Original Article

Synthesis, characterization, and biological screening of Co(II), Ni(II), Cu(II), Pd(II), and Pt(IV) complexes of a novel hydrazide-hydrazone ligand derived from gallic acid

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Abstract

This work involves the synthesis of the Co(II), Ni(II), Cu(II), Pd(II), and Pt(IV) complexes (M1-M5) with a new Schiff base ligand by the condensation reaction of 3,4,5-trimethoxybenzohydrazide with 2-hydroxy-4-methoxybenzaldehyde to yield N'-(2-hydroxy-4-methoxybenzylidene)-3,4,5-trimethoxybenzohydrazide (L). The structures of the new ligand and its complexes were examined by fourier-transform infrared (FT-IR), ultraviolet-visible (UV-visible), proton nuclear magnetic resonance (1H NMR), 13C nuclear magnetic resonance (13C NMR) spectroscopy, mass spectrometry, microelement analysis, atomic absorption flame (AAF) spectrophotometry, conductivity, and magnetic susceptibility tests. The spectroscopic and elemental analysis results revealed tetrahedral geometry around the Co(II) ion, square-planar geometry around Ni(II), Cu(II), and Pd(II), and octahedral geometry around Pt(IV). The ligand (L) and its complexes (M1-M5) were subjected to in vitro studies to test their antibacterial activities against Staphylococcus aureus as gram positive and Escherichia coli as gram negative bacteria, the (MIC) values were measured against standard antibiotics. Furthermore, the new compounds were also tested as antifungal agents against Aspergillus Flavus and Penicillium spp.

1. Introduction

Gallic acid (GA) is a benzoic acid with three -OH groups at the 3,4,5-positions that is phenolic carboxylic acid. It is a naturally occurring substance (secondary metabolite) that may be discovered in a range of fruits, vegetables, nuts, and other plants [1-3]. Three phenolic hydroxyl groups are reported to be present in GA, which is proven to have strong anti-inflammatory and antioxidant properties [4]. In addition, a variety of pharmacological effects, including those against bacteria, tumors, diabetes, obesity, and myocardial ischemia, have been observed [5-7]. In many classes of organic compounds with diverse biological activities, hydrazide hydrazone serves as an important structural scaffold. Many of these compounds have been reported to have antibacterial [8], antifungal [9], anticonvulsant [10], antitubercular [11], anticancer [12], and antiviral [13].

By condensation of carboxylic acid hydrazides with aldehydes of ketones to form imine linkage (Schiff base), the CO-NHN=C moiety of the hydrazide hydrazone is created. This straightforward chemistry facilitates the synthesis of such bioactive compounds while allowing flexibility in the design of new molecules with specific structural requirements [14].

The improvement of their cellular permeability and pharmacokinetic properties is made possible by the invention of novel hydrazone derivatives [15].

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One of the efficient strategies used to combat the issues brought on by the crisis of antibiotic resistance is the utilization of metal complexes since these substances have a range of antibacterial activity mechanisms [16]. When organic molecules are chelated with metal, both the biological properties of the metal and the ligand moiety are significantly altered [17]. According to reports, the development of compounds' antibacterial activity may be enhanced by the presence of a phenyl ring with electron-donating groups [18].

We introduce a new hydrazone ligand with its metal complexes as a promising antimicrobial agent in this article as part of our interest in the synthesis of new ligands and metal complexes [19, 20]. The new compounds are designed to have 3,4,5-trimethoxyphenyl and 2-hydroxy-4-methoxyphenyl rings connected with hydrazone bridge that contain the chelating groups that coordinate with the metal ions to form the target complexes.

2. Experimental

2.1. Materials and Instrumentation

Gallic acid, hydrazine hydrate 80 percent, and the salts required to generate the complexes were all acquired from commercial sources and utilized in this investigation exactly as they were given. On melting point equipment, the melting points were recorded (Stuart SMP30). The 1H and 13C NMR spectra were measured using Bruker Bio-Spin GmbH at 400 MHz and 100 MHz, respectively. The Tensor II spectrophotometer from Bruker was used to collect the infrared spectra using the ATR method. Using a WTW Cond 7300 digital conductivity meter, the electrolytic conductivity values of the metal complexes (10-3M solutions in DMF) were determined at 25°C. Using a SHIMADZU 1800-UV spectrophotometer, the electronic spectra of the ligands and their metal complexes were captured in the 200–1100 nm range. A Sherwood Scientific Magnetic Susceptibility Balance was used to gauge the complexes' magnetic susceptibilities. The generated complexes' metal content was evaluated using (FL Aspect LS 131 RC1). On Vario ELV5 CHNS Mode, S. No. 11086109, the elemental analyses were captured.

2.2. Preparation of 3,4,5-trimethoxybenzoic acid (H1)

Gallic acid monohydrate (13 g, 0.069 mol) has been added to a scold solutionsof sodium hydroxide (20 g, 0.5 mmol) in water (125 mL) in a three-necked round bottom flask that is between 25 and 30 degrees Celsius. When the acid has entirely dispersed, the flask is immediately shut and periodically swirled. After adding dimethyl sulfate (0.18 mol, 16.5 ml), the flask has been stirred for a further 20 minutes. The mixture is kept at a 30-35°C temperature, and the covers is sometimes opened to releases any pressure. Following that, the samesamount of dimethyl sulfates is added, and stirringsis continued forsan additional 20 minutess. During the secondsedition, the temperaturesmight rise to 40–45 °C. Then, the flaske is fitted with a reflux condensser, and the mixture is boileds for two hours while being stirred. After that, the boiling process continued for an additional two hours while 5 g of sodium hydrroxide was dissolved in 7.5 mL of waters. A 5% percent hydrochloric acid solution is added to the reaction mixture when it has cooled to room temperature. Before the precipitate is recrystallized from the ethanol (H1), it is filtered and washed with cold water [21]..

2.3. Preparation of methyl 3,4,5-trimethoxybenzoate (H2)

thin-layer chromatography (TLC) was used to track the reaction as it occurred during the 8-hour reflux of H1 (7 g, 0.032 mmol) in 100% methanol (30 mL) while also adding a few drops of concentrated sulfuric acid. Following cooling, a precipitate was created. The excess methanol was evaporated, and the resulting solid was collected, cleaned with a solution of sodium bicarbonates, filtered, rinsed with colds water, drieds, and recrystallized froms hot ethanol to produce compound H2[22].

2.4. Preparation of 3,4,5-trimethoxy benzohydrazide (H3)

(TLC) was used to monitor the reaction after adding hydrazine hydrate (80%, 20 mL) to H2 (10 gm, 0.044 moles) in 40 mL of ethanol. The mixturswas then heatedsto reflux with constant stirring for 13 hourss. The solid product was eliminated using filtration, after which it was rinsed with ice-cold water, dried, and recrystallizeeds from ethanol to produice compound H3[23].

2.5. Synthesis of the Schiffbase ligand N'-(2-hydroxy-4-methoxybenzylidene)-3,4,5-trimethoxybenzohydrazide[L]

By combining (0.226 g, 0.001 moles) of H3 and (0.162 g, 0.001 moles) of (2-hydroxy-4-methoxybenzaldehyde in 30 mL of ethanol, the Schiff base ligand was created. The finished product was refluxed for 8 hours if there are 5–6 drops of glacial acetic acid present. Using thin-layer chromatography, the reaction was seen (TLC). When the reaction was finished, the solution was poured upon the ice that had been crushed. The substance was extracted from the ethanol and crystallized again [24].

2.6. Synthesis of the metals complexess of liganed

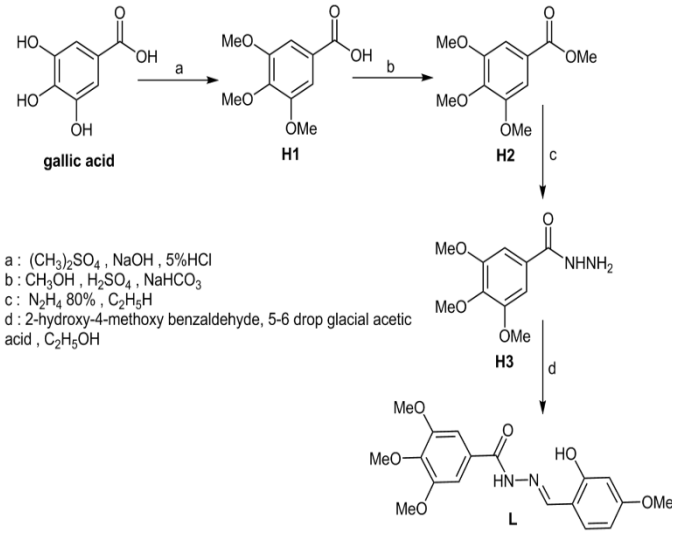
Thescomplexes werescreated bysmixingss20 mL (1 mmol) of the metal ion (CoCl2.6H2O, CuCl2.2H2O, NiCl2.6H2O, H2PtCl6.6H2O, and PdCl2) in a 1:2 mol

ratio with 2 mmol (0.720 g) of the ligand in ethanol absolute. The mixture was maintained at reflux for 4-6 hours, following which the solid complexes were cooled and 5–10 mL of diethyl ether was added to the precipitate Following filtering, ethanol and cold water were used to wash the crystals. Then it was dried, and ethanol was used to recrystallize it.

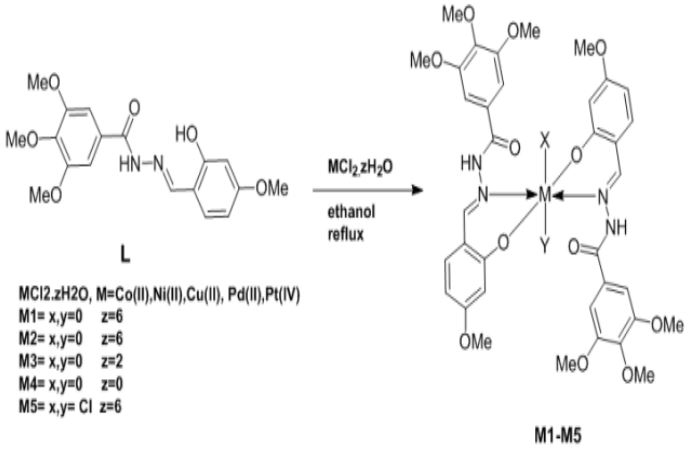
3. Results and Discussion

(L) was produced from gallic acid, and the first step involved methylating the hydroxyl groups to produce (H1). The second step involved esterifying H1 to produce (H2), and the third step involved converting H2 to (H3). The final step involved reacting H3 with 2-hydroxy-4-methoxybenzal dehyde (L). The processes in (L)synthesis are shown in Scheme 1. The intermediates' FT-IR data, melting temperatures, and other physical characteristics were discovered to be exact replicas of the stated date and real samples. By using's FT-IR, 1H NMR, 13C NMR, and elemental analysis to validate the structures of the novel ligands L . After creating the new Schiff bases ligand L, it was then possible to create the metal complexes by reacting L with the appropriate meta, salt to produce the matching complexes M1–M5, as illustrated in Scheme 2.

The results show that complexes with a metals: ligands ratio of 1:2 may be produced. The evidence Table 1 displays the physical measurements and chemical characteristics of the Schiff bases ligand and their metal complexes. Based on C.H.N., flame atomic absorption, conductivity tests, magnetic susceptibility, and spectrum data, the chemical formulae of the studied compounds were proposed. shows that the ligand being used is a neutral bidentate. Although they are soluble in DMFs and DMSOs, the complexes are insoluble in the majority of organic solvents.



Scheme 1: Synthesis of the Schiff bases Ligand (L)



Scheme 2: Synthesis of the metal complexes M1-M5.

3.1. Infrared studies

The IR spectra of the (L) and those of its metal complexes are contrasted to investigate how the hydrazine-hydrazone ligand bonds to metals ions. The L ligand's metal complexes, together with their assigned assignments, are listed in Table 2 Figure 1's IR spectrum results. An analysis of the (L) spectra reveals that some fundamental weak broad bands at 3616- 3416 cm-1 attributable to stretching vibrations of adsorbed(O H) disappear in those of the complexes, by deprotonation of the phenol group (Supplementary Information). [25,26], In ligand spectra at 3254cm-1 and metal complex spectra at 3005-3201 cm-1, (N-H) of the uncoordinated NH groups appeared as a shoulder [27], 3099-2841 (=C-H), 3099-2841 (=C-H), 3099-2841 (=C-H), 3099-2841 (=C-H), and 3099-2841 (=C-H) (-C- H), The unique (C=N) (azomethine

group) bands of the free ligand's IR spectra at 1630 cm-1 are displaced to lower frequencies in the spectra of metal complexes (1600-1609 cm-1) [28, 29]. A ν(C=N) shift of around 21–30 cm-1 is seen in all complexes, indicating the presence of azomethine nitrogen in the coordination sphere with the metal ions. The nitrogen of the azomethine group (CH=N) and the Schiff base (L) function as a mono-basic bidentate ligand, respectively, in the IR spectra (OH phenolic). However, the coordination bonds (M-N), (M-O), and (M-Cl) are associated with the novel weak bands around 550(-200) cm-1 [30,27].

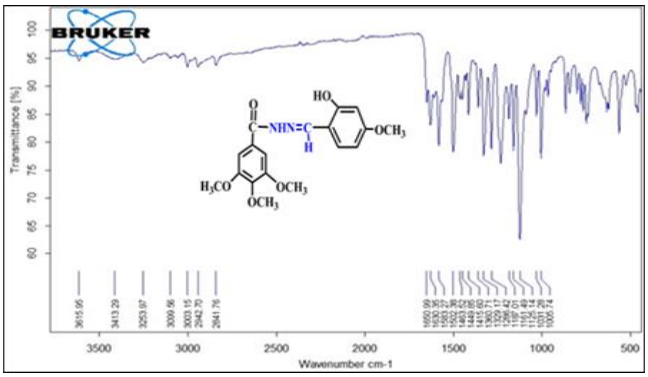


Figure 1: FT-IR spectrium of Schiff bases ligand [L]

Table 1: Elemental analyses and physical characteristics of the Schif base ligand (L) and its metal complexes.

Compound	Formula	Color	M.Wt. (g/mol)	Yield %	M.P. ºC	Elemental analysis %*			Metal content*
						C	H	N	
L1	$C_{18}H_{20}N_2O_6$	off white	360.37	89	119-122	60.08 (59.93)	5.98 (5.54)	8.13 (7.76)	-----
M1	$[Co(C_{18}H_{19}N_2O_6)_2].H_2O$	blue	795.673	67	280-282	56.04 (54.29)	5.11 (5.03)	7.59 (7.03)	8.107 (7.406)
M2	$[Ni\ (C_{18}H_{19}N_2O_6)_2]$	green	777.433	64	334-336	55.94 (55.56)	5.21 (4.88)	7.49 (7.20)	6.892 (7.549)
M3	$[Cu[(C_{18}H_{19}N_2O_6)_2]$	green	782.286	55	293-295	55.82 (55.22)	5.32 (4.85)	7.66 (7.15)	7.507 (8.123)
M4	$[Pd\ (C_{18}H_{20}N_2O_6)_2]$	brown	825.16	64	250-253	53.09 (52.35)	4.96 (4.60)	7.02 (6.78)	13.73 (12.896)
M5	$[Pt\ (C_{18}H_{19}N_2O_6)_2Cl_2]$	dark brown	984.818	77	300-302	44.11 (43.87)	3.92 (3.66)	5.84 (5.69)	15.927 (16.653)

Table 2. The characteristics absorptionsbands in the FT-IR spectras of ligand L, M1-M5.

Compound	υ(O-H)	υ(C=N)	υ(N-H)	υ(M-N)	υ(M-O)	υ(M-C)
L1	3616	1630	3245	---	---	---
M1	---	1607	3080	534	457	---
M2	---	1600	3073	537	465	---
M3	---	1608	3078	536	474	---
M4	---	1609	3201	529	474	---
M5	---	1608	3005	536	458	316

3.2. NMR Studies

The 1H NMR spectrum of L showed all the predicted signals, it showed two singlet signals at11.84 ppm (s, 1H) and 11.59 ppm (s, 1H) ppm corresponding to the -NH and -OH protons respectively, and signal at 8.55 ppm (s, 1H) corresponds to the azomethine proton, signals at 7.42 ppm (d, J = 8.4 Hz, 1H), 7.25 ppm (s, 2H) and 6.56 – 6.47 ppm (m, 2H) which correspond to the aromatic protons, and signals at 3.85 ppm (s, 6H), 3.76 ppm (s, 3H), and 3.72 ppm (s, 3H) correspond to the protons of the methyl groups. The 1H NMR of M4 was found to be identical to the spectrum of L except for the disappearance of the signal at 11.59 ppm which corresponds to the -OH proton in L, this confirms the formation of the metal complex via coordination with the oxygen atom of the deprotonated -OH, Figure 1 shows 1H NMR spectra for L and M4.[31]

The 13C NMR spectrum of L showed all the expected 15 signals for the different types of carbons in the structure, it showed signals at 162.52, 162.50, 159.82, 153.18, 149.02, 140.97, 131.43, 128.41, 112.26, 106.90, 105.62, 101.62, 60.57, 56.53, and 55.74 ppm, Figure 2 shows the 13C NMR spectrum of L [32] . The signals can be assigned as follows: 162.50 ppm which is corresponding to the carbon atom of the carbonyl group; 149.02 ppm which is corresponding to the carbon atom of the azomethine (imine) group; ten signal in the area 101.62 to 162.52 ppm which are corresponding to the aromatic carbon atoms; 60.57, 56.53, and 55.74 ppm are corresponding to the four methoxy groups (two of them are identical).

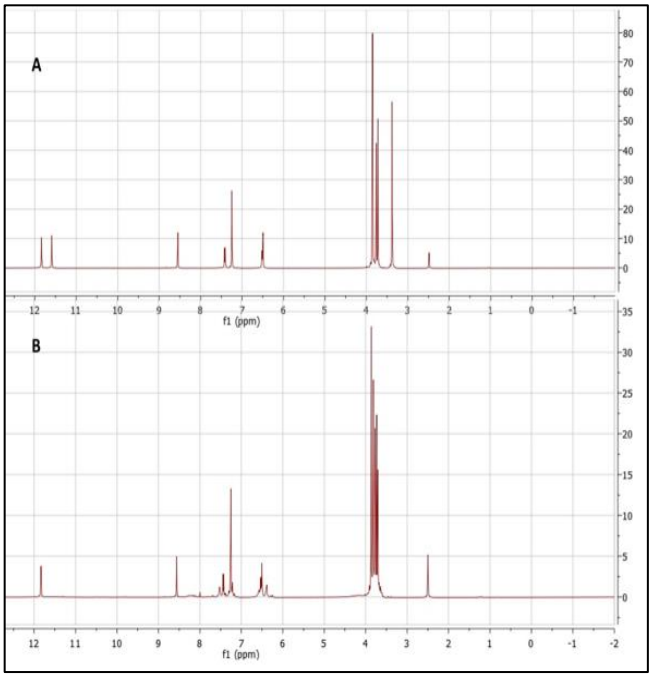


Figure 2: 1H NMR spectra of L (A) and M4 (B).

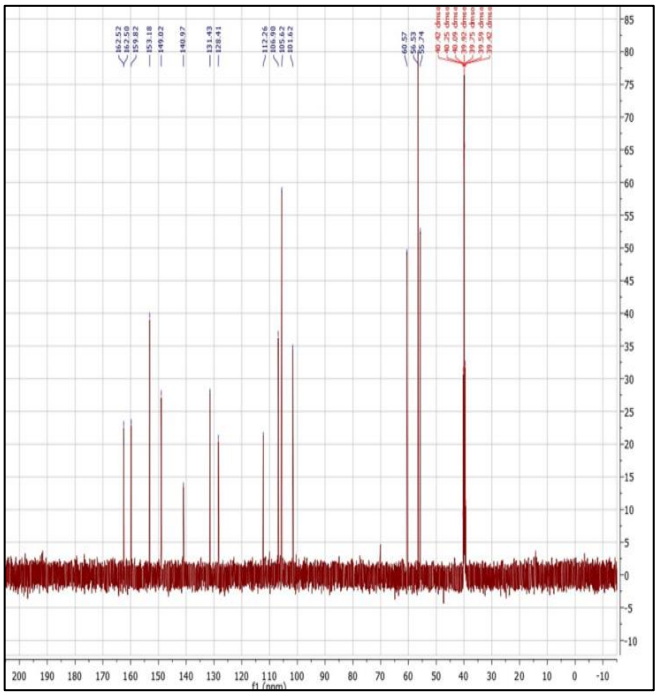


Figure 3: 13C NMR spectrum of L.

3.3. Eleectronic absorptions spectra, Magnetic ssusceptibility, and Conductivity measurementts

The (U.V.- Viss) spectra of the (L) in 100% ethanolsrevealed (p®p*) and (n-®π*)transitions, which were attributed to two absorption bands at (301 nm, 33222cm-1), and (333 nm, 30030 cm-1), respectively [33]. In the presence of metal ions, (L) complexes form additional visiblesand UV bands. These bands were attributeds to ligand field transitions and M-L charge transfer. Table 3 includes a description of the bands of maximum complex absorption in chloroform as well as their allocations. The potential geometries of synthetic complexes are shown in Figure 3.

[CoL]: The blue cobalt complex's (M1) magnetic moment value was 4.72B.M, suggesting that it is a high-spin kind of complex [34]. In the electronic spectra of the chloroform solvent, a band in the range of (20000-13986) cm-1 was seen [35]. These bands are responsible for the transition 4A2 → 4T1 (P) (ν3), whereas the transition 4A2 → 4T2 (F) (ν1) was seen at 3404 cm-1 in the infrared spectrum, but the transition of (ν2) anticipated in the range (5000–6000) cm-1 could not be measured [36]. For the (d7) configuration, the Tanabe-Sugano diagram was used to calculate the various ligand field parameters (10Dq, B-, and ν2), which were then determined to be (3373, 740, and 5624) respectively. The calculated nephelauxetic factor was (0.76), indicating a high degree of covalence in the binding of ligand donor atoms to cobalt (II) ions [36]. The molar conductance demonstrated the compound's non-ionic nature.

[NiL]: In the electronic speectra of the Ni complex (M2), onesbands at 425 nm, 23529 cm-1, is a location for 1A1g→Eg (P) transitions [39,40]. The calculated magnetic moment values for this band suggest that the Ni(II)ion is surrounded by a square planer (0.43 B.M). This is in line with the theory that diamagnetic behavior is complex and non-conducting[41].

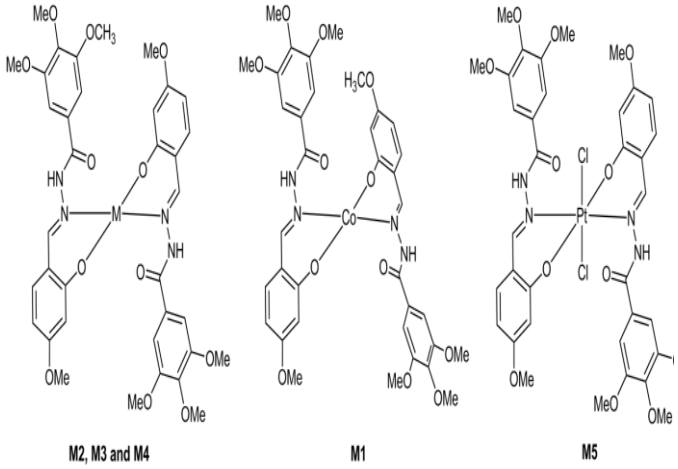
[CuL]: The 2B1g → 2A1g transition is represented by a single broadband at 675 nm, 14814 cm-1], in the electronicsspectrasof the Cu complexs(M3) [40,42]. The position of this band resembles a square planer arrangement quite a bit. At roomstemperatures, the magneticsmomentswas found to be 1.86 B.M., which is in good agreement with the distorting square planer shape around the Cu (II) complex[43]. The conductance tests in DMF reveal that this chemical is non-ionic.

[PdL]: Two bands at (435 nm, 22988cm-1) and (407 nm, 24570cm-1) that are associated with the 1A1g→1B1g and 1A1g→1Eg transitions, respectively, can be seen in the electronic spectra of the Pd complex (M4)[36,44]. The surrounding ligands and the complex's central metal ion may coordinate squarely in space. Magnetic moment statistics for the Pd(II) complex (0.08 B.M) show that this complex is diamagnetic. This is in line with the Pd(II) ion's square-planar structure [45]. The conductance tests in DMF reveal that this chemical is non-ionic.

[PtL]:In the electronic spectra of the Pt complex, one band at (464nm,21551cm-1), assigned to 1A1g→3T1g [36,42], can be detected (M5). Pt(IV) has a magnetic value of 0.11B.M, which is consistent with the octahedral geometry surrounding the ion[41,45]. The surrounding ligands in the complexes may act as octahedrally coordinated ligands for the core metal ion. The conductance measurements reveal this compound's ionic behavior.

Table 3. Electronic spectra, Conductivity, and magnetic of metal complexes

Compound	λ max nm (υ cm^{-1})	Assignment	ohm$^{-1}cm^2mol^{-1}$	μ B.M. found	Suggested Structure
L	333(30030) 301(33222)	n-→π* π→π*	----	----	---
M1	3404	$^4A2 \rightarrow {}^4T2(F)$	8.54	4.72	Tetrahedral
	5624(cal)	$^4A2 \rightarrow {}^4T1(F)$			
	16548	$^4A2 \rightarrow {}^4T1(P)$			
M2	425(23529)	$^1A_1g \rightarrow Eg_{(P)}$	11.44	Zero(0.43)	Square planer
M3	675(14814)	$^2B_1g \rightarrow {}^2A_1g$	10.22	1.86	Square planer
M4	435(22988) 407 (24570)	$^1A_1g \rightarrow {}^1B_1g$ $^1A_1g \rightarrow {}^1Eg$	14.03	Zero(0.08)	Square planer
M5	464(21551)	$^1A_1g \rightarrow {}^3T_1g$	12.61	Zero(0.11)	Octahedral



Scheme 3:The geometry of the metal complexes M1-M5.

3.4. Study of Biological Activities

The antibacterial and antifungal activity of the synthesized compounds were studied against Escherichia coli (gram negative bacteria), Staphylococcus aureus (gram positive bacteria) Aspergillus flavus and Penicillium spp. Fungi. DMSO was used as a solvent and as a control, and the concentrations of the compounds in the solvent for both approaches were 0.001M. The first protocol was the disc sensitivity test in which the growth inhibition zones are measured for microorganisms treated with discs of the test compounds on agar plate [46]. The plates were incubated at 37 oC for 24 hours, and the zone of bacterial growth inhibition around the disc was detected. The minimum inhibitory concentration (MIC) of each of the test compounds was measures,

this was done by using the Tube Dilution Method [47]. The minimum inhibitory concentration (MIC) of L, M1-M5 against each bacteria was determined by incubating tubes containing different concentrations of L, M1-M5 at 37 oC for 45 hours. Two well-known antibiotics (Ampicillin and Amoxicillin) were used as standard antibiotics.

The new ligand (L) and its metal complexes were evaluated for their antifungal activity against pathogenic fungi (Aspergillus flavus and Penicillium spp.) cultivated on potato dextrose agar

medium and incubated at 30 oC for 72 hours. Solutions of 10-3M concentration in DMSO were used, the solvent was considered as a control. The inhibition percentage of fungal growth (based on the growth in test plates relative to the appropriate control plates) was calculated according to the following equation [48]:

Inhibition % = 100 (C – T) / C

Where C is the diameter of fungal growth on the control plate and T is the diameter of fungal growth on the test plate.

Table. 4 also shows the Antibacterial and antifungal activities for free ligand (L) and its metal complexes M1-M5, it is clear that the metal complexes are more effective than the ligand, M5 is the most effective complex against E. coli, on the other hand against Staph. Aureus all the complexes showed similar antibacterial activity values.

The results of the antifungal activity studies of the novel ligand and its metal complexes are also shown in Tables.4, the metal complexes were found to be more effective than the ligand (L) against the same microbes and under the same experimental circumstances. The influence of the metal ion on the normal cell process might explain the increase in antifungal activity of metal complexes. Tweed's Chelation Theory [49] states that chelation decreases the polarity of the metal atom mostly due to the partial sharing of its positive charge with the donor groups of the ligand, which facilitates complex penetration through the lipid layer of the cell membrane [50].

Table 4: Anti-bacterial and anti-fungale activities for free ligand (L) and its metals complexes M1-M5 (10-3µgm.ml-1)

Comp. No.	E. coli *	Staph . Aureus*	Asp. Flavusspp**	Penci . spp**
DMSO	---	---	---	---
L	2	6	31	34
M1	6	10	25	28
M2	8	8	23	26
M3	12	10	15	17
M4	10	8	21	24
M5	16	10	14	15

*6-8 (+), 8-10(++) and > **10 (+++).**
**10-20 (+++++), 20-30 (++++) and 30-40 (+++).

Table.5 displays the results of the (MIC) investigations. Since all of them showed lower MIC values, it is obvious that L and M1-M5 are more effective than the commonly used standard antibiotics ampicillin and amoxicillin. M5 has the lowest MIC values (0.025 gm. ml-1) against Escherichia coli, while M2, M4, and M5 were found to have the lowest MIC value (0.05 gm. ml-1

Table 5. Minimum inhibitory concentrations (MIC) of L and M1-M5

Compound	*Escherichia coli*								*Staphylococcus aureus*							
	Concentration (µgm. ml^{-1})								Concentration (µgm. ml^{-1})							
	0.025	**0.05**	**0.1**	**0.25**	**0.5**	**1**	**2.5**	**5**	**0.025**	**0.05**	**0.1**	**0.25**	**0.5**	**1**	**2.5**	**5**
L	+	+	+	MIC	-	-	-	-	+	+	MIC	-	-	-	-	-
M1	+	+	MIC	-	-	-	-	-	+	+	+	MIC	-	-	-	-
M2	+	+	MIC	-	-	-	-	-	+	MIC	-	-	-	-	-	-
M3	+	+	MIC	-	-	-	-	-	+	+	+	+	MIC	-	-	-
M4	+	+	MIC	-	-	-	-	-	-	MIC	-	-	-	-	-	-
M5	MIC	-	-	-	-	-	-	-	+	MIC	-	-	-	-	-	-
Ampicillin	+	+	+	+	+	MIC	-	-	+	+	+	+	+	MIC	-	-
Amoxicillin	+	+	+	+	+	+	MIC	+	+	+	+	+	+	MIC	-	-

(+): growth, (MIC):99%, (-): No growth

4. Conclusion

New ligand (L) and its Co(II), Ni(II), Cu(II), Pd(II), and Pt(IV) complexess (M1-M5) have beens synthesized and characterized by different techniques. The results obtained from spectra and elemental analyses indicated the tetrahedral geometry around Co(II) ion, square-planner for Ni(II), Cu(II), and Pd(II), and octahedral geometry around Pt(IV). The new ligands have excellent anti-bacterial and anti-fungal activities, they can be considered promising antimicrobial agents.

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