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Synthesis and characterization of some Schiff base complexes derived from 2, 5-dihydroxyacetophenone with transition metal ions and their biological activity

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Abstract

Schiff base complexes derived from 2, 5-dihydroxyacetophenone as ligand and some metal cations: Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} have been prepared and investigated. The structures of the synthesized complexes were characterized by different techniques such as elemental analysis, UV-Vis, IR and ^{1}H NMR spectra. The biological activity of the ligand and its complexes was studied using five microorganisms representing different microbial categories. The synthesized compounds showed good biological activity.

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1. Introduction

Schiff bases and their complexes are of high interest among the researchers because of their biological activity including anti-tumor, antibacterial, fungicidal, antidepressants, antiphlogogistic, nematocide, anti-carcinogenic and catalytic activity [1-3]. Schiff bases are the compounds containing azomethine group (-HC=N-) which were first reported by Hugo Schiff in 1864 and formed by condensation of a primary amine with an active carbonyl compound, and generally take place under acid, base catalysis or with heat [4]. Schiff bases have been widely used as ligands because of high stability of their coordination compounds and their good solubility in common solvents [5]. Schiff base and their metal complexes are very popular due to their diverse chelating ability [6]. The Schiff bases are very important tools of the inorganic chemists as these are widely used to design molecular ferromagnets [7]. In order to widen the scope of investigations on the coordination behavior of various donor ligands including Schiff base towards organo-metallics, we carried out the investigations and established their bioactivities [8-11]. The microorganisms adsorb metal ions on their cell walls and through respiration process of cells these ions are disturbed, and the process of protein synthesis is blocked which is the requirement for further growth of organisms.

In the present work, hydroquinone diacetate and 2, 5 – dihydroxyacetophenone are prepared as start materials to prepare new Schiff base derivatives and their metal complexes. The prepared compounds are investigated using elemental analysis (C, H, N), IR, ^{1}H-NMR and UV-Vis spectra and the biological activity of ligands and their metal complexes are also studied.

2. Experimental

Materials and reagents

All chemicals used were of the analytical reagent grade (AR) and of highest purity available and they were used without further purification.

Preparation of hydroquinone diacetate

A drop of concentrated sulphuric acid was add to a mixture of 55 g (0.5 mol) of hydroquinone and (95 mL, 1 mol) of acetic anhydride in 500 mL conical flask. The mixture was stirred gently until complete dissolution. Then, the reaction mixture solution was poured on to crushed ice to give precipitate, which was then filtered off and washed with water. The product was crystallized with 50 % aqueous ethanol to give 89 g of pure hydroquinone diacetate (m.p. 122, (91 %) [12].

Preparation of 2, 5-dihydroxyacetophenone

A mixture of 40 g (0.2 mol) of dry hydroquinone diacetate and 87 g (0.65 mol) of anhydrous aluminum chloride was finely grounded in mortar and introduced into a 500 mL round bottomed flask, fitted with an air condenser protected by a calcium chloride tube and connected to a gas absorption trap. The reaction blend was slowly heated so that at the end of about 30 minutes the temperature of the sand reached 110-120°, at which point the evolution of hydrogen chloride began. The temperature was then slowly raised to 160-165 °C and maintained at that point for about 3 h. The flask was removed from the sandy bath and allowed to cool to room temperature. The excess aluminum chloride was decomposed by treating the reaction mixture with 250 g of crushed ice followed by 20 mL of concentrated hydrochloric acid. The resulting solid was filtered and washed with two 80 mL portions of cold water. The product was recrystallized from water to give green silky needles (m.p 202-203° C) [12].

Synthesis of Schiff bases

The Schiff bases under study were prepared according to the recommended method for Schiff base compounds [13].

To a stirred and heated (60 °C) ethanolic solution of 2,5-dihydroxy acetophenone (152 g,1mol), an ethanolic solution of p-toluidine (107.16 g,1mol), o-aminophenol (109.13 g,1mol) and o-anizidine (123.16 g, 1mol) was immediately added. The reaction mixture was heated at reflux on a water-bath for 6 h. By time the color of reaction mixture was changed to obtain the Schiff base compounds. The reaction mixture was concentrated to 30 mL and after cooling, the produced Schiff bases were filtered off, washed with ethanol (70 %) and crystallized from the proper solvent.

Synthesis of metal complexes

The solid chelates with a stoichiometric ratio of (1:1) (M:L) were prepared by mixing the metal ion solutions with a hot ethanolic solution of the ligands. The mixture was then refluxed on a water bath for about 8 h and then allowed to cool where the solid chelates were separated and recrystallized from absolute ethanol, dried and preserved in a desiccator over dried silica gel [14].

Instruments and measurements

All the absorption spectral measurements were carried out using Jasco V-530 (UV-Vis.) spectrophotometer (Japan) with scanning speed 400 nm/min, band width 2.0nm and equipped with 10.0 mm matched quartz cells.

The ^1H NMR spectra of the ligands were performed using a Varian EML 390 spectrometer at 200 MHz using TMS as a reference in Demso –d_6 solvent. The elemental analysis of C, H and N were performed in the Micro Analytical Center of Cairo University, Egypt.

Conductance measurements were carried out using conductometic type HI 8033 HANA instruments with cell constant k= 1.0.

IR spectra were recorded on Beckman IR 4280 double beam spectrophotometer in the range 4000-400 cm^{-1}.

Biological activities of the Schiff base compounds and their complexes

The well–plate methods (diffusion methods) were followed with some modifications [15]:

1-The spore suspension of the test organisms (three-gram negative bacteria, Escherichia coli, Klebsiell pneumonia and Proteus vulgarius and one-gram positive bacteria, Staphylococcus aureus) and yeast (Candida albicans), was prepared in 9.0 mL sterile saline (0.9% NaCl) solution.

2- A solid medium containing nutrient agar for bacteria (g/L) (beef extract 3.0, peptone 10.0, agar-agar 20.0, Distilled water 1000 mL) and for yeast Sabouraud dextrose agar (peptone 10.0, dextrose 40.0, agar-agar 15.0) was sterilized and divided while warm (45:40 °C) in 15 mL portions among sterile petri-dishes of 9.0 cm diameters.

3- 0.1 mL of the spore suspension was surface placed on the surface of the solid media in the petri-dish and spread all over the surface.

4- Different concentrations of the tested substance were prepared using DMSO solvent.

5- In petri-dishes containing the culture of one of the tested microorganisms, one well of 5 mm diameter was made in the center of the dish. It was marked on the bottom of the dish, and filled with 20 µL from each concentration of the tested substance. The petri-dishes were incubated at 5 °C for 2 h. To permit good diffusion and then transferred to an incubator at 37 °C for tested bacteria and at 30 °C for 72 h for tested yeast.

3. Results and discussion

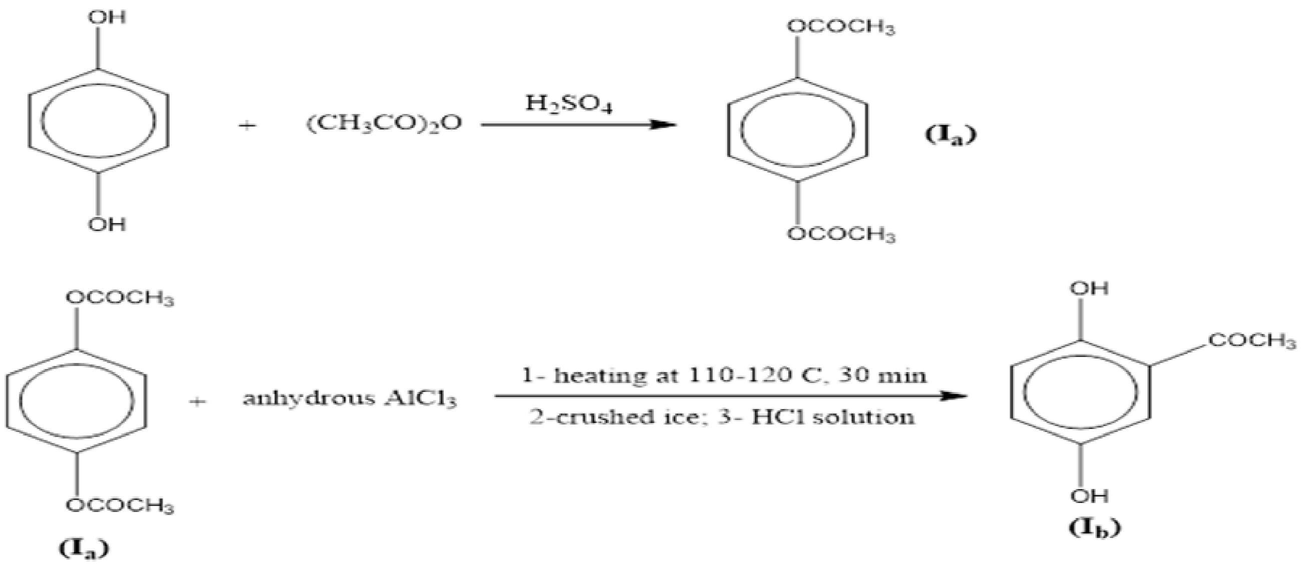
Synthesis and characterization of hydroquinone diacetate (I_a):

Hydroquinone diacetate, (I_a), Scheme (3), was synthesized by the reaction of a mixture of hydroquinone and acetic anhydride in presence of sulphuric acid. Moreover, the elemental analysis data show an excellent agreement with the proposed formula (molecular formula, $C_{10}H_{10}O_4$), Table (1).

Synthesis and characterization of 2, 5-dihydroxyacetophenone (I_b):

The product **(I_b)** was prepared by reacting hydroquinone diacetate and anhydrous aluminum trichloride as outlined in scheme (1).

The purity of the product **(I_b)** was first checked by the melting point constancy (202-203°C) which is in complete agreement with the reported ones. The obtained elemental analysis data given in Table (1) show an excellent agreement with the proposed molecular formula ($C_8H_8O_3$).



Scheme (1): Preparation of hydroquinone diacetate *(Ia)*2, 5-dihydroxyacetophenone, *(I_b)*.

Table (1): Physical data of the prepared compounds.

compounds	Molar ratio, M:L	%C	%H	%N	%Cl	Proposed Chemical formula	Molar conductivity ($\Omega^{-1}cm^2mol^{-1}$)	Spin only magnetic moment
		Found (Calc.)	Found (Calc.)	Found (Calc.)	Found (Calc.)			
I_a	-	61.72 (61.85)	5.05 (5.15)	-	-	$C_{10}H_{10}O_4$	-	-
I_b	-	63.12 (63.15)	5.85 (5.9)	-	-	$C_{10}H_8O_3$	-	-
L_1	-	74.6 (74.67)	6.25 (6.27)	5.79 (5.81)	-	$C_{15}H_{15}NO_2$	-	-
Cu^{2+}-L_1	1:1	37.351 (37.276)	6.102 (6.214)	2.925 (2.892)	7.471 (7.353)	$[CuL_1(H_2O)_4].Cl.4H_2O$	79.5	1.93
Zn^{2+}-L_1	1:1	37.796 (37.871)	5.412 (5.473)	5.924 (5.893)	-	$[ZnL_1(H_2O)_3NO_3].3H_2O$	15.8	diamagnetic
Cd^{2+}-L_1	1:1	33.924 (33.841)	5.797 (5.648)	2.584 (2.635)	6.713 (6.678)	$[CdL_1(H_2O)_4].Cl.4H_2O$	65.7	diamagnetic
Hg^{2+}-L_1	1:1	28.987 (29.038)	4.759 (4.845)	2.432 (2.265)	5.824 (5.729)	$[HgL_1(H_2O)_4].Cl.4H_2O$	70.6	diamagnetic
L_2	-	69.1 (69.12)	5.35 (5.39)	5.75 (5.76)	-	$C_{14}H_{13}NO_3$	-	-
Cu^{2+}-L_2	1:1	33.421 (33.395)	5.798 (5.963)	2.692 (2.786)	7.124 (7.067)	$[CuL_2(H_2O)_3].Cl.6H_2O$	66.9	1.91
Zn^{2+}-L_2	1:1	33.824 (33.917)	5.376 (5.257)	5.572 (5.653)	-	$[ZnL_2(H_2O)_2NO_3].5H_2O$	21.3	diamagnetic
Cd^{2+}-L_2	1:1	30.359 (30.448)	5.578 (5.437)	2.378 (2.539)	6.589 (6.438)	$[CdL_2(H_2O)_3].Cl.6H_2O$	69.1	diamagnetic

Continued,

Complexes	Molar ratio, M:L	%C	%H	%N	%Cl	Proposed Chemical formula	Molar conductivity ($\Omega^{-1}cm^2mol^{-1}$)	Spin only magnetic moment
		Found (Calc.)	Found (Calc.)	Found (Calc.)	Found (Calc.)			
Hg^{2+}-L_2	1:1	25.298 (26.241)	4.597 (4.683)	2.239 (2.185)	5.398 (5.549)	$[HgL_2(H_2O)_2 Cl].6H_2O$	19.3	diamagnetic
L_3	-	70 (70.02)	5.89 (5.88)	5.45 (5.44)	-	$C_{15}H_{15}NO_3$	-	-
Cu^{2+}-L_3	1:1	42.175 (42.218)	5.214 (5.163)	3.197 (3.285)	8.398 (8.327)	$[CuL_3 (H_2O)_3].Cl.H_2O$	69.2	1.95
Zn^{2+}-L_3	1:1	41.179 (37.769)	4.679 (4.583)	6.497 (6.417)	-	$[ZnL_3 (H_2O)_2NO_3].H_2O$	13.5	diamagnetic
Cd^{2+}-L_3	1:1	37.769 (37.872)	4.694 (4.638)	3.012 (2.947)	7.582 (7.475)	$[CdL_3 (H_2O)_3].Cl.H_2O$	70.2	diamagnetic
Hg^{2+}-L_3	1:1	32.089 (31.945)	3.915 (3.962)	2.413 (2.486)	6.286 (6.327)	$[HgL_3 (H_2O)_3].Cl.H_2O$	83.8	diamagnetic

I_a: hydroquinone diacetate

I_b: 2, 5-dihydroxyacetophenone

L_1, L_2, L_3: Schiff bases

Infrared spectrum of hydroquinone diacetate

The IR spectrum of the compound **(I_a)**, as KBr disk, exhibite bands at 3090 cm^{-1}, and 3100 cm^{-1} for aromatic C-H stretching and -OH stretching of adsorbed water molecules [16]. The bands at 1750 cm^{-1} (C=O stretching for ester), 1500 cm^{-1} (C=C ring stretching),1360 and 1220 cm^{-1} (bending -CH_3), and 1180 cm^{-1} (asymmetric C-O-C stretching) [17]. Comparing the structure of the compound

The infrared spectrum of 2, 5-dihydroxyacetophenone

The IR spectrum of compound **(I_b)** revealed the presence of the following bands at**:** 3230 cm^{-1} (phenolic -OH stretching) [17], 3060 cm^{-1} (aromatic -C-H stretching), 1640, and 1620 cm^{-1} (C=O stretching) [17,18], 1580 and 1490 cm^{-1} (aromatic -C=C- stretching) [10], 1405 and 1375 cm^{-1} (-CH_3 bending) [17],1300 cm^{-1} (bending -OH) [17], and 1210 cm^{-1} (-C-O- stretching) [16-18].

Table (2): Some selected bands of diagnostic importance for the IR spectra of I_a and I_b

Compound		Assignment
I_a	I_b	
-	3230	ν(OH) phenolic
3100	-	ν(OH) absorbed water
3090	3060	ν(C-H) assym (Ar)
1750	1640,1620	ν(C=O)
1500	1580,1490	ν (C=C) (Ar)
1360, 1220	1405,1375	ν (CH_3)
-	1300	(bending -OH)
1180	-	ν(C-O-C) assym
-	1210	(-C-O-)

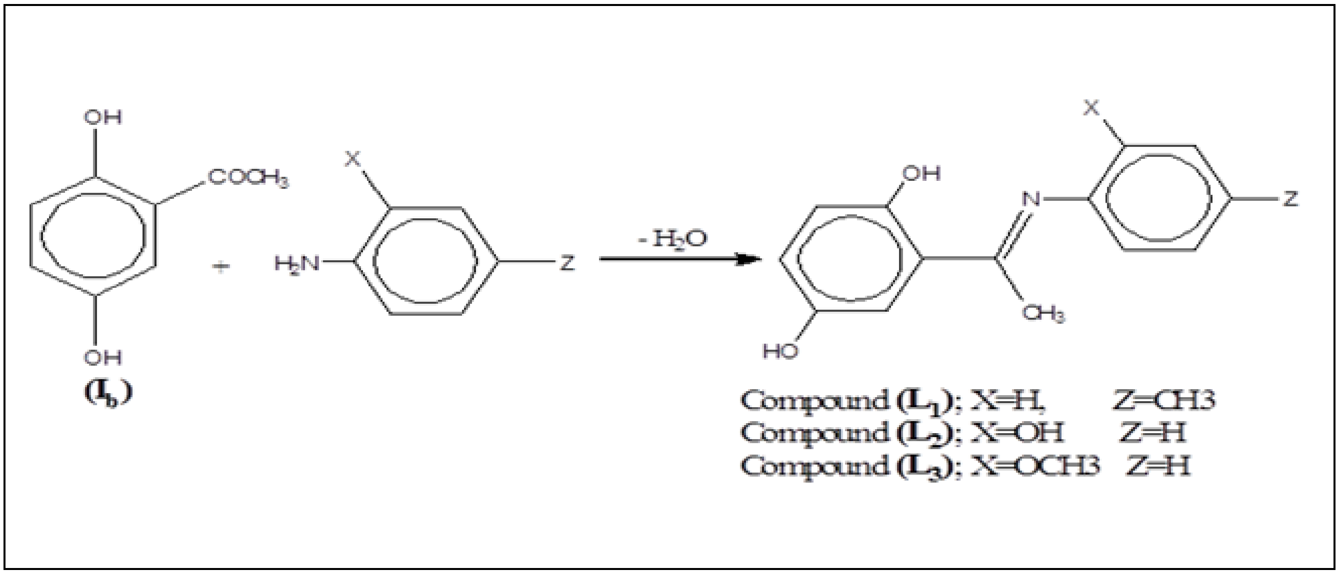
^{1}H-NMR spectrum of 2, 5-dihydroxyacetophenone compound (I_b)

The ^{1}H-NMR spectrum (in d_6-DMSO) expressed in δ ppm scale, revealed the presence of six signals due to $\mathbf{I_b}$ compound. The first one is a singlet at $\delta = 2.6$ ppm (s, 3 H, -CH_3) due to the -CH_3 group. There is one doublet at $\delta = 6.8$ ppm (d, 1H, H_a) due to the proton labeled H_a of the benzene ring. There is another one doublet at $\delta = 7.0$ ppm (d, 1H, H_b) which is due to the proton that is labeled H_b of the benzene ring and there is more one doublet at 7.2 ppm due to one proton labeled H_c. There is one broad singlet at $\delta = 9.2$ ppm (s, 1H, -OH) due to the labeled OH^2 group [20]. There is one singlet signal at $\delta = 11.3$ ppm (s, 1H, -OH) which is due to the phenolic OH^1 group. [19]

Synthesis and characterization of some Schiff bases

The Schiff bases under study were prepared by condensation of 2,5-dihydroxyacetophenone, *(I_b)*, with different amine derivatives (paratoluidine, orthoaminophenol, and orthoanizidine, separately) according to Scheme (2). The percentage obtained of yields were in the range from 78.1 % to 85.3 %.

The prepared Schiff bases were characterized by melting point, elemental analysis, IR spectrum, and ^{1}H-NMR spectrum.



Scheme (2). Schematic representation for the synthesis of Schiff bases: L_1, L_2, and L_3.

Melting points and elemental analysis:

Sharp m.p. range was obtained indicating the high purity of the prepared Schiff bases. The C, H, and N% obtained from elemental analysis are in excellent agreement with the proposed molecular formulas. The data of the elemental analysis along with m.p. values are cited in Table (1).

FT-IR spectra of L_1, L_2 and L_3 Schiff bases

In the FT-IR spectra of the synthesized Schiff bases as shown in Table (3) a medium intensity band observed in the range of 3589–3245 cm^{-1} is attributed to OH stretching whereas, a medium to high intensity bands observed at 1618, 1581–1612, 1575 cm^{-1} are attributed to C=N stretching. The absorption at about 3060 cm^{-1} can be assigned to aromatic -CH stretching. Various absorption bands observed at about 1490 cm^{-1} are assigned to C=C stretching vibrations whereas, bands observed in the range of 786–780 cm^{-1} are assigned to C-H out of plane bending of aromatic rings. Other absorption bands observed in the range 710-749 cm^{-1} are assigned to hydrogen bonded out of plane O-H [16, 17, 20].

Table (3): Representive results for analysis of IR spectra of the Schiff base ligands and their metal chelates (cm^{-1}).

Compound	ν(OH^1)	ν(OH^2)	ν(OH^3)	(C-H)	ν (C=C)	ν (CH$_3$)	(bending - OH)	ν (C=N)	δ (C=N)	ν(M-O)	ν(M-N)
L$_1$-free	3249	-	-	3063	1493	1407, 1367	1302,1212	1618, 1575	641	-	-
Cu^{2+}-L$_1$	-	-	3263	2929	1498	1408, 1304	1304,1213	1617, 1579	637	531	444
Zn^{2+}-L$_1$	-	-	3238	3059	1493	1381	1299, 1210	1616, 1574	647	522	442
Cd^{2+}-L$_1$	-	-	3250	2927	1489	1406, 1373	1302,1211	1615, 1579	650	529	435
Hg^{2+}-L$_1$	-	-	3261	3064	1496	1407, 1369	1302,1212	1607, 1575	646	527	450
L$_2$-free	3302	3376	3589	3056	1495	1404, 1370	1298,1205	1612, 1581	615	-	-
Cu^{2+}-L$_2$	-	3323	3580	2932	1498	1402	1307	1585	610	537	410
Zn^{2+}-L$_2$	-	3352	3583	3059	1497	1381	1299	1609, 1580	630	530	420
Cd^{2+}-L$_2$	-	3361	3574	3058	1500	1402	1298	1606, 1579	625	536	426
Hg^{2+}-L$_2$	-	3365	3576	3063	1497	1400, 1366	1301	1605, 1579	635	533	446
L$_3$-free	3245	-	-	3063	1493	1403, 1367	1301,1211	1614, 1572	644	-	-
Cu^{2+}-L$_3$	-	-	3254	2361	1498	1375	1303	1610	640	538	466
Zn^{2+}-L$_3$	-	-	3246	3059	1490	1380	1301	1601, 1576	642	535	458
Cd^{2+}-L$_3$	-	-	3241	3064	1494	1402, 1368	1300	1621, 1574	653	526	451
Hg^{2+}-L$_3$	-	-	3261	3063	1495	1408, 1371	1304	1622, 1577	656	523	460

^{1}H-NMR spectra of L$_1$, L$_2$ and L$_3$ Schiff bases

The ^{1}HNMR spectra (in d$_6$-DMSO) of the synthesized Schiff bases have signals at about: 2.7 (s, 3 H, CH$_3$) (labeled CH$_3^{(4)}$ for L$_2$ and L$_3$ and labeled CH$_3^{(3)}$for L$_1$), 2.3 (s, 3 H, CH$_3^{(4)}$) (L$_1$), 3.8 (s, 3 H, -OCH$_3$) (L$_3$), 6.4 (m, 3H, H$_e$, H$_f$ and H$_g$) (L$_3$), 6.4 and 6.6 ppm (m, 4H, H$_d$, H$_e$, H$_f$ and H$_g$) (L$_2$), 6.8 [(d, 2H, H$_e$ and H$_f$) (L$_1$), and (d, 1H, H$_b$) (L$_3$)], 7.0 [(d, 2H, H$_g$ and H$_d$) (L$_1$), H$_c$ (L$_2$), H$_c$ (L$_3$)], 7.2[(d, 2H, H$_b$ and H$_c$) (L$_1$), (s, 1H, H$_a$) (L$_2$), (d, 2H, H$_a$ and H$_d$) (L$_3$), 7.3 (s, 1H, H$_a$) (L$_1$), 8.9 (s, 1H, labeled OH$^{(1)}$) (L$_2$), 9.18 (s, 1H, OH$^{(1)}$), 9.2 [(s, 1H, OH$^{(3)}$) (L$_2$), [(s, 1H, OH$^{(1)}$) (L$_3$)], 11.3 [(s, 1H, OH$^{(2)}$ (L$_1$), (L$_2$), (L$_3$),) these data are collected in Table (3) [20].

Table (4): Assignment and chemical shift (ppm) of different types of protons of ligands under investigation.

Ligand (L$_1$) /chemical shift ppm) of protons	Assignment	Ligand (L$_2$) /chemical shift ppm) of protons	Assignment	Ligand (L$_3$) /chemical shift ppm) of protons	Assignment
2.3	H(4)	2.7	H(4)	2.7	H(4)
2.7	H(3)	6.4	H$_d$, H$_e$	3.8	H(3)
6.8	H$_e$, H$_f$	6.6	H$_f$, H$_g$	6.4	H$_e$, H$_f$ and H$_g$
7.0	H$_g$, H$_d$	6.8	H$_b$	6.8	H$_b$
7.2	H$_b$, H$_c$	7.0	H$_c$	7.0	H$_c$
7.3	H$_a$	7.2	H$_a$	7.2	H$_a$ and H$_d$
9.18	H(1)	8.9	H(1)	9.2	H(1)
11.3	H(2)	9.2	H(3)	11.3	H(2)
-	-	11.3	H(2)	-	-

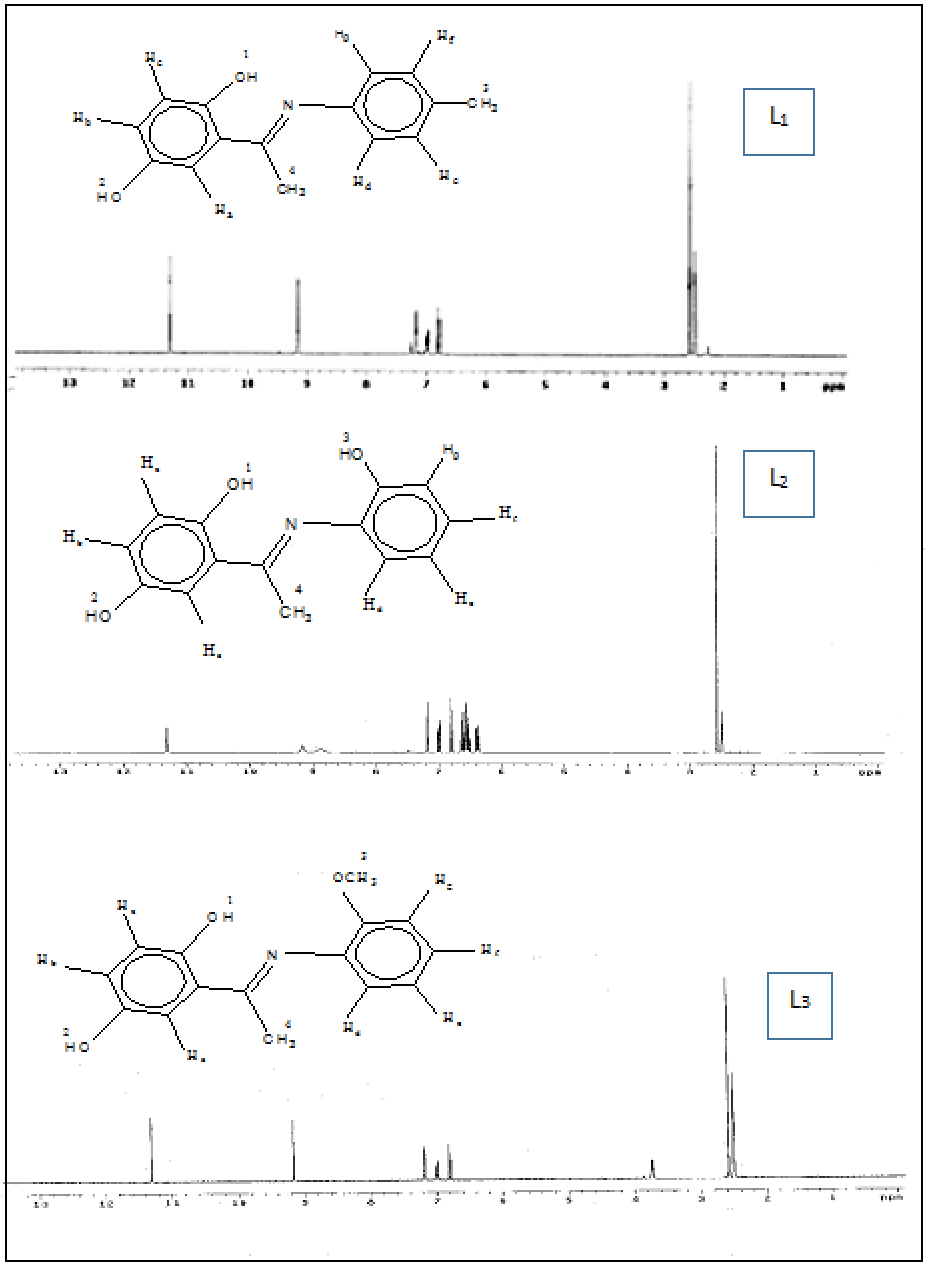


Fig. (1) ^{1}H-NMR spectra of the prepared ligands

Synthesis and characterization of Schiff base complexes

Solid metal-Schiff base complexes were synthesized by allowing the synthesized Schiff base ligands to react with some metal salts. In the present work the metal chlorides for Cu^{2+}, Cd^{2+} and $Hg^{2+}$are used, while in case of Zn^{2+} ion the nitrate salt was used. The synthesized Schiff base complexes were characterized using elemental analysis, molar conductivity, FT-IR, magnetic moment analysis, and UV–Vis spectroscopy. The elemental analyses of complexes are in excellent agreement with the proposed molecular formulas as shown in Table (1).

FT-IR spectra of Schiff base complexes

Assignments of significant infrared bands of the free ligands and their metal complexes are compiled in Table (3). According to the obtained IR data each prepared ligand coordinates with the four metal cations under study, as mono-negative ONO tridentate ligand. The ligand coordinates through N atom of (C=N) $_{\text{azomethine}}$, oxygen atom of the deprotonated OH^1, and oxygen atom of undeprotonated OH^2. The mode of chelation is supported by (i) disappearance of the bands attributed to ν (OH^1), (ii) the shift of ν $(C=N)_{\text{azomethine}}$ to lower frequencies, and (iii) the shift of bands assigned to ν (OH^2).

New bands observed in all complexes at 520-538 and 410-466 cm^{-1} can be assigned to the ν (M-O) [21] and ν (M-N) [22] respectively.

Electronic spectra and magnetic measurements of the solid complexes

The electronic spectra of the ligands show two bands in the range 27000- 28000 cm^{-1} and 28000-29000 cm^{-1} for the prepared ligands. The first one can be attributed to π-π* transition of the benzene ring. The second band may be assigned to the n-π*transition associated to the azomethine. The bands undergo shift to higher energy suggesting and confirming the coordination of the imino nitrogen atom and oxygen atom of the hydroxyl group with the central metal ion.

However, Zn complexes showed bands at ca 28000 and 27000 cm^{-1}; Cd complexes exhibited bands at ca 28000, 26000 cm^{-1}; and Hg complexes revealed bands at ca 27000 and 26000 cm^{-1}, which might be due to L-M charge transfer transition.

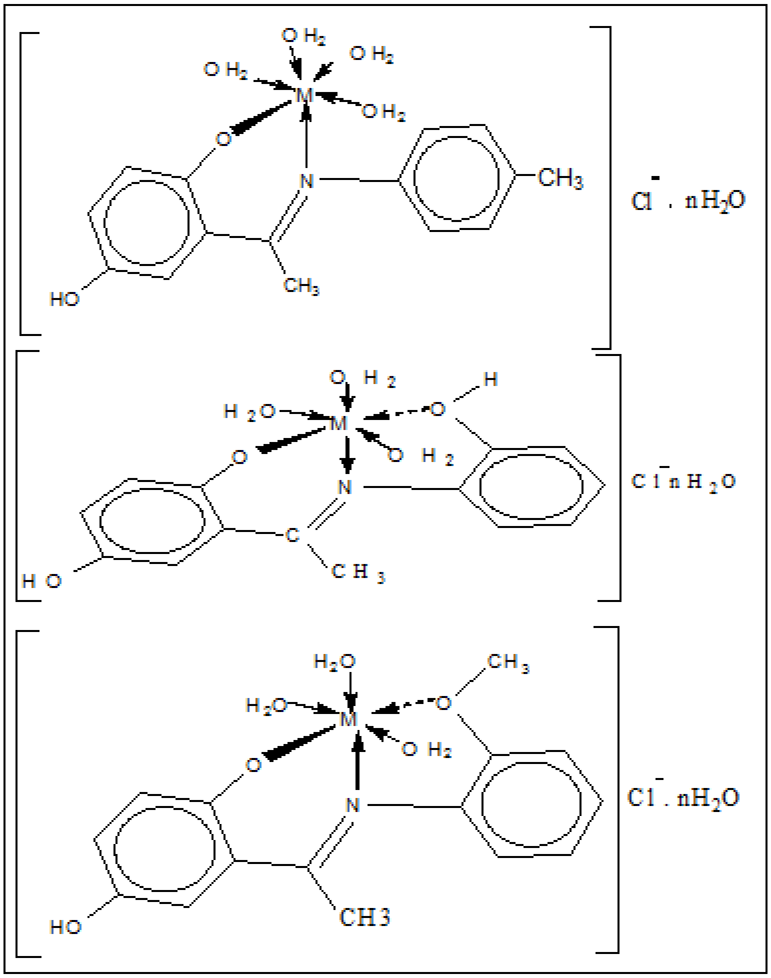
On the other hand, the electronic spectra of Cu (II) complexes showed only one broad band at ca (24000-29000) cm^{-1} assigned to $^2E_g \longrightarrow {}^2T_{2g}$ which confirmed the octahedral geometry of the solid copper complexes under investigation. Moreover, the magnetic susceptibility measurement, confirmed the conclusion deduced from the electronic spectra of the prepared complexes. The Zn(II), Hg(II) and Cd(II) complexes were found to be diamagnetic as expected for d^{10} configuration. However, the magnetic moment values of the prepared Cu(II) complexes were found to be around 1.9 B.M which suggest a distorted octahedral geometry around the copper ion, Table (1).

Table (5): Electronic absorption spectral data of the synthesized ligands and their complexes

Compound	Ratios M:L	Band maxima (cm^{-1})		Assignment
		DMF	Nujol mull	
Free L$_1$	-	27932, 27173	26041, 24630	π-π^*, n-π^*
Cu^{2+}-L$_1$	1:1	29239	26178, 23474	$^2E_g \longrightarrow {}^2T_{2g}$
Zn^{2+}-L$_1$	1:1	28011, 27322	31055, 23529	n-π^*,CT
Cd^{2+}-L$_1$	1:1	28248, 27027	29444, 21645	n-π^*,CT
Hg^{2+}-L$_1$	1:1	28089, 27397	31152, 26737	n-π^*,CT
Free L$_2$	-	28248, 26954, 22779	26246, 23923	π-π^*, n-π^*
Cu^{2+}-L$_2$	1:1	24154	24271	$^2E_g \longrightarrow {}^2T_{2g}$
Zn^{2+}-L$_2$	1:1	28328, 24038, 23148	25773, 23255	π-π^*, n-π^*,CT
Cd^{2+}-L$_2$	1:1	28409, 26455, 23201	25000, 22727	π-π^*, n-π^*,CT
Hg^{2+}-L$_2$	1:1	27100, 24330, 22935	25510, 22624	π-π^*, n-π^*,CT
Free L$_3$	-	28169, 26881	25839, 23419	π-π^*, n-π^*
Cu^{2+}-L$_3$	1:1	29154	22321	$^2E_g \longrightarrow {}^2T_{2g}$
Zn^{2+}-L$_3$	1:1	28248, 27072	26455, 20661	n-π^*,CT
Cd^{2+}-L$_3$	1:1	28011, 27173	26315, 23364	n-π^*,CT
Hg^{2+}-L$_3$	1:1	28490, 26595	26385, 23584	n-π^*,CT

Conductivity measurements

The molar conductivities of the solid complexes in DMF are listed in Table (1). The molar conductivity values for Zn^{2+} metal ion complexes of the ligands, L$_1$ - L$_3$ and Hg^{2+}-L$_2$ complex fall in the range 13.5-21.3 Ω^{-1}cm^{2}mol^{-1} indicating the non-electrolytic and neutral character of such complexes. On the other hand, the values of the molar conductivity for Cu^{2+}, Cd^{2+}and Hg^{2+}complexes of ligand L$_1$; Cd^{2+}-L$_2$, and Cu^{2+}, Cd^{2+}and Hg^{2+} complexes of ligand L$_3$ fall within the range 65.7- 83.8 Ω^{-1}cm^{2}mol^{-1} indicating that these complexes have an ionic nature and each molecule ionizes in solution to two ions



Scheme (3). Proposed schematic representation of M-L complexes.

Biological activity studies of free ligands and their complexes

In this study, five microorganisms representing different microbial categories (three gram negative bacteria, ***Escherichia coli, Klebsiell pneumonia*** & ***Proteus vulgarius)***, one and gram positive bacteria (***Staphylococcus aureus)*** *and one yeast* (***Candida albicans***) were used. The study includes 15 compounds, 3 ligands (L_1-L_3) and 12 complexes of different metal ions (Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+})

The diffusion agar technique was used to evaluate the antibacterial activity of the prepared ligands and their chelates [23]. The results of the microorganisms screening of the synthesized Schiff base compounds and their complexes are listed in table (5) and shown in Figure (2).

The data presented in Table (6) reflected that ligand L_1 showed antibacterial activity against Klebsiell pneumonia (G-) and Candida albicans (yeast).

Metal complexes for L_1 are found to inhibit most of tested bacteria at different rates and the biological activity has the order Hg- L_1 >Cd- L_1 >Cu- L_1 >Zn- L_1 for gram -negative bacteria and Hg-L_1>Cd- L_1 for gram- positive bacteria and yeast. It is also obvious that Hg- L $_1$, Cd-L_1 complexes at higher concentrations have higher biological activity than ligand L_1.

As about ligand L_2, the data given in Table (6) reflected that this compound showed antibacterial activity against gram- negative, gram-positive in addition to yeast. Also, ligand L_2 has higher sensitivity to gram- positive than gram-negative bacteria. And its metal complexes are found to inhibit almost all the tested bacteria and yeast at different rates and different concentrations. The biological activity which has the order Hg-L_2> Cd-L_2 > Zn-L_2> Cu-L_2 for gram-positive bacteria and Hg-L $_2$>Cd-L_2 > Cu-L_2 >Zn-L_2 for gram-negative bacteria. In addition, Hg-L_2 and Cd-L_2 have nearly higher activities towards yeast while Cu-L_2 showed slightly inhibition to yeast. It was obvious that Hg-L_2, Cd-L_2, and Cu-L_2 complexes have higher biological activity than the ligand L_2 against all the tested organisms. Ligand L_3 did not exhibit any remarkable activity, as shown in Table (6), only Cu-L_3 showed slight activity against both gram-negative bacteria (E. coli and K.pneumoniae), while Hg-L_3 complex exhibited high biological activity against all tested organisms which increased with increasing the concentration.

Moreover, the antimicrobial results evidently show that the activity of the Schiff base compounds became more pronounced when coordinated to the metal ions in most cases. Also, the antimicrobial activity of the Schiff base compounds and their complexes show a positive relationship with concentration. It is suggested that the complexes having antimicrobial activity may act either by killing the microbe or by inhibiting multiplication of the microbe by blocking their active sites [24]. Such increased activity of the metal chelates can be explained on the bases of chelation theory. On chelation the polarity of the metal ion will be reduced to a great extent due to the overlap with the ligand orbital. Further, chelating increases the delocalization of the π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes, this increased lipophilicity leads to breakdown of the permeability barrier of the cell and thus retards the normal cell process [25].

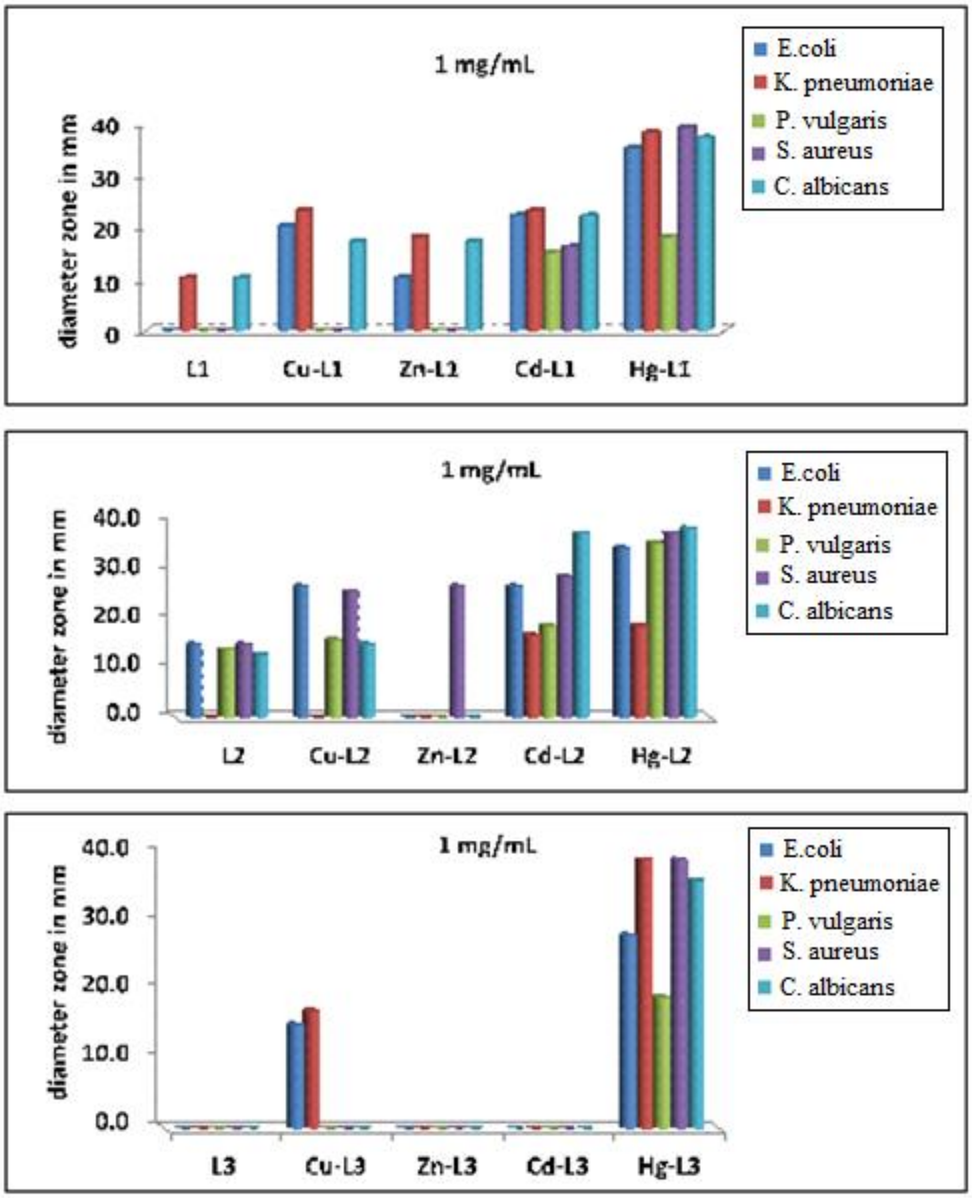


Fig. (2): Biological activity histogram for all ligands and their metal complexes.

Table (6): Responses of various microorganisms to the Schiff bases and their complexes in vitro culture (numbers in the table represent the extent of the inhibition zone diameters in mm).

compounds	Concentration (mg/mL)	E.coli	K. pneumoniae	P. vulgaris	Staph. aureus	C.albicans
L_1	0.25	-	-	-	-	-
	0.50	-	-	-	-	-
	1.00	-	10	-	-	10
Cu^{2+}-L_1	0.25	-	11	-	-	11
	0.50	12	15	-	-	13
	1.00	20	23	-	-	17
Zn^{2+}-L_1	0.25	-	11	-	-	11
	0.50	-	15	-	-	13
	1.00	10	18	-	-	17
Cd^{2+}-L_1	0.25	13	12	-	-	-
	0.50	16	14	12	12	18
	1.00	22	23	15	16	22
Hg^{2+}-L_1	0.25	-	19	-	19	18
	0.50	25	34	13	32	33
	1.00	35	38	18	39	37
L_2	0.25	-	-	-	-	-
	0.50	10	-	10	11	-
	1.00	15	-	14	15	13
Cu^{2+}-L_2	0.25	15	-	-	-	-
	0.50	23	-	12	21	-
	1.00	27	-	16	26	15
Zn^{2+}-L_2	0.25	-	-	-	13	-
	0.50	-	-	-	22	-
	1.00	-	-	-	27	-
Cd^{2+}-L_2	0.25	13	-	-	16	22
	0.50	23	12	15	22	33
	1.00	27	17	19	29	38
Hg^{2+}-L_2	0.25	15	-	15	17	18
	0.50	25	13	26	25	28
	1.00	35	19	36	38	39
L_3	0.25	-	-	-	-	-
	0.50	-	-	-	-	-
	1.00	-	-	-	-	-
Cu^{2+}-L_3	0.25	-	-	-	-	-
	0.50	-	-	-	-	-
	1.00	15	17	-	-	-
Zn^{2+}-L_3	0.25	-	-	-	-	-
	0.50	-	-	-	-	-
	1.00	-	-	-	-	-
Cd^{2+}-L_3	0.25	-	-	-	-	-
	0.50	-	-	-	-	-
	1.00	-	-	-	-	-
Hg^{2+}-L_3	0.25	-	18	-	-	18
	0.50	18	33	12	33	26
	1.00	28	39	19	39	36

Numbers in the table represent the extent of the inhibition zone in mm.

(-): no inhibition (10mm: 19mm): Slightly active (20mm: 29mm): Moderately active (30mm: 40mm): highly active

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