes.

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Table 1: Some physical properties of prepared ligand (H₂L) and its complexes

Compounds	Empirical Formula	Yield %	M.P C ⁰	Colour	%)Calc.) Found					
					C	H	N	Cl	Metal	
H ₂ L	C ₂₆ H ₂₀ N ₂ O ₂	392.45	70	260	Yellow	(79.57) 63.67	(5.14) 4.40	(7.14) 9.21	-	-
[Co ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	C ₅₀ H ₃₈ Cl ₂ Co ₂ N ₆ O ₄	975.65	60	278	Dark yellow	(61.55) 57.20	(3.93) 4.50	(8.61) 7.50	(7.27) 7.07	(12.08) 11.20
[Ni ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	C ₅₀ H ₃₈ Cl ₂ N ₆ Ni ₂ O ₄	975.17	55	290	Greenish yellow	(61.58) 62.64	(3.93) 3.21	(10.38) 10.60	(7.27) 6.89	(12.04) 14.30
[Mn ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	C ₅₀ H ₃₈ Mn ₂ Cl ₂ N ₆ O ₄	967.66	56	287	Pale brown	(62.06) 62.45	(3.96) 3.21	(8.68) 8.14	(7.33) 6.97	(14.37) 14.35
[Cu ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	C ₅₀ H ₃₈ Cl ₂ Cu ₂ N ₆ O ₄	984.87	53	300	brown	(60.98) 61.69	(3.89) 3.32	(10.75) 10.56	(7.20) 6.98	(12.90) 15.80
[Hg ₂ (phen) ₂ (L)(H ₂ O)Cl]	C ₅₀ H ₃₈ Cl ₂ Hg ₂ N ₆ O ₄	1127.91	51	270	brown	(47.70) 41.89	(3.04) 2.86	(10.89) 10.69	(5.63) 5.28	(31.87) 31.11

Table 2: ¹H-NMR Chemical Shifts for Ligand (H₂L) (ppm in D₂O)

HC=N	C ₅ H _{6aromatic}	COH
3.4	7.2-7.9	9.69

Table 3: ¹³C-NMR Chemical shifts for Ligand (H₂L) (ppm in D₂O)

C ₅ H _{6aromatic}	HC=N	C-OH
123-127	133	170

Table 4: Infrared spectral data (wave number ν⁻¹) cm⁻¹ for the ligand (H₂L), and its mixed ligand complexes

Compounds	νOH	νC-H _{arom}	νC-H _{aliph}	νC=O	ν(C=N) _{imine}	νC-O	δOH _{ro} king	M-N M-O
H ₂ L=C ₂₆ H ₂₀ N ₂ O ₂	3446	3053	2998	1691	1612	1315	-	-
1,10-phenanthroline	-	3034	-	-	1620	-	-	-
[Co ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	3564	3049	2937	-	1616 1600	1365	842	640 452
[Ni ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	-	3055	2991	-	1614 1600	1363	-	644 420
[Mn ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	-	3072	2987	-	1615 1602	1355	-	636 428
[Cu ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	-	3064	2999	-	1617 1598	1365	-	644 448
[Hg ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	3562	3049	2937	-	1616 1593	1363	846	638 442

Table 5: Electronic spectral data of the ligand (H₂L) and its mixed ligand complexes

Compound	μ _{eff}	λ _m 2 S.Cm ⁻¹ molar ⁻¹	λnm	ν-wave number cm ⁻¹	(εmax molar ⁻¹)	Assignments	geometry
H ₂ L	-	-	256	39062	878	π→π*	-
			334	29940	1562	n→π*	
1,10-phenanthroline	-	-	202	49504	789	π→π*	-
			228	43859	1992	π→π*	
			264	37878	1345	n→π*	
[Co ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	5.47	18	283	35335	1584	C.T	Octahedral
			626	15974	62	⁴ T _{1g(F)} → ⁴ A _{2g(P)}	
			675	14814	71	⁴ T _{1g(F)} → ⁴ A _{2g(P)}	
			917	1090	18	⁴ T _{1g(F)} → ⁴ T _{2g(F)}	
[Ni ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	3.33	13	259	38610	152	C.T	Octahedral
			410	24390	279	³ A _{2g(F)} → ³ T _{1g(P)}	
			681	14684	56	³ A _{2g(F)} → ³ T _{1g(F)}	
[Mn ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]	5.67	17	261	38314	2021	L.F	Octahedral
			338	29585	1989	C.T	
			370	27027	1245		
			409	24449	487	⁶ A _{1g} → ⁴ T _{2g(D)}	
			447	22371	311	⁶ A _{1g} → ⁴ T _{2g(G)}	
[Cu ₂ (phen) ₂ (L)(H ₂ O) ₂ Cl ₂]		15	282	35460	2187	L.F	Distorted

	1.86		343	29154	1528	C.T	Octahedral
			375	26666	1196		
			787	12706	46	${}^2B_{1g} \rightarrow {}^2A_{2g}$	
$[Hg(phen)_2(L)(H_2O)_2]^{+2}$	-	14	290	34482	2174	L.F	Octahedral
			349	28653	2132		
			368	27173	1331	C.T	

Table 6: Diameter of zone of inhibition (mm)

Comp.	<i>Escherichia. Coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus</i>	<i>pseudomonas</i>
H ₂ L	5	8	7	8
$[Co_2(phen)_2(L)(H_2O)Cl]$	12	10	10	13
$[Ni_2(phen)_2(L)(H_2O)Cl]$	11	16	11	14
$[Mn_2(phen)(H_2O)Cl]$	17	12	14	12
$[Cu_2(phen)_2(L)(H_2O)Cl]$	15	14	16	17
$[Hg_2(phen)_2(L)(H_2O)Cl]$	17	18	12	10

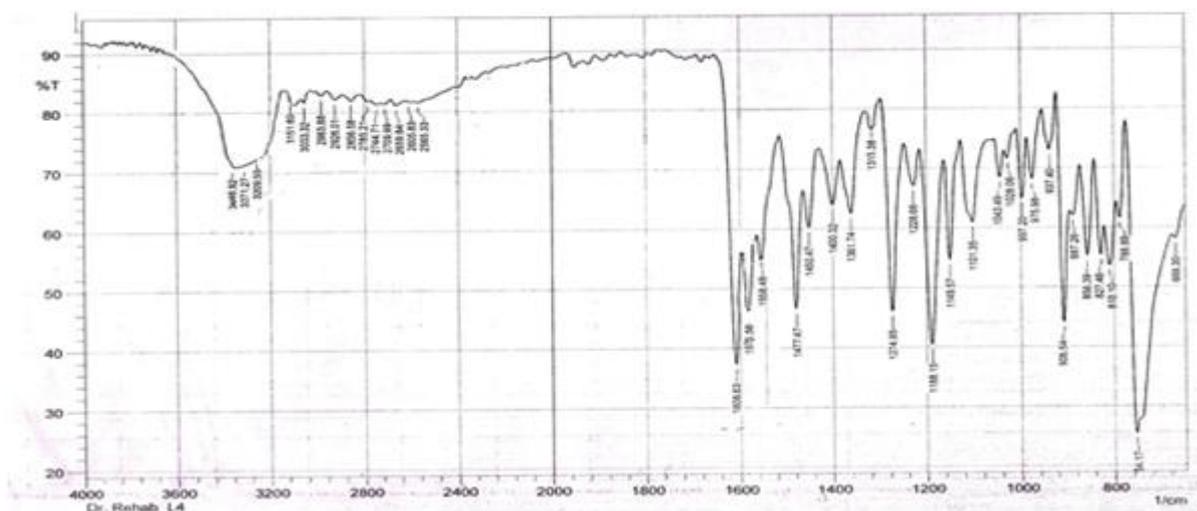


Figure 1: IR Spectrum of Ligand [H₂L]

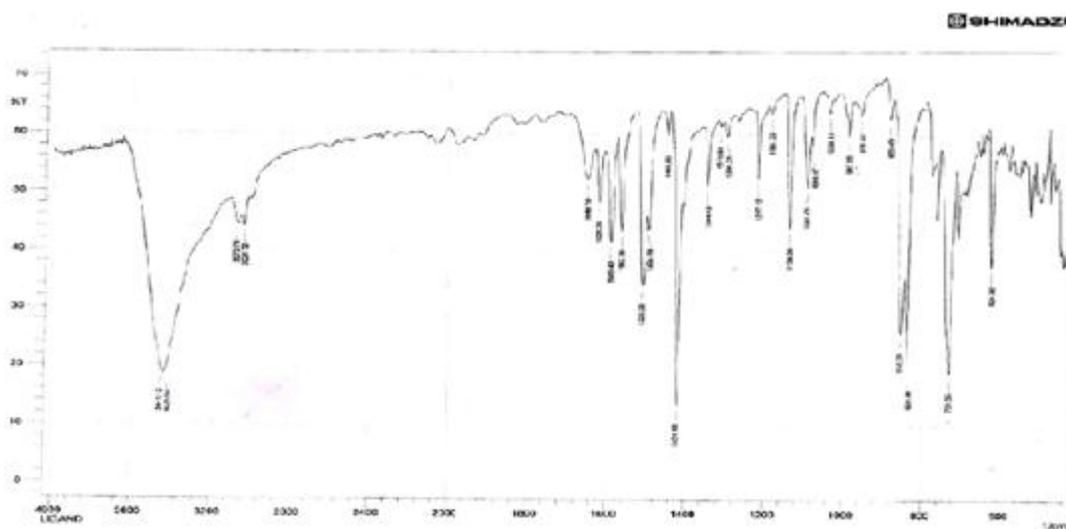


Figure 2: The IR spectrum of 1,10-phenanthroline ligand.

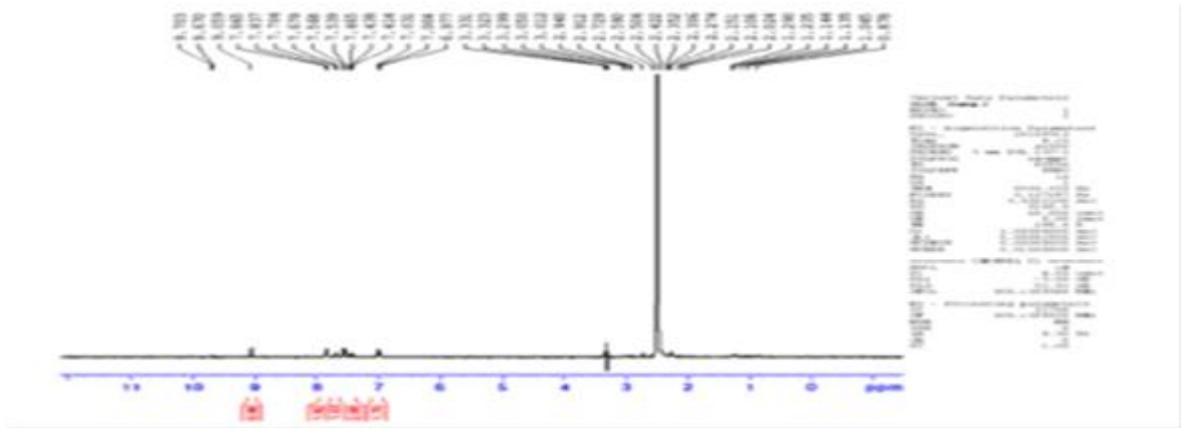


Figure 3: ¹H NMR spectrum of Ligand(H₂L)

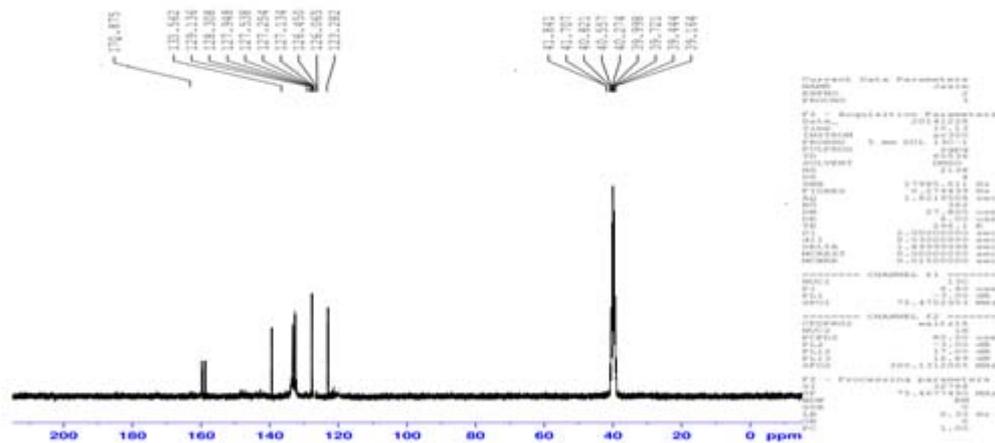


Figure 4: ¹³C NMR spectrum of Ligand(H₂L)

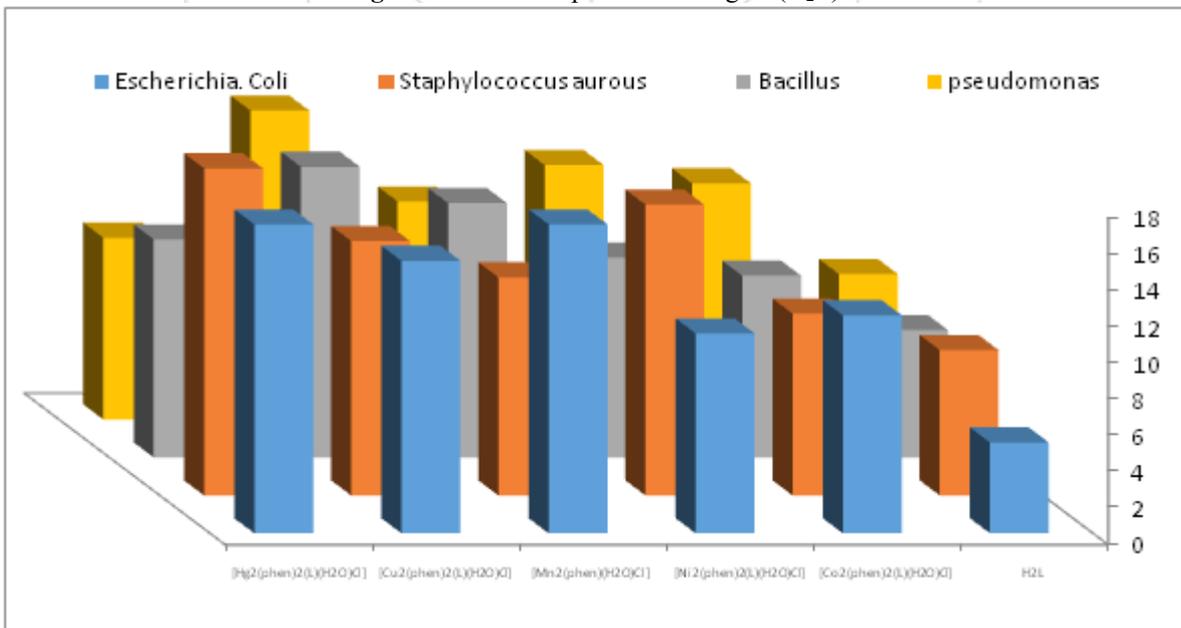


Figure 5: Difference between the antimicrobial activity of ligand(H₂L) & metal complexes