

Synthesis, Characterization, Biological, and Antioxidant Activity of New Metal Ion Complexes with Schiff Base Derived from 2-Hydroxybenzohydrazide

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Abstract: The study involved the synthesis of new complexes with tetradentate ligand (LH). The general formula of complexes was $[M(LH)(H_2O)_2]$ with M of Ni^{+2} , Co^{+2} , Cu^{+2} , and Zn^{+2} . The ligand was synthesized by treating the 2-hydroxybenzohydrazide with salicylaldehyde. The structural characteristics of ligands and complexes were analyzed using various techniques, including elemental analyses, magnetic susceptibility, molar conductivity, infrared, ultraviolet absorption, mass, and NMR spectroscopy studies. The physical measurements indicated that the prepared complexes are non-electrolyte and showed that the ligand is tetradentate when coordinated with metal ions through the nitrogen of azomethine ($-C=N-$), two oxygen atoms of O-H phenolic, and an oxygen atom of carbonyl ($C=O$) for benzohydrazide. It was found that Co^{+2} , Cu^{+2} , and Zn^{+2} complexes are octahedral, while Ni^{+2} complexes are square planar. The biological screening of the complexes demonstrates that the Schiff base metal complexes exhibit remarkable efficacy in combating microorganisms by utilizing Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria, as well as *Candida albicans* fungi. Hence, their results were good in inhibition. Then, the potential of these prepared compounds as antioxidants was determined by inhibiting free radicals.

Keywords: antioxidant; biological activity; 2-hydroxybenzohydrazide; Schiff base metal complexes

■ INTRODUCTION

The Schiff bases' reaction was studied by Hugo Schiff in 1984. Condensation of primary amines and active carbonyl groups can form Schiff bases [1-3]. Schiff bases are widely used in coordination chemistry because of their high coordination number and ability to form complexes with a variety of metal ions, including those from transition metals [4-6]. Imines, sometimes referred to as compounds with the general formula $R_1-CH=N-R_2$, consist of linear or cyclic alkyl or aryl groups that may have different substitutions [7]. These compounds contain an azomethine group ($-N=C-$). Schiff bases exhibit diverse uses in several fields, including inorganic chemistry, physical chemistry, analytical chemistry, biochemistry, and biology [8-10]. Extensive studies of Schiff bases have been conducted for heterocyclic metal complexation, such as catalysis and antibiotics [11-12].

Recently, Schiff base ligands that contain nitrogen (N), oxygen (O), and sulfur atoms have become accessible for structural analysis in various forms. The inclusion of electron-rich pairs, such as N and O atoms, in transitional metals enhanced their significance due to their diverse range of physical, chemical, biological, medicinal, and other activities [13-14]. The focus of research has shifted from creating novel chemicals to developing new applications. The liquid crystal property of some metal complexes is utilized by numerous industries. These include the digital clock business, computer and television screens, and the aerospace sector [15]. Compared with free Schiff bases, the complexation of Schiff bases with metals results in improved antibacterial and antifungal activities [16]. Schiff base derivatives are used in industries, medicines and pharmaceuticals [17]. They can also be used as raw

materials for the production of anticancer, antioxidant, antitumor, and antibiotics [18-19].

Hydrazine compounds can react with aldehydes and ketones and produce pure derivatives [20]. A multitude of hydrazones have been employed as antibacterial agents, commonly employed in the treatment of many biological processes [21-22]. This research aims to elucidate the process of synthesizing a new Schiff base ligand and use it as a ligand to create possible donor sites and build complexes with Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} . The ligand and its complexes have undergone comprehensive characterization, along with biological activity and antioxidant tests.

■ EXPERIMENTAL SECTION

Materials

All the chemicals and reagents used for the present study were sourced from (Sigma Aldrich) and met the standards of analytical quality. Methyl salicylate (99%), hydrazine monohydrate (99%), salicylaldehyde (99%), dimethyl formamide (DMF), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, and ZnCl_2 (99%), were obtained from BDH and used upon receipt.

Instrumentation

The Department of Chemistry, College of Science, University of Baghdad utilized a Shimadzu FTIR Spectrometer with discs made of KBr for bonds and CsI for complexes to record the FTIR spectra of bonds and their complexes. The electronic spectra were acquired at a temperature of 25 °C using a Shimadzu UV-1650 PC spectrophotometer. Conductivity measurements of the complexes were taken at a temperature of 25 ± 2 °C for a solution of the samples in DMF with a concentration of 1×10^{-3} M. The measurements were conducted using a WTW meter. Magnetic susceptibility measurements were performed at ambient temperature using a device known

as John Mathey. The molecular weight of the ligand was measured using GCMS-QP2010PLUS. The melting points were calculated using Gallen Kamp's equipment at the College of Science for Women, University of Baghdad.

Procedure

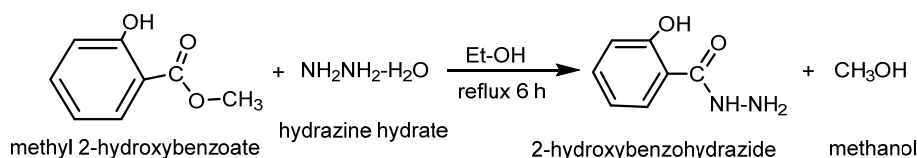
Ligand preparation

The preparation method for 2-hydroxybenzohydrazide (A) is described as follows. First, 5 g (0.039 mol) of A is dissolved in 10 mL of absolute ethanol and 10 mL (0.00984 mol) of hydrazine monohydrate is added gradually by dropping and stirring continuously into the mixture followed by the addition of 3–5 drops of glacial acetic acid. The mixture is kept refluxing at 80 °C, until it homogenizes for 5–6 h. After the reaction, the mixture is filtered and washed with ethanol. The product is recrystallized using a methanol:ethanol (40:60) mixture and is then filtered and dried for 24 h (Scheme 1).

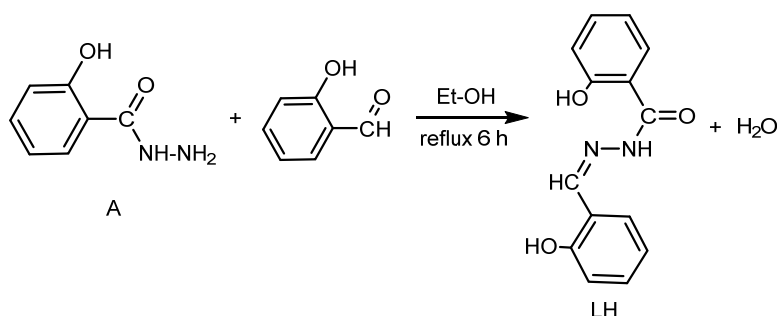
In a 100 mL round-bottom flask, 0.72 g (0.00390 mol) of A was dissolved in 10 mL of ethanol EtOH absolute, and a drop of DMF with continuous stirring and heating to perform the dissolution of the primary substance. Then, 0.57 g (0.00466 mol) of salicylaldehyde is added to the primary substance solution with reflux and continuous stirring for about 4 to 6 h. Finally, after the addition of 2 drops of glacial acetic acid to obtain Schiff base ligand, the white product was precipitated, recovered by filtering, dried for 24 h after being cleaned with absolute ethanol, and then recrystallized from heated absolute ethanol. After being filtered and dried for 24 h, the re-crystallized powder had a melting point (m.p.) of 280–282 °C and a yield of 80%. The route is shown in Scheme 2.

Preparation of the metal complexes

In a 250 mL circular glass vial with a flat bottom, 0.1 g (0.000399 mol) of the prepared ligand (LH) was



Scheme 1. Preparation of 2-hydroxybenzohydrazide (A)



Scheme 2. Preparation ligand (Schiff base)

Table 1. Equivalent weights of metal salts

Complex symbol	Salt formula	M.wt., for salt (g/mol)	Quantity (g)
[Co(LH)(H ₂ O) ₂]	CoCl ₂ ·6H ₂ O	237.93	0.0699
[Ni(LH)]·H ₂ O	NiCl ₂ ·6H ₂ O	237.69	0.0698
[Cu(LH)(H ₂ O) ₂]	CuCl ₂ ·2H ₂ O	170.48	0.0501
[Zn(LH)(H ₂ O) ₂]	ZnCl ₂	136.29	0.0601

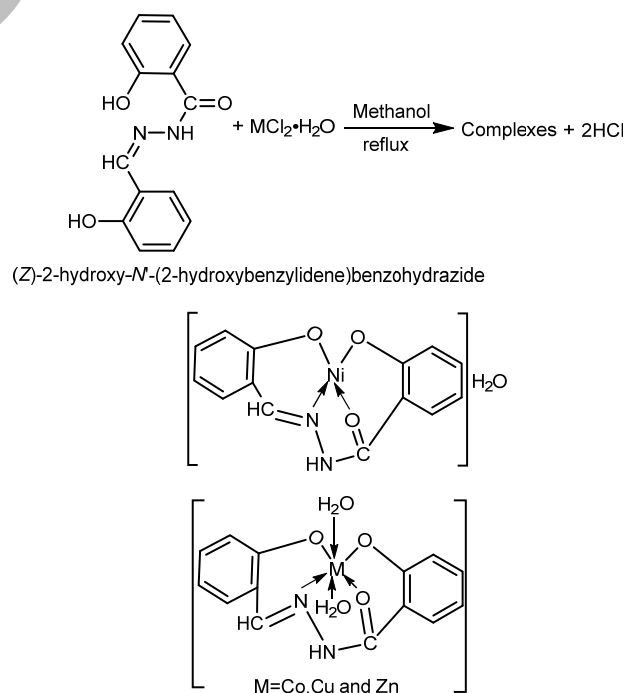
*LH = C₁₄H₁₂N₂O₃

dissolved in 10 mL of ethanol with agitation. Six drops of DMF and two drops of triethylamine were then added to the solution. According to the equivalent weights of each salt listed in Table 1, the metal chloride hydrate was dissolved in 10 mL of methanol. This metal solution was then added to the ligand solution, followed by vigorous agitation until complete dissolution was achieved. The mixture was subjected to the reflux procedure for 8 h until a colorful precipitate indicated the formation of a complex. The reaction was then halted, and the product was allowed to settle at room temperature before being filtered and rinsed with clean cold ethanol. The product was filtered and desiccated at 50 °C. Then, the m.p. was measured. The results provide exceptional to excellent yields of 75–80%. Scheme 3 shows the route of general metal complexes synthesis.

Biological activity analysis

The effectiveness of ligands and their metal complexes against pathogenic bacteria was assessed using the diffusion method under aerobic conditions. The inhibitory activity was evaluated against various pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans* fungi on Mueller-Hinton agar plates. The plates were inoculated with bacteria at a

concentration of 1.5×10^8 CFU/mL and with mold and yeast at 1.5×10^6 CFU/mL, using 0.5 McFarland turbidity standards. Wells (6 mm in diameter) were cut into the Mueller-Hinton agar plates, and 100 mL of the ligand solutions and their metal complexes were added to each well. For bacterial tests, the plates were incubated



Scheme 3. General synthesis of metal complexes

at 37 °C for 24 h, while fungal tests were incubated at 28 °C for 72 h. The inhibitory activity was evaluated based on the diameter of the inhibitory zones [23].

Total antioxidant capacity

The total antioxidant capacity of the fractions was determined by the phosphomolybdate method using ascorbic acid as a standard. A 0.1 mL liquor of sample solution was mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). For 90 min, the tubes were incubated in a water bath at 95 °C. At 765 nm, we measured the absorbance of the mixture against a blank after the samples had cooled to room temperature. Under the same conditions, a blank contained 1 mL of the reagent solution and the appropriate volume of solvent [24-25]. The ability of the ligand and its complexes to act as antioxidants was measured by preparing them at three different concentrations (50, 100, and 150 mg/L) and comparing them with ascorbic acid as a reference. The value of antioxidant efficacy was extracted from the Eq. (1).

$$\text{Antioxidant effect(\%)} = \left[\frac{\text{control}_{\text{abs.}} - \text{sample}_{\text{abs.}}}{\text{control}_{\text{abs.}}} \right] \times 100 \quad (1)$$

Table 2. Physical properties of the ligand and complexes

Compound formula	Color	M.p. (°C)	M.wt. (g/mol)	Yield %
LH	White bright	280–282	256.3	80
[Co(LH)(H ₂ O) ₂]	Cream	292–295	348.9	59
[Ni(LH)]·H ₂ O	Yellowish green	300	330.7	55
[Cu(LH)(H ₂ O) ₂]	Light green	300	335.5	76
[Zn(LH)(H ₂ O) ₂]	Light Yellow	290–292	355.4	71

Table 3. C, H, N and metal analysis of the compounds

Compound	Elemental content in % based on the experiment and calculation			
	C%	H%	N%	M
LH	65.32 (65.62)	4.74 (4.74)	11.19 (10.93)	-
[Co(LH)(H ₂ O) ₂]	47.90 (48.15)	3.75 (4.04)	8.68 (8.02)	17.68 (16.88)
[Ni(LH)]·H ₂ O	50.03 (50.81)	3.83 (3.65)	9.37 (8.46)	18.65 (17.73)
[Cu(LH)(H ₂ O) ₂]	46.84 (47.53)	3.34 (3.99)	8.25 (7.92)	17.23 (17.96)
[Zn(LH)(H ₂ O) ₂]	48.11 (47.28)	3.53 (3.97)	8.58 (7.88)	17.58 (18.38)

RESULTS AND DISCUSSION

The reaction between 1 mole of A and 1 mole of salicylaldehyde resulted in the excellent yielding of production of the tetradentate ligand. The produced ligand and its complexes were identified using the FTIR spectrum, microscopic examination of the elements, the proton NMR spectrum, MS, and the molar conductivity. Table 2 shows the physical properties of the ligand and complexes.

Microanalysis of the Elements

Micro-elemental analysis, namely for carbon-hydrogen and nitrogen, was conducted on all produced compounds. The results of these analyses may be found in Table 3. The experimentally measured values closely matched the theoretically calculated values, confirming the accuracy of the proposed formulation of the compounds [26].

¹H-NMR Spectral of Schiff Bases Ligand

NMR spectroscopy is a technique used to ascertain the chemical environment of organic molecules. Fig. S1(a)

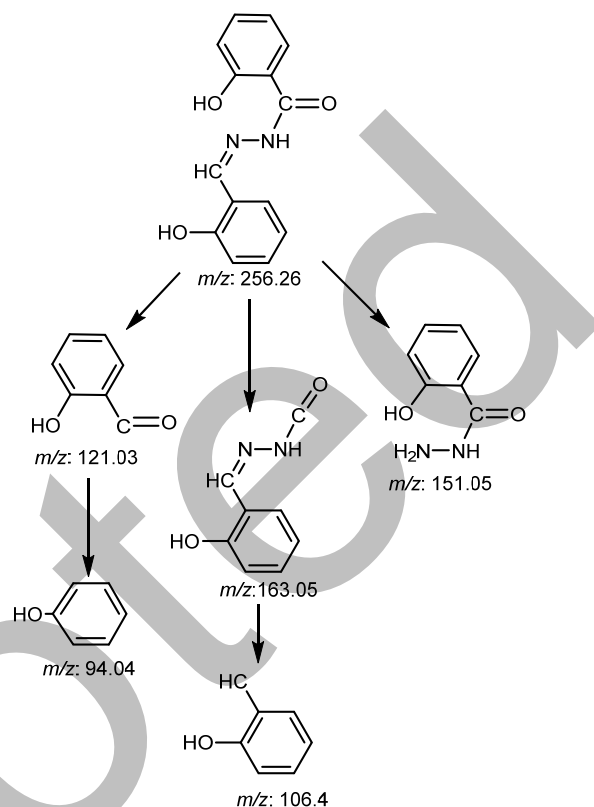
displays the $^1\text{H-NMR}$ spectra of LH dissolved in dimethyl sulfoxide ($\text{DMSO-}d_6$), using tetramethyl silane as the internal reference standard. The $^1\text{H-NMR}$ spectrum exhibited all the necessary peaks to validate the chemical composition of LH. The protons of the aromatic ring display multiple peaks in the chemical shift range of 6.87–7.69 ppm, indicating the presence of different chemical environments within the ring structure. The proton of the azomethine group (HC=N) exhibits a singlet peak at 8.69 ppm. The peak is significantly displaced due to the proton being bonded to an unsaturated carbon atom adjacent to a highly electronegative nitrogen atom inside the aromatic system [27]. The hydrazine proton exhibits a singlet peak at 11.19 ppm. Finally, the benzaldehyde proton exhibits a solitary peak at 11.76 ppm, while the O–H group emerges at 12.01 ppm [28–29].

$^{13}\text{C-NMR}$ Spectral of Schiff Bases Ligand

The $^{13}\text{C-NMR}$ spectrum is utilized to determine the chemical structure of the produced ligand by determining the chemical shifts corresponding to each carbon atom. The chemical composition of the synthesized materials is verified using $^{13}\text{C-NMR}$ spectroscopy. The spectroscopy revealed eight well-defined peaks with clear boundaries at corresponding chemical shifts, as depicted in Fig. S1(b). Six carbon atoms of the heteroaromatic unit display four peaks at 128.88, 129.70, 131.92, and 134.27 ppm. The chemical shifts of the 2 carbon atoms connected to the O–H aromatic rings are 157.78 and 159.266 ppm, respectively. The carbon atom in the azomethine group shows a peak at 149.16 ppm. Finally, the carbon atom of carbonyl group displays a peak at 164.76 ppm.

Mass Spectral of Schiff Bases Ligand

In addition to determining the molecular weight, the mass spectrum can identify fragmentation associated with the substance under investigation. Schiff base LH mass spectra were in agreement with the proposed structural formula, $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_3$. The calculated value of 256.26, as depicted in Fig. S2, corresponds to the molecular ion peak observed at 256.89, confirming the accuracy of their formula weight for the ligand. As it underwent serial fragmentation, distinct peaks corresponding to LH became apparent in the mass spectra of Scheme 4.



Scheme 4. The proposed mass fragmentation of LH

FTIR Spectra Analysis of Ligand and Complexes

The LH FTIR band allocations can be found in Table 4 and Fig. S3. In Fig. S3, we observe a prominent absorbance band at 1618 cm^{-1} , which is characteristic of the azomethine group [30]. At 3220 cm^{-1} , there is a peak that corresponds to N–H. It has been shown that the stretching frequencies in all the complexes shift to higher frequencies when linked by N atom, which is different from the frequencies in LH [31–32]. As a result of the donation of N electrons to the partially filled d -orbitals of the metal ions (+2) [33], A set of $\nu(\text{C=O})$ is shifted in the complexes at 1620 and 1650 cm^{-1} [34]. The disappearance of the OH peak, along with the appearance of new absorbent bands corresponding to $\nu(\text{M-O})$ and $\nu(\text{M-N})$ at 563 – 595 and 445 – 466 cm^{-1} , respectively [35–36]. Some complexes have 3456 , 752 , and 682 cm^{-1} absorption bands, indicating that the water molecules are within the coordination field.

Molar Conductivity Measurements

The measured molar conductivity of $\text{M}^{+2} = \text{Co, Ni}$,

Table 4. FT-IR Spectra analysis of ligand and metal complexes

Compound	$\nu(\text{OH})$	$\nu(\text{H}_2\text{O})$	$\nu(\text{N-H})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	Twisting $\nu(\text{H}_2\text{O})$	Wagging $\nu(\text{H}_2\text{O})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$
LH	3434	–	3245	1631	1618	–	–	–	–
[Co(LH)(H ₂ O) ₂]	–	3456	3259	1650	1600	756	680	568	462
[Ni(LH)]·H ₂ O	–	3440	3251	1623	1600	759	698	597	433
[Cu(LH)(H ₂ O) ₂]	–	3433	3107	1625	1598	754	690	588	445
[Zn(LH)(H ₂ O) ₂]	–	3431	3218	1620	1602	757	698	592	430

Cu, and Zn complexes in DMF solutions at a temperature of 25 °C are listed in Table 5. The absence of Cl⁻ ions in all complexes indicates that they are non-electrolytes, as no dissociation into ions occurs in the solution [37].

UV-vis Ligand and Complexes

Fig. S4 shows that the electronic spectrum of the electronic spectra of LH and their complexes were measured in DMF (1×10^{-3} M) solution in the range 190–1100 nm. The ligand absorption spectra appeared at 290 nm to be due to the transfer of electrons from $\pi \rightarrow \pi^*$ and transition $n \rightarrow \pi^*$ peaks around 338 nm [38]. Complexes have revealed changes in the absorption bands that indicate coordination with metal ions through the functional set azomethine nitrogen, and charge transfer peaks also appear, as do $d-d$ transition peaks [39]. Peaks appeared in the Co

complex in the $d-d$ region transition at 404, 499, 677, and 903 nm, which are related to the electronic shift of C.T, ${}^4T_{1g} \rightarrow {}^4T_{1g(p)}$, ${}^4T_{1g} \rightarrow {}^4A_{2g(F)}$, and ${}^4T_{1g} \rightarrow {}^4T_{2g(F)}$, respectively [40]. The Cu complex appeared in spectrum bands at 414 and 641 nm that are related to the electronic transition of C.T and ${}^2E_g \rightarrow {}^2T_{2g}$, respectively. The Ni complexes revealed the electronic absorption of three bands in the visible region were observed at about 410, 489 and 659 nm, attributed to the spin-allowed transitions of C.T, ${}^1A_{1g(F)} \rightarrow {}^1A_{2g}$, and ${}^1A_{1g(F)} \rightarrow {}^1B_{1g}$, respectively. As for Zn complexes [41], no transitions occur due to the stability and saturation of the outer shell. All the explanations can be found in Table 5, as the UV-vis spectra of the band location were compared with those of the ligand alone [42]. Conductivity measurements provide insights into the movement of electrolytes.

Table 5. Electronic spectra for Schiff base ligand and metal complexes

Compound	Molar conductivity ($\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$)	μ_{eff} (B.M)	Electronic arrangement	λ_{max} (nm)	Assignments	Geometry
LH	9.0	–	–	290 338	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	–
[Co(LH)(H ₂ O) ₂]	6.1	4.9	d^7	269 404 499 677 903	$\pi \rightarrow \pi^*$ C.T ${}^4T_{1g} \rightarrow {}^4T_{1g(p)}$ ${}^4A_{2g(F)} \rightarrow {}^4T_{1g}$ ${}^4T_{1g} \rightarrow {}^4T_{2g(F)}$	octahedral
[Ni(LH)]·H ₂ O	13	0.0	d^8	410 489 659	C.T ${}^1A_{1g(F)} \rightarrow {}^1A_{2g}$ ${}^1A_{1g(F)} \rightarrow {}^1B_{1g}$	square planer
[Cu(LH)(H ₂ O) ₂]	9.3	1.8	d^9	319 414 641	$\pi \rightarrow \pi^*$ C.T ${}^2E_g \rightarrow {}^2T_{2g}$	octahedral
[Zn(LH)(H ₂ O) ₂]	7.7	0.0	d^{10}	306 329 389	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ C.T(M→L)	octahedral

Table 6. Antibacterial properties of Schiff base ligands and metal complexes

Compound	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
LH	9	10	9	9	13
[Co(LH)(H ₂ O) ₂]	19	31	15	14	15
[Ni(LH)]·H ₂ O	13	17	12	10	25
[Cu(LH)(H ₂ O) ₂]	16	15	10	11	22
[Zn(LH)(H ₂ O) ₂]	17	20	32	15	18
Tetracycline	9	9	13	13	9

Table 7. Total antioxidant capacity (%) of compounds in different concentrations

Compounds	150 mg/L	100 mg/L	50 mg/L
LH	18.37	15.60	11.70
[Co(LH)(H ₂ O) ₂]	24.58	19.88	18.71
[Ni(LH)]·H ₂ O	20.06	16.82	13.84
[Cu(LH)(H ₂ O) ₂]	19.09	17.27	14.58
[Zn(LH)(H ₂ O) ₂]	22.73	19.05	12.92
Ascorbic acid	20.11	18.75	17.31

Biological Efficacy Against Gram-positive and Gram-negative Bacteria

These species have been studied for their importance in medicine, and tests have been performed on them. Drilling techniques are used to examine the antibacterial properties of chemical compounds, known as biological activity [43-46]. The ligand's *in vitro* antibacterial activity was assessed against both Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*Pseudomonas* and *E. coli*). The findings of the antibacterial activity, as presented in Table 6, unequivocally demonstrated the physiological activity of the produced ligand and its complexes. The results indicated that several of these compounds had beneficial effects on the species being studied. The Co⁺² complex exhibited the greatest efficacy against *S. aureus*, while the Zn⁺² complex demonstrated the highest activity against *B. subtilis*, *E. coli*, and *P. aeruginosa* bacteria. The antifungal activity of the ligand and its complexes was tested against *C. albicans* fungi. Ni⁺² complex exhibited the highest level of activity against *C. albicans*, and tetracycline was used as a reference to determine pharmacological resistance to the bacteria [47-49].

Phosphomolybdate Assay (Total Antioxidant Capacity)

The value of antioxidant efficacy is presented in Table

7. The highest antioxidant activity for Cu⁺² and Zn⁺² complexes was 24.58 and 22.73%, respectively, in 150 mg/mL. This included the higher antioxidant activity of the synthesized Schiff bases and complexes due to the presence of the hydroxyl group.

CONCLUSION

The synthesis and characterization of M⁺² (Co, Ni, Cu, and Zn) complexes of the Schiff base ligand produced via the condensation reaction of 2-hydroxybenzohydrazide and salicylaldehyde are reported in this study. Various analytical and spectroscopic techniques, the data indicate that the complexes have a composition of a certain type (M:L ratio is 1:1). All complexes are coordinated to two oxygen atoms of O-H phenolic, oxygen atom (C=O) for benzohydrazide and N atoms of azomethine. All complexes were octahedral except for the Ni complex's square planer geometry. These compounds exhibited significant activity against all the tested microorganisms. Then, the potential of these prepared compounds as antioxidants was determined by inhibiting free radicals.

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CONFLICT OF INTEREST

The authors state that a conflict of interest does not exist.

AUTHOR CONTRIBUTIONS

Ayyat Jawad Kadhim developed this idea based on Naser Shaalan's expressions, carried out the experiment,

wrote the manuscript, and performed the analysis. Naser Shaalan supervised the project. The results were discussed, and all authors approved the final manuscript.

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