



Antimicrobial evaluation of newly synthesized tri nuclear complexes

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Abstract

In this study, we describe the synthesis, spectroscopic characterization and antimicrobial evaluation of novel Schiff base 1 and its trinuclear complexes. Schiff base 1 obtained by condensation of Protocatechualdehyde (3,4-dihydroxybenzaldehyde, PCA) with o-phenylene-diamine in 2:1 molar ratio, respectively. Reaction of ligand 1 with chlorides of Co(II), Ni(II) and Cu(II) as well as nitrates of Fe(III) and Zn(II) resulted in the formation of the corresponding metal complexes 2-6. Structural elucidations of ligand and its complexes were based on compatible analytical and spectroscopic evidences. The data reveals that the ligand molecule coordinate through three coordination centers {phenolic(O,O), imine(N,N) and another phenolic (O,O)}. Electronic spectra and magnetic susceptibility measurements were corroborative of octahedral geometry for iron complex, Tetrahedral for Cobalt, Copper and Zinc complexes as well as square planar for Nickel complex. The synthesized ligand, along with its metal complexes, were screened for their in vitro antimicrobial activity against two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*), two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains, unicellular fungi (*Candida albicans*) and filamentous fungi (*Aspergillus niger*). Complex 6 shows highest antimicrobial activity. The minimum inhibitory concentration (MIC) values of the compounds against the growth of microorganisms were determined by means of standard serial dilution method.

Key words: Metal chelates, Spectral characterization, biological evaluation and MIC

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1. Introduction

Metal ions play a vital role in a vast number of widely different biological processes. The interaction of these ions with biologically active ligands, for example in drugs, is a subject of considerable interest. Some of the biologically active compounds act via chelation [1], but for most of them little is known about how metal binding influences their activity. Schiff bases enjoy promising properties which attract growing interest both from preparative and biological viewpoints [2-4]. The C=N linkage in these bases is essential for biological activity where several azomethines were reported to possess remarkable antibacterial, antifungal, agrochemical and other biological activities [5]. Besides, Schiff bases can coordinate via the azomethine nitrogen to metal ions yielding complexes which can act as successful models of biological compounds [6]. Thus, they are found in potential anticancer drugs [7] and their activity is markedly enhanced in comparison to their free ligands [8]. Besides, Schiff base complexes can be found at key points in the development of inorganic biochemistry, catalysis and optical materials [9]. Recently, we reported synthesis, characterization and

biological evaluation of Fe (III), Co (II), Ni(II), Cu(II), and Zn(II) with Schiff bases derived from protocatechualdehyde with 2-amino phenol [10] and p-phenylene diamine [11], the ligands and their metal complexes show high biological activities. Therefore, here we interest in studying the complexing ability and the biological activity of new bifunctional azomethine Schiff base (1) and its metal complexes with Fe (III), Co (II), Ni(II), Cu(II), and Zn(II).

2. Materials and methods

2.1. Materials and reagents

The chemicals used 3,4-dihydroxy benzaldehyde, 1,2-phenylene diamine, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ are Merck or Aldrich and organic solvents used (methanol, ethanol, diethyl ether and acetone) were HPLC or extra pure grades and were used without further purification.

2.2. Instruments

Percentages of C, H and N were determined in the Micro analytical Laboratory, Cairo University, Giza, Egypt. IR

spectra were recorded using KBr pellets on a Perkin-Elmer 1430 Spectrometer in the region (4000-200 cm^{-1}) at Tanta University, Gharbia, Egypt. Electronic spectra were measured in UV/Vis range (195-1100 nm) using a Perkin-Elmer lambda 35 UV/Vis at Al-Azhar University, Cairo, Egypt. The $^1\text{H-NMR}$ spectra were recorded on DEITAZ NMR 500 MHZ Spectrometer at the National Research Centre, Dokki, Giza, Egypt. The mass spectra were recorded on GC-MSA-QP 5050A Shimadzu at the National Research Centre, Dokki, Giza, Egypt. Magnetic susceptibility measurements were carried out at room temperature on a Sherwood Scientific Magnetic Balance at Mansora University, Egypt. Antimicrobial activity measurements were carried out at Fermentation Biotechnology and Applied Microbiology Center, Al-Azhar University, Cairo, Egypt.

2.3. Synthesis of the Schiff base ligand (1)

Schiff base ligand **1** prepared by drop wise addition of hot ethanolic solution (20ml) of 3,4-dihydroxybenzaldehyde (1.381g, 0.010mole) to ethanolic solution (20ml) of *o*-phenyldiamine (0.5405g, 0.005mole). The reaction mixture was heated under reflux for 3h. Evaporation of solvent allowed the appearance of fine brown crystals which were filtered, washed with ethanol, acetone and diethyl ether then air dried. M.p.: 220 $^{\circ}\text{C}$; M.wt.: 348.0; Anal. Calcd. for $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_4$: C, 68.95; H, 4.58; N, 8.0 %. Found: C, 68.65; H, 4.24; N, 7.96%. Main IR peaks (KBr, cm^{-1}): $\nu(\text{OH})$ 3381; $\nu(\text{CH, ar})$ 3052; $\nu(\text{C=N})$ 1620; $\nu(\text{Ph-O})$ 1279 and $\nu(\text{Ph-N})$ 1133.

2.4. General procedure for Preparation of the metal complexes 2-6.

The metal complexes were prepared by dropwise addition of an ethanolic solution of the metal salt to an ethanolic solution of the ligand in a 3:1 molar ratio. The reaction mixture was refluxed for 3h and the obtained precipitate was filtered, washed with ethanol, acetone and diethyl ether then air dried.

2.4.1. The complex, $\text{Fe}_3\text{L}(\text{H}_2\text{O})_7(\text{NO}_3)_5$; 2

Black solid. M.p. >300 $^{\circ}\text{C}$; M.wt.: 947.4; Anal.Calcd. for $\text{C}_{20}\text{H}_{26}\text{Fe}_3\text{N}_7\text{O}_{26}$: C, 25.34; H, 2.75; N,10.2; Fe,17.65%. Found: C, 26.50; H, 3.42; N, 10.08; Fe, 17.1; Main IR peaks (KBr, cm^{-1}): $\nu(\text{CH, ar.})$ 3068; $\nu(\text{C=N})$ 1602; $\nu(\text{Ph -O})$ 1190 and $\nu(\text{Ph -N})$ 1118.

2.4.2. The complex, $\text{Co}_3\text{L}(\text{H}_2\text{O})_4(\text{Cl})_2.2\text{H}_2\text{O}$; 3

Dark brown solid. M.p. >300 $^{\circ}\text{C}$; M.wt.: 699.7; Anal.Calcd. for $\text{C}_{20}\text{H}_{24}\text{Co}_3\text{N}_2\text{O}_{10}\text{Cl}_2$: C, 34.32; H, 3.43; N, 4.00; Co, 25.25%. Found: C, 34.37; H, 3.36; N, 3.59; Co, 24.7%. Main IR peak (KBr, cm^{-1}): $\nu(\text{CH, ar.})$ 3022; $\nu(\text{C=N})$ 1611; $\nu(\text{Ph -O})$ 1195 and $\nu(\text{Ph -N})$ 1122.

2.4.3. The complex, $\text{Ni}_3\text{L}(\text{H}_2\text{O})_4(\text{Cl})_2.2\text{H}_2\text{O}$; 4

Yellowish brown solid, M.p. >300 $^{\circ}\text{C}$; M.wt.699.13; Anal.Calcd. for $\text{C}_{20}\text{H}_{24}\text{Ni}_3\text{N}_2\text{O}_{10}\text{Cl}_2$: C, 34.32; H, 3.43; N, 4.00; Ni, 25.20%; Found: C, 34.95; H, 3.52; N, 3.05; Ni, 24.9%; Main IR peak (KBr, cm^{-1}): $\nu(\text{CH, ar.})$ 3035; $\nu(\text{C=N})$ 1613; $\nu(\text{Ph -O})$ 1193 and $\nu(\text{Ph -N})$ 1117.

2.4.4. The complex, $\text{Cu}_3\text{L}(\text{H}_2\text{O})_4(\text{Cl})_2.2\text{H}_2\text{O}$; 5

Black solid. M.p.: 255 $^{\circ}\text{C}$; M.wt.: 713.5; Anal.Calcd. for $\text{C}_{20}\text{H}_{24}\text{Cu}_3\text{N}_2\text{O}_{10}\text{Cl}_2$: C, 33.70; H, 3.36; N, 3.92; Cu, 26.69%. Found: C, 34.09; H, 3.5; N, 3.42; Cu, 26.2%. Main IR peak (KBr, cm^{-1}): $\nu(\text{CH, ar.})$ 2920; $\nu(\text{C=N})$ 1598; $\nu(\text{Ph -O})$ 1215 and $\nu(\text{Ph -N})$ 1110.

2.4.5. The complex, $\text{Zn}_3\text{L}(\text{H}_2\text{O})_4(\text{NO}_3)_2.2\text{H}_2\text{O}$; 6

Brown solid. M.p.: 270 $^{\circ}\text{C}$; M.wt. 754.0; Anal.Calcd. for $\text{C}_{20}\text{H}_{22}\text{Zn}_3\text{N}_4\text{O}_{15}$: C, 31.8; H, 2.92; N, 7.76; Zn, 25.95%; Found: C, 32.5; H, 2.1; N, 8.2; Zn, 25.10; Main IR peak (KBr, cm^{-1}): $\nu(\text{CH, ar.})$ 3075; $\nu(\text{C=N})$ 1612; $\nu(\text{Ph -O})$ 1188 and $\nu(\text{Ph -N})$ 1122.

3. Results and Discussion

Schiff base ligand **1** (Fig.1) obtained by the reaction of PCA and 1,2-phenylene diamine. The structure of formed Schiff base was established by IR, $^1\text{H-NMR}$, mass and UV-Vis spectra as well as elemental analyses. The metal complexes were prepared by direct reaction between Schiff base ligand **1** and the appropriate metal salt in 1:3 molar ratio, respectively. The obtained complexes (Figures.2,3 and 4) are stable in air and have melting points above 250 $^{\circ}\text{C}$. They are insoluble in organic solvents such as diethyl ether and acetone, but soluble in DMF and DMSO. The elemental analysis data of the Schiff base and complexes are in well agreement with the expected structures. The molar conductance values of the synthesized complexes were determined by using 10 $^{-3}$ M concentration in DMF as solvent, in the range of 0.83 -1.65 $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$. These values suggest non-electrolyte nature for these complexes [12].

3.1. Characterization of the ligand

The infrared spectrum of Schiff base ligand **1** showed a medium absorption band at 1620 cm^{-1} assigned to the C=N stretching vibration, indicating the formation of the Schiff base linkage. Furthermore, the spectrum showed the absence of C=O and -NH₂ stretching vibrations related to aldehyde and amine, respectively, indicating the completion of the Schiff base formation [13]. The spectrum showed a broad medium intensity band at 3381 cm^{-1} assigned to νOH . Stretching vibration due to $\nu\text{Ph-O}$ was observed at 1279 cm^{-1} . The two-weak intensity bands at 3052 and 2938 cm^{-1} corresponding to $\nu(\text{C-H})$ ar. and $\nu(\text{C-H})$ aliph stretching vibration. $\nu\text{Ph-N}$ gave medium intensity band at 1133 cm^{-1} . The $^1\text{H-NMR}$ spectrum of the Schiff base ligand (Fig.5) showed broad signals lying at 8.9-9.7ppm due to resonance of the hydroxyl groups; the signals of OH groups lying at higher field side could be attributing to the contribution of the OH group intramolecular and intermolecular hydrogen bonds. The resonance of azomethine proton downfield shifted to 9.6 ppm due to the strong deshielding effect of the hydroxyl groups. Also the multiple lying in the range 6.3-7.6 ppm is attributed to resonance of aromatic protons. The electronic spectrum of the ligand in DMF displayed bands below 330nm which were attributed to intraligand $\pi-\pi^*$ and $n-\pi^*$ transitions of the phenyl rings and azomethine groups, respectively.

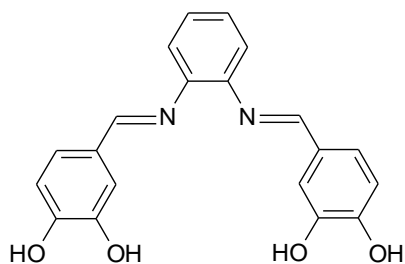


Fig.1. Structure of ligand 1

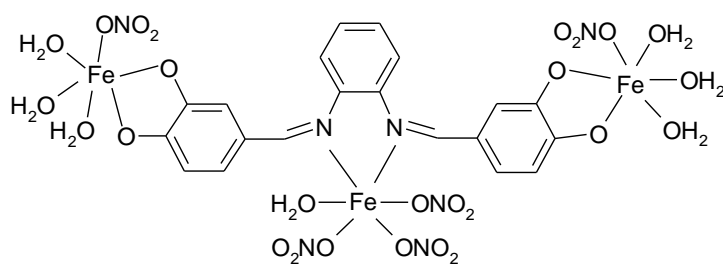


Fig.2. Octahedral structure of complex (2)

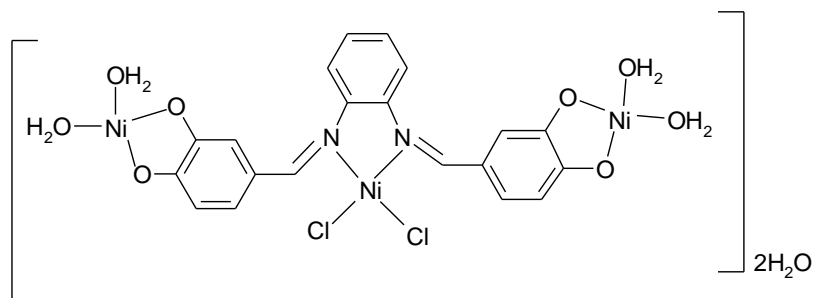
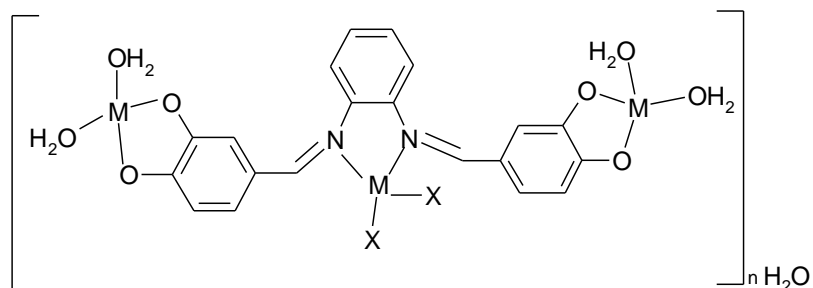


Fig.3. Square planer structure of complex (4)



M= Co and Cu X=Cl n=2
M=Zn X=NO3 n=1

Fig.4. Tetrahedral structure of complexes (3, 5 and 6)

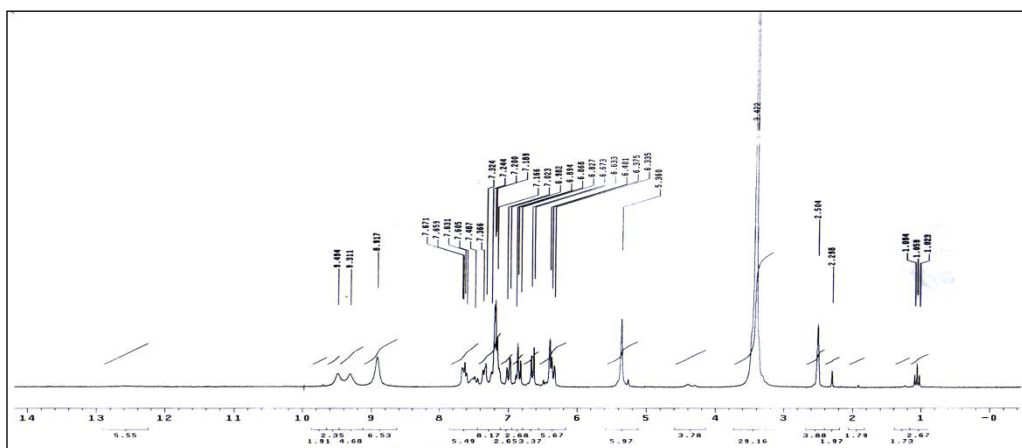


Fig.5. The $^1\text{H-NMR}$ spectrum of 1 in (DMSO)

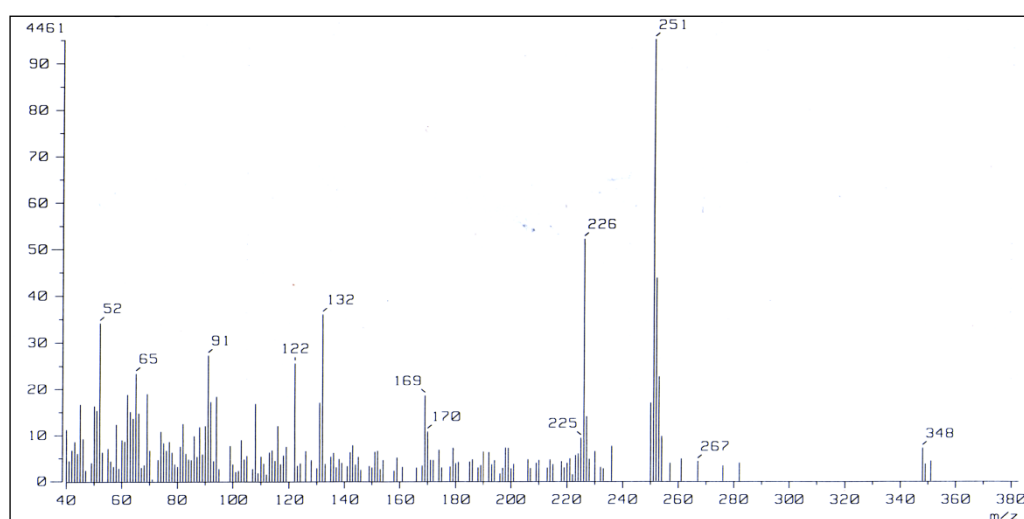


Fig.6. The mass spectrum for 1

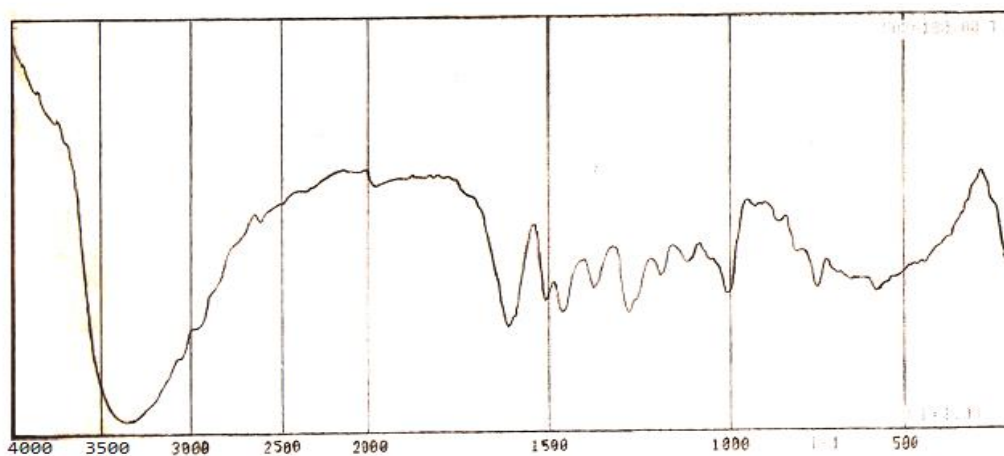


Fig.7. IR spectrum of complex (4)

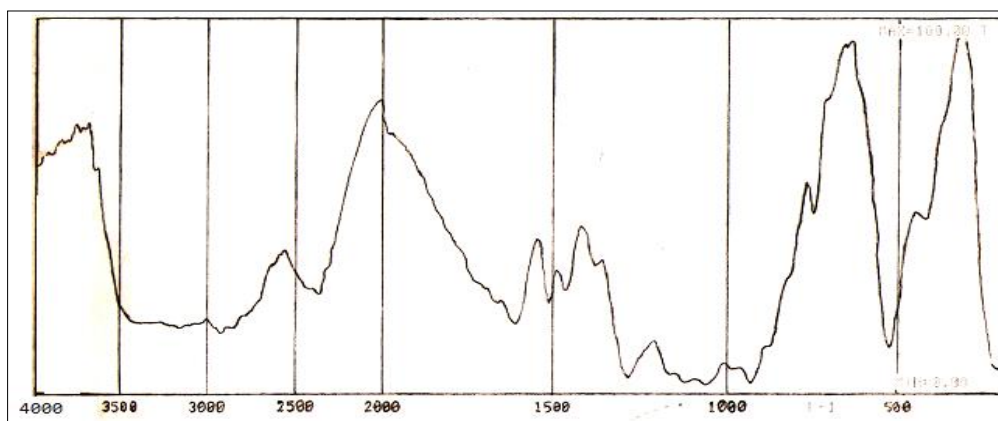


Fig.8. IR spectrum of complex (6)

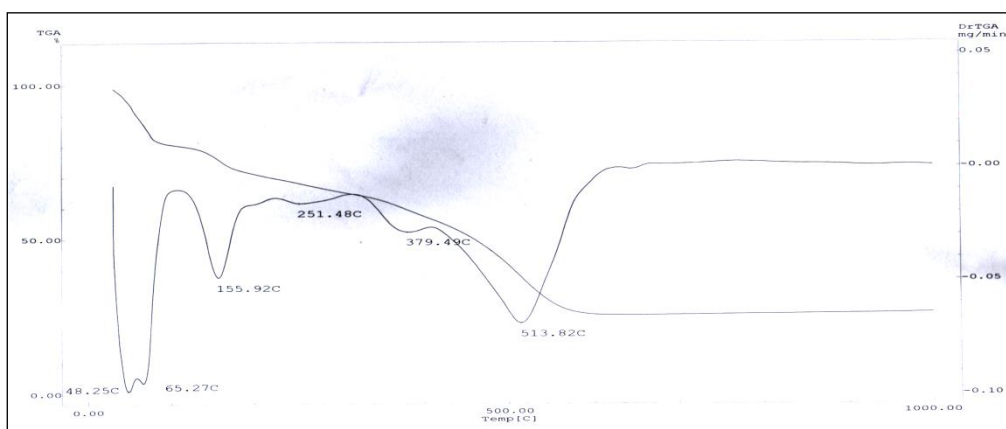


Fig.9. TGA of complex (4)

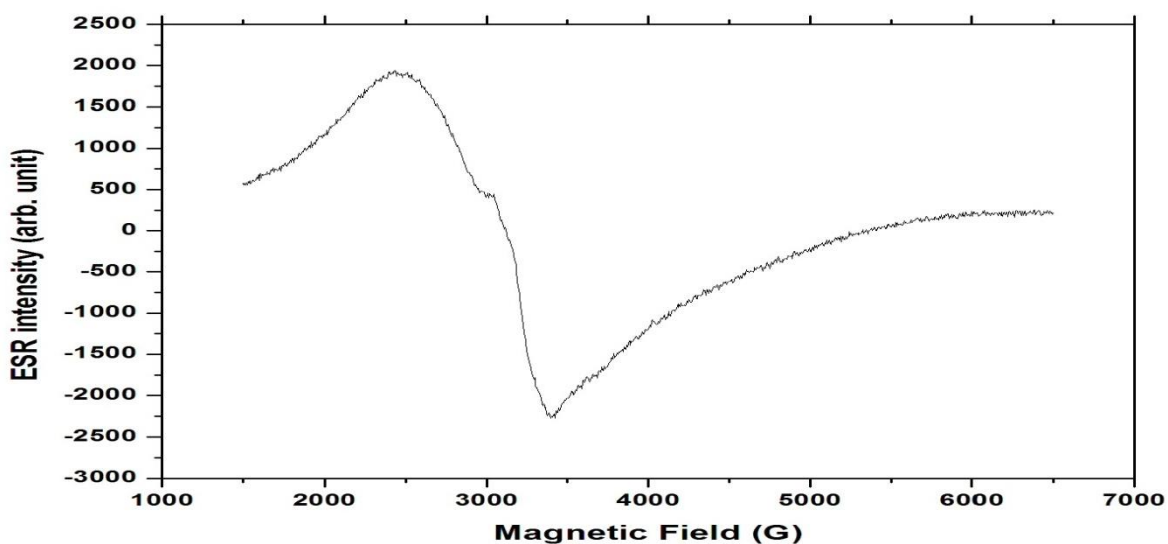


Fig.10. ESR Spectrum of complex (5)

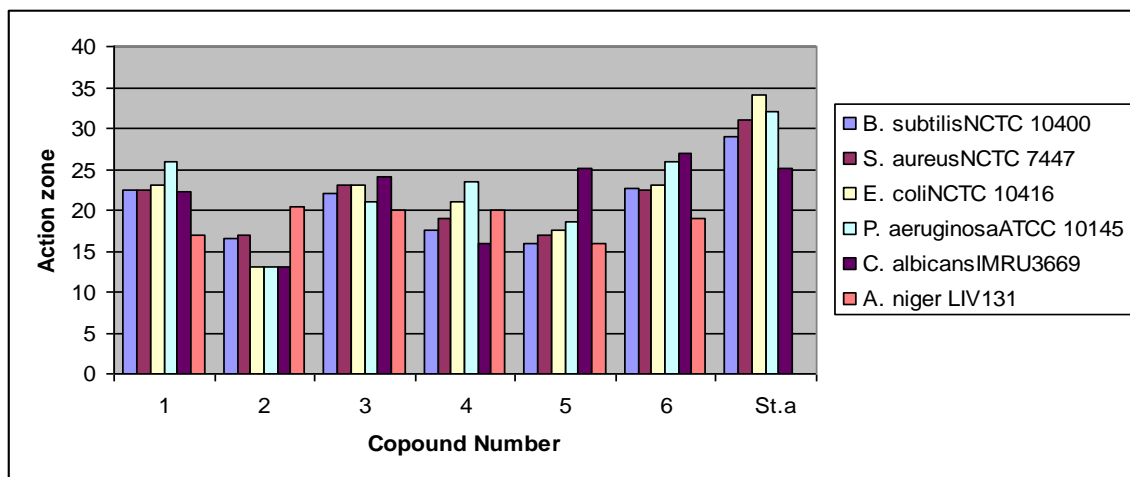


Fig.11. Biological evaluation of ligand 1 and its metal complexes

Table 1: The IR Spectra data for compounds (1-6)

Compound	$\nu(\text{H}_2\text{O})$	$\nu(\text{OH})$	$\nu(\text{CH})$ ar.	$\nu(\text{CH})$ aliph.	$\nu(\text{C}=\text{N})$	$\nu(\text{C}-\text{OH})$	$\nu(\text{Ph}-\text{N})$	$\nu(\text{M}-\text{O})$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{Cl})$
1	-	3381	3052	2938	1620	1279	1133	-	-	-
2 ^a	3390	-	3068	2928	1602	1190	1118	495	345	-
3	3395	-	3022	2954	1611	1195	1122	583	440	311
4	3392	-	3035	2934	1613	1193	1117	582	335	278
5	3427	-	2920	2850	1598	1215	1110	580	332	267
6 ^a	3425	-	3075	2925	1612	1188	1122	529	424	-

^anitrate complexes

Table 2: The antimicrobial activity of the compounds 1-6

Cpd. No.	Recorded zone diameter (mm)					
	For each test microorganism					
	Bacteria				Fungi	
	Gram +ve		Gram -ve		Unicellular	Filamentous
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
1	22.5	22.5	23	26	22.3	17
2	16.5	17	13	13	13	20.5
3	22	23	23	21	24	20
4	17.5	19	21	23.5	16	20
5	16	17	17.5	18.5	25	16
6	22.7	22.5	23	26	27	19
St. ^a	29	31	34	32	25	0

^aSt= Standard antibiotic (Amikacin)

Table 3: Minimum inhibitory concentration of compounds (1-6)

Comp No.	Bacteria			Fungi		
	Gram +ve		Gram -ve		Unicellular	Filamentous
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
1	1.25	1.25	0.625	1.25	1.25	1.25
2	5	5	1.25	5	5	2.5
3	5	2.5	0.625	1.25	5	2.5
4	1.25	5	1.25	2.5	5	2.5
5	1.25	1.25	2.5	1.25	1.25	1.25
6	2.5	0.625	2.5	2.5	0.625	1.25
St.	0.0195	0.0097	0.0195	0.00976	0.039	---

The mass spectrum of the ligand (Fig.6) showed its molecular ion peak at $m/z=348$ which was coincidence with its molecular formula.

3.2. Characterization of the complexes

3.2.1. Infrared spectra

The infrared spectra (Table 1) of the complexes provide the information about the bonding in the complexes. The band present in the infrared spectrum of the ligand at 1620 cm^{-1} found to be shifted to lower frequencies in the range ($1598\text{-}1613\text{ cm}^{-1}$) in the spectra of the complexes, indicating coordination *via* the azomethine nitrogen [14,15], confirming this, the appearance of new band in the far infrared spectra of the complexes in the range ($332\text{-}445\text{ cm}^{-1}$) assigned to the $\nu\text{M-N}$. Deprotonation of all phenolic functions was confirmed by the lack of phenolic O-H stretching bands at 3381 indicating the participation with the metal ion as $-\text{O}^-$. On the other hand very broad bands are observed in the spectra of the complexes in the range $3390\text{-}3427\text{ cm}^{-1}$ which provide considerable support to the presence of water molecules in the complexes [16]. The band at 1279 cm^{-1} in the free ligand ascribed to the phenolic C-O stretching vibration, this band was shifted to lower frequencies ($1188\text{-}1215\text{ cm}^{-1}$) due to O^- metal coordination [15]. The weak bands that appeared in the far IR spectra between ($495\text{-}583$) cm^{-1} were attributed to $\nu\text{M-O}$. The infrared spectra of complexes 4 and 6 presented in Figure (7) and figure (8), respectively. The infrared spectra of complexes 2 and 6 exhibited bands around 1543 , 1289 and 1125 cm^{-1} due to $\nu(\text{N=O})$, $\nu_{\text{asym}}(\text{NO}_2)$ and $\nu_{\text{sym}}(\text{NO}_2)$, respectively which were ascribed to nature of coordinated monodentate nitrate group[17].

3.2.2. Electronic spectra

The information regarding geometry of the complexes is obtained from their electronic spectral data and magnetic susceptibility measurements. The electronic spectrum of Fe(III) complex 2 in DMF, exhibits band at 512 nm and 330 nm assignable to the spin allowed

electronic absorption of $\text{T}_{2g}(\text{F}) \rightarrow 5\text{E}_g$ transition in octahedral configuration and charge transfer, respectively. The magnetic moment (5.60 B.M) infer the presence of octahedral geometry around the central Fe^{+3} ion[18]. The electronic spectrum of Co(II) complex 3 in DMF showed the low intensity shoulders at 607 and 683 . The former bands is probably due to ${}^4\text{A}_2(\text{F}) \rightarrow {}^4\text{T}_1(\text{P})$ and ${}^4\text{A}_2(\text{F}) \rightarrow {}^4\text{T}_1(\text{F})$ which indicating tetrahedral geometry of this complex[19]. The magnetic moment of 4.27 BM sustained this configuration. The spectrum of the Ni(II) complex 4 in DMF showed a very broad bands at 530 and 440 nm indicating ${}^1\text{A}_{1g} \rightarrow {}^1\text{A}_{2g}$ and ${}^1\text{A}_{1g} \rightarrow {}^1\text{B}_{1g}$ transitions of square planar structure. A lack of any electronic transition at longer wave lengths indicates a large crystal field splitting and is consistent with the square planar geometry of Ni(II) complexes. The diamagnetic properties of this complex confirm the square planar geometry of complex 4 [20]. The spectrum of Cu(II) complex 5 in DMF gave broad band at 512 nm , hence, the copper complex appear to be in tetrahedral geometry. The μ_{eff} value (1.7 B.M) was corresponding to tetrahedral geometry of complex 5 [21]. The electronic absorption spectrum of Zn(II) complex 6 in DMF showed only charge transfer transition which can be assigned to charge transfer from the ligand to the metal and vice versa and no d-d transition are expected for d^{10} Zn(II) complexes[22].

3.2.3. The ${}^1\text{H-NMR}$ spectra

The ${}^1\text{H-NMR}$ spectrum of the zinc complex is recorded in DMSO-d_6 at room temperature. In the spectrum of the complex the phenolic proton signals observed at $\delta 8.9\text{-}9.7$ ppm in the spectrum of the free ligand were absent, confirming subsequent involvement of deprotonated hydroxyls in chelation to the metal ions as $-\text{O}^-$. The spectrum shows very broad signal appeared at $\delta 4.5$ ppm which not found in the ${}^1\text{HNMR}$ spectrum of the ligand due to resonance of coordinated and lattice water protons.

3.2.4. Thermal analysis

The thermogravimetric analysis (TGA) curve; (Fig .9), for complex 4, was obtained at a heating rate of

10°C/min and flowing nitrogen atmosphere over a temperature range of 20–100°C. The decomposition temperature and the weight losses of the complexes were calculated from TGA data. The curve shows five steps of decomposition. The first step of decomposition shows loss of the two crystalline water, one of coordinated water and one mole Cl₂; calc(17.86%),found(17.27%). The second step of decomposition indicate the loss of the subsequent three coordinated water molecules; calc(7.75%),found(8.92%). The third, fourth and fifth steps of decomposition show the decomposition of the remain organic part of the ligand leaving 2.5 NiO as residue; calc(26.71%),found(26.87%).

3.2.5. ESR Spectra

The ESR spectrum of complex 5 (Fig.10) is axial in shape and having symmetric bands with two "g" value, $g_{\parallel} = 2.25$, $g_{\perp} = 2.075$, while $g_{\parallel} > g_{\perp} > 2.0027$ are characteristic of a complex with according to the equation: $g_{av} = 1/3[g_{\parallel} + 2g_{\perp}]$, which found equal=2.13. The g_{\parallel} is a moderately function for covalency. Complex 5 exhibit $g_{\parallel} < 2.3$, suggesting covalent character of copper-ligand in the present complex, while, if $g_{\parallel} > 2.3$ is character of ionic metal-ligand. The axial parameter "G" is shown to be a measure of the exchange interaction between Cu (II) centers in solid complex. If G value is greater than 4, the exchange interaction between Cu(II) centers in the solid state is negligible, whereas when is less than 4, a considerable exchange interaction is indicated in the solid complex. The G value is calculated from equation: $G = (g_{\parallel} - 2.0023)/(g_{\perp} - 2.0023)$. The complex 5 having $G = 3.33$, So there are exchange coupling between Cu(II) centers in the solid state [23].

3.3. Antimicrobial Activity

Screening for the antimicrobial activity of the ligand 1 and its complexes was tested. The testing was carried out using the classical agar diffusion method[24]. Antimicrobial disk diffusion was performed as described by the National Committee for Clinical Laboratory Standard [25, 26]. The data in Table (2) show that all tested compounds have an appreciable activity against Gram-positive bacteria represented by *Bacillus subtilis* and *Staphylococcus aureus*. The most active of them was compound 3 (recorded 22 & 23 mm inhibition zone diameter respectively) followed by compounds 1 and 6. Also, these compounds showed a good activity against Gram-negative bacteria represented by *Escherichia coli* and *Pseudomonas aeruginosa*. In this case, the compounds 1 and 6 recorded the highest activity (23 & 26 mm zone diameter respectively). In turn, compounds 1 and 6 have a good and the highest activity against unicellular fungi represented by *Candida albicans* (compound 6 recorded 27 mm zone diameter) and filamentous fungi represented by *Aspergillus niger* (compound 1 recorded 20.5 mm zone diameter). In summation, the most active compound among the six tested compounds against the six tested microorganisms was compound number 6 due to its wide spectrum of activity Fig. 11.

3.4. Minimum inhibitory concentration (MIC) of compounds

The antimicrobial screening concentrations of the compounds to be used were estimated from minimum inhibitory concentration (MIC) value, i.e., the lowest concentration of drug which completely inhibits bacteria and fungi growth of compounds. The MIC value of ligand 1 and its complexes were determined by means of standard serial dilution method in microgram per milliliter and the data were collected in table (3). The compound 1, 4 and 5 were appeared lowest inhibitory concentration against Gram-positive bacteria represented by *Bacillus subtilis* and *Staphylococcus aureus* and recorded MIC value = 1.25 µg/ml and complex 5 was recorded lowest MIC against *S. aureus*; MIC=0.625 µg/ml. The lowest inhibitory concentration of compounds 1 and 3 against Gram-negative bacteria represented by *Escherichia coli* and recorded MIC value = 0.625 µg/ml. Compounds 1,3 and 5 were recorded lowest MIC against *Pseudomonas aeruginosa*; MIC= 1.25 µg/ml. The compounds 1, 5 and 6 showed lowest inhibitory concentration against unicellular fungi (Yeast) represented by *Candida albicans* and recorded MIC value = 1.25 µg/ml of 1 and 5 and 0.625 µg/ml of ZnL¹. Finally, the compounds 1, 5 and 6 showed lowest inhibitory concentration against filamentous fungi represented by *Aspergillus niger* and recorded MIC value = 1.25 µg/ml. The values of MIC showed that the 1 and 5 compounds were found to be the lowest inhibitory concentration against six microorganisms as compared to the other studied compounds.

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