Short Communication:

Synthesis, Spectral Identification, Thermal Studies, Antioxidant Properties, and Biological Effects for Some Metal Ion Complexes with New Schiff Base Ligand

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Abstract: *The tetradentate N_2O_2 Schiff base ligand, which is produced via the condensation reaction of 2-hydroxynaphthaldehyde with phthalohydrazide, is prepared in this work with a fair yield. The prepared ligand was characterized using a microanalysis technique (C.H.N), UV-vis, FTIR, ^{1}H-,^{13}C-NMR, mass spectrometry, and thermal gravimetric analysis (TGA). New complexes were synthesized by a reaction between ligand (N′1E,N′2Z)-N′1,N′2-bis((1-hydroxynaphthalen-2yl)methylene)phthalohydrazide and metal chloride of Co^{+2}, Ni^{+2}, and Zn^{+2} ions in absolute ethanol. The present complexes are also characterized by techniques such as C.H.N, UV-vis, FTIR, TGA, molar conductivity, atomic absorption, and magnetic moment measurements. The* in vitro *antimicrobial activity of the prepared compounds was tested against two Gram-positive bacteria (*Staphylococcus aureus *and* Bacillus*) and two Gram-negative bacteria (*Escherichia coli *and* Pseudomonas aeruginosa*), as well as* Candida albicans *as a fungal species, by diffusion technique in addition to antioxidant properties. The spectroscopy measurement showed that the ligand coordinated with the metal ion as a tetradentate ligand via oxygen and nitrogen in addition to the chloride ion to form octahedral shapes. All compounds under study had a positive effect against antibacterial, antifungal, and antioxidant activities.*

Keywords: *condensation reaction; antioxidant activities; antimicrobial activity; tetradentate*

■ INTRODUCTION

Generally, the Schiff bases are easy to produce by condensation of an aldehyde with a primary amine. They can create stable coordination complexes (via azomethine group chelation) [1] with a wide variety of metal ions. Scientific researchers have also been interested in the poly- or tetra-dentate ligands for Schiff bases via their metallic complexes and the N_2O_2 coordination system because of their superior complexation capacity [2-3]. They are therefore employed in the analytical area to selectively separate and purify metal ions from mixtures and also to eliminate some heavy pollutants from liquid media, including Ag(I) and Hg(II), among other uses [4]. Unique organic compounds, which come in a range of coordination numbers and oxidation states, have motivated scientists to produce and use them as optical detectors for copper ion detection and as modified electrode sensors for aliphatic alcohols [5-6].

N_2O_2 Schiff base ligands complexes have been applied in the biological field for DNA split in the existence of hydrogen peroxide, antifungal, antioxidant, anticancer, and antibacterial against Gram-positive and Gram-negative bacteria. Tetra-dentate Schiff base ligands have been working in catalytic processes as catalysts for phenol oxidation and as a suitable way to extend the chain length of imines by regulating radical polymerization [7-8]. Furthermore, because of their strong fluorescence, some N_2O_2 Schiff base ligands' chelated complexes show the capabilities of their photoactive materials [9]. Therefore, this study aims to

synthesize and characterize a tetradentate N_2O_2 Schiff base ligand and its metal complexes, investigate their antimicrobial and antioxidant activities, and examine their structural properties and coordination behavior with transition metal ions. The study aims to explore the potential of these complexes as therapeutic agents against bacterial and fungal infections and to assess their antioxidant efficacy. The tetradentate N_2O_2 Schiff base ligand was synthesized through the condensation reaction of 2-hydroxynaphthaldehyde with phthalohydrazide. New metal complexes were synthesized by reacting the ligand with Co(II), Ni(II), and Zn(II) chlorides in absolute ethanol. The antimicrobial activity of the ligand and its metal complexes was evaluated using the diffusion technique against both Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus*), and the fungal species *Candida albicans*. Additionally, the antioxidant properties of the compounds were also investigated.

■ EXPERIMENTAL SECTION

Materials

All chemicals were purchased from commercial sources and were used without further purification from Sigma-Aldrich. Dibutyl phthalate (99%), hydrazine monohydrate (99%), 2-hydroxy-1-naphthaldehyde (99%), C_2H_5OH (BDH, 99%), DMF (99%), DMSO (99%), $CoCl_2{\cdot}6H_2O$ (99%), $NiCl_2{\cdot}6H_2O$ (99%), and $ZnCl_2$ (98%) were utilized in this study.

Instrumentation

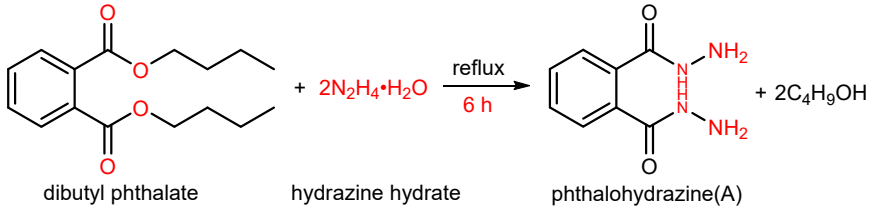
The Shimadzu FT-IR-8100 spectrometer was used to record the FTIR spectra, and the Euro Vector EA 3000 A was used to quantify the amounts of carbon, hydrogen, and nitrogen. Tetramethylsilane (TMS) was considered as an internal standard to evaluate the ligand ^{1}H- and ^{13}C-NMR spectra in DMSO-d_6 solvent on a Bruker 300 MHz. Using UV1650 PC Shimadzu spectrophotometer, the electronic spectra were measured at 25 °C. Electrical conductivity measurements of the complexes were taken at 25 ± 2 °C for 1×10^{-3} M solution of the samples in DMF using a Philips PW-digital conductivity meter. Using Gouy balance, magnetic susceptibility data were also obtained at 25 °C in the solid state. The GCMS-QP2010 PLUS DI analysis Shimadzu, Japan spectrometer was utilized in the University of Samarra's lab to determine the generated ligand's molecular weight. All melting point readings were taken at the University of Baghdad's College of Science for Women using the Gallen Kamp melting devices.

Procedure

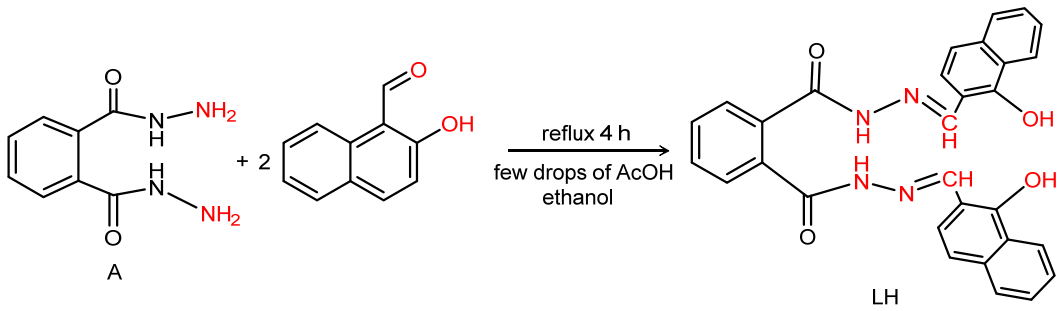
Ligand preparation

The following description includes the procedure for the preparation of phthalohydrazide by reaction between dibutyl phthalate (10 g, 0.036 mol) was dissolved in 15 mL of C_2H_5OH and still at 25 °C in a round bottom flask. Aqueous hydrazine monohydrate (3.604 g, 0.072 mol) was then added dropwise in a 1:2 molar ratio while continuously stirred, and the mixture was then refluxed for 6 h. The precipitate was filtered when the reaction was stopped and cooled at room temperature, and it was then washed with methanol and dry ether [10]. Recrystallization of a white precipitate from 100% ethanol produced a yield of 87% (melting point 294 °C), as present in Scheme 1.

Phthalohydrazide (1 g, 0.005 mol) was dissolved in 10 mL ethanol in a 25 mL round bottom flask with 1.245 g (0.010 mol) of 2-hydroxy-1-naphthaldehyde 1:2 molar ratio followed by 3–4 drops of anhydrous acetic acid. The mixture was then refluxed for 4 h under stirring



Scheme 1. Synthesis of phthalohydrazine (A)



Scheme 2. The prepared Schiff bases ligand (LH)

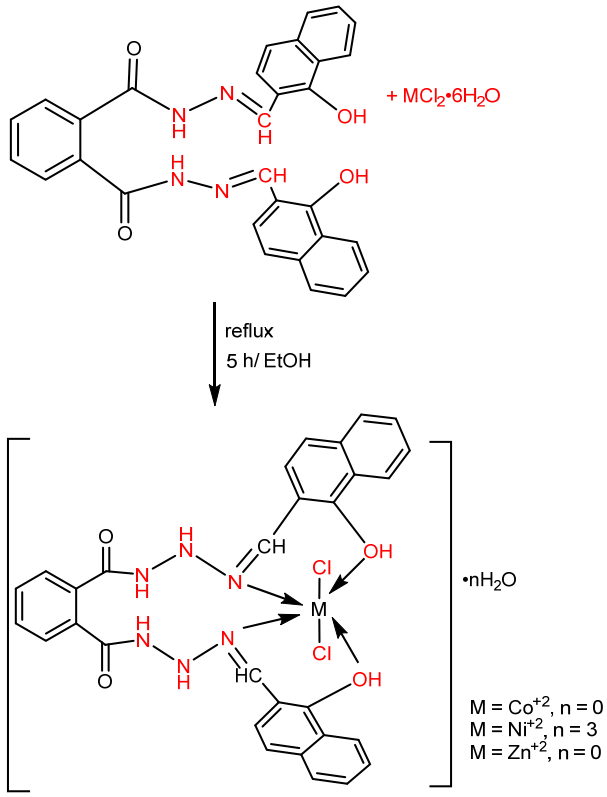
and heating at 50 °C. Yellow crystal was created, filtered, washed, re-crystallized, and dried. The yield was 85%, and the melting point was 281–283 °C, as shown in Scheme 2. The synthesized ligand was characterized by several instrumentations, such as FTIR, UV-vis, mass, ^{1}H-NMR, ^{13}C-NMR, C.H.N elemental analysis, and TGA.

Synthesis of metal complexes

Complexes of the obtained Schiff base (LH) have been prepared using the following approach [11]. First, in a 25 mL round bottom flask, the ligand (1.00 g, 0.002 mol) was dissolved in 10 mL of absolute ethanol. From Table 1, the metal chloride, M = Co^{+2}, Ni^{+2}, and Zn^{+2}, as much as 0.532, 0.395, and 0.475 g, respectively, was dissolved in 10 mL of absolute ethanol and then added to the flask containing ligand solution in a 1:1 molar ratio. After 5 h of reflux heating to 40 °C, the mixture's color changed, as shown in Table 2. After filtering the precipitate, it was cleaned with ethanol and dried at room temperature for 24 h. The yields were good, at 82%, according to Scheme 3.

Biological activity

The bioactivity of ligands and complexes was investigated against both Gram-positive (*S. aureus* and *Bacillus*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria. The diffusion method was used to examine the antibacterial activity *in vitro* towards both kinds of bacteria. Tetracycline has been used as an antibiotic. Test samples and antibiotics were prepared using a DMF solution (1 mg/mL). A microbe suspension (1/100 mL from the middle) was mixed with sterilized agar and liquefied before being put on a Petri plate to a depth of approximately 3 mm. The wells contained test and antibiotic samples were used further. After assembling



Scheme 3. The prepared complexes of Schiff bases ligand

Table 1. Equivalent weights of metal salts

Complex symbol	Salt formula	M.wt, for salt (g/mol)	Quantity (g)
$[CoLHCl_2]$	$CoCl_2{\cdot}6H_2O$	237.93	0.532
$[NiLHCl_2]{\cdot}3H_2O$	$NiCl_2{\cdot}6H_2O$	237.69	0.395
$[ZnLHCl_2]$	$ZnCl_2$	136.29	0.475

the wells in a hardened medium, the dishes were stored in the refrigerator for 1 h at 5 °C then incubated for 1 d at 37 °C for 18 h. Zones of inhibition of bacterial growth caused by test and antibiotic samples were evaluated [12].

Antioxidant activity

The phosphomolybdenum test was utilized to estimate the total antioxidant capacity by reducing Mo(VI) to Mo(V) by the compounds in the sample under investigation. The reaction in an acidic environment produces green-blue complexes of reduced phosphomolybdate, and these complexes are evaluated spectrophotometrically at 765 nm. The antioxidants' measured characteristics may be represented as standard equivalents to ascorbic acid. First, 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was combined with 0.1 mL of the sample solution. After being sealed, the tubes were left to stand for 90 min at 95 °C in a water bath. The absorbance of the combination at 765 nm was measured in comparison to a blank once the samples had reached room temperature. After that, 1 mL of reagent solution and the equivalent volume of solvent made up a standard blank, which was incubated in the same manner. The antioxidant capacity was calculated using Eq. (1) [13-14].

$$\text{Antioxidant effect (\%)} = \frac{\text{Control}_{abs} - \text{Sample}_{abs}}{\text{Control}_{abs}} \times 100\% \quad (1)$$

■ RESULTS AND DISCUSSION

The 2-hydroxybenzohydrazide (2 mol) was mixed with 1 mol of compound A to produce the tetradentate ligand with a high yield. The IR spectrum, microscopic analysis, ^{1}H-NMR spectrum, mass spectrometry, and molar conductivity were used to identify the generated ligand and its complexes. The ligand and complexes' physicochemical characteristics are displayed in Table 2.

Element Micro Analysis

Element micro analysis C, H, N, Cl, and physical properties were performed for ligand and Co^{+2}, Ni^{+2}, and Zn^{+2} complexes. As shown in Tables 2 and 3, it was found that the hypothesis and the actual results agreed. Using metal salts in a 1:1 of M:L reaction, the job approach identified all complexes as non-electrolytes based on their molar conductivities [15].

^{1}H-NMR

NMR spectroscopy can be used to identify the environment of chemical and organic substances. Using TMS as the internal reference standard, the ^{1}H-NMR of LH in DMSO-d_6 produced ligand's chemical structure was

Table 2. Physical characteristics of the synthesized compounds

Compound	Color	m.p. (°C)	M.wt (g/mol)	Conductivity	Yield%
Ligand (LH)	Yellow	280–284	502.52	-	80
$[CoLHCl_2]$	Dark-brown	255–258	632.36	15.6	77
$[NiLHCl_2]\cdot 3H_2O$	Orange	280–283	686.10	19.0	71
$[ZnLHCl_2]$	Shiny-yellow	280–Dec	638.30	6.5	83

Table 3. The element micro analysis C, H, N, X, and M of the synthesized compounds

	C% cal (exp)	H% cal (exp)	N% cal (exp)	X% cal (exp)	M% cal (exp)
$C_{30}H_{22}N_4O_4$ (LH)	72.00	4.40	11.14	-	-
	(72.45)	(4.49)	(10.32)	-	-
$[CoLHCl_2]$	56.92	3.50	8.85	11.22	9.32
	(56.00)	(3.90)	(7.85)	(10.85)	(9.02)
$[NiLHCl_2]\cdot 3H_2O$	52.47	4.08	8.20	10.34	8.55
	(52.77)	(4.07)	(7.20)	(9.94)	(8.11)
$[ZnLHCl_2]$	56.40	3.44	8.77	11.20	10.23
	(56.22)	(2.80)	(8.52)	(11.20)	(10.53)

confirmed by the ^{1}H-NMR, which showed all the required peaks [16]. The LH displayed a singlet peak observed at 2.50 and 3.40 ppm, belonging to the solvent DMSO and H_2O group. The protons of the aromatic ring unit show multiple peaks at regions between 7.32–8.32 ppm, while the proton of the azomethine group (HC=N) shows a single peak at 10 ppm. The hydroxyl group's (O–H) proton shows a singlet peak at 14.32 ppm [17]. Only one peak is visible at 11.5 ppm related to the N–H, as shown in Fig. S1.

^{13}C-NMR

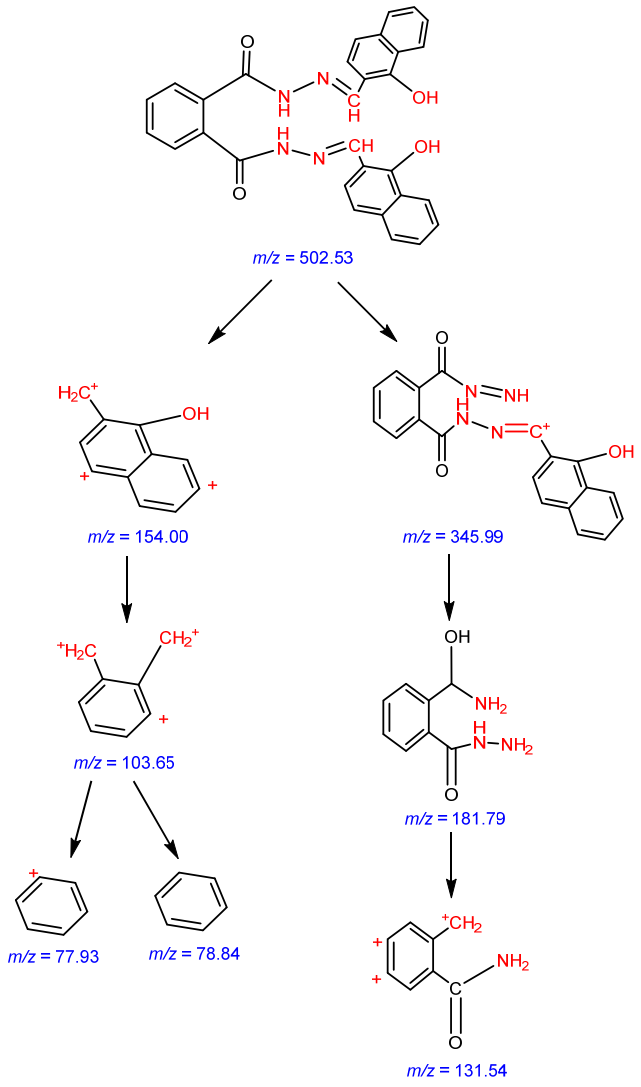
A ^{13}C-NMR spectrum is used to establish the chemical structure of the synthesized ligand by identifying chemical shifts that correspond to each of its carbons, as shown in Fig. S2. The ^{13}C-NMR was studied using DMSO-d_6 as the solvent. The carbon atom of the two-carbonyl group (C=O) exhibits a peak at a chemical shift of 165.40 ppm. The ligand spectrum showed that δ 120–128 ppm belongs to the carbon of the aromatic ring, Ar–OH appears at 161.6 ppm [18], and the ligand has been successfully synthesized with a high level of purity.

Mass Spectral of Schiff Bases Ligand

Mass spectroscopy is an effective structural characterization method commonly utilized in coordination chemistry—equivalents for the free Schiff base ligand's mass spectrum fragmentation. LH ligand was suitable compared to its structure in Scheme 4, as shown in Fig. S3, characterized by the intense peak at 502.28 *m/z* that approximately matches its calculated molecular weight of 502.52 [19].

FTIR Studies of Ligand and Related Complexes

The FTIR spectra bands assignment of the compounds is appeared in Table 4. In complexes, the imine group ν(C=N) band in the ligand 1622 cm^{-1} goes to lower frequencies in all the complexes. The shifting indicates that it is connected by the nitrogen atom of C=N in coordination with the metal [20]. The presence of the bands within the range 3463 cm^{-1} belonging to the –OH of ligand shifting in all complexes to lower frequencies is evidence for chelation [21]. A new band appeared in the region of 603–520 cm^{-1} due to the vibrations of the group



Scheme 4. Fragmentation pattern of Schiff base

Table 4. FTIR detection of the ligand and complexes

Symbol	O–H phenolic	H_2O lattice	N–H	C=N	M–O	M–N	M–Cl
Ligand	3463	-	3325	1622	-	-	-
$[CoLHCl_2]$	3433	-	3082	1608	530	420	314
$[NiLHCl_2]\cdot 3H_2O$	3450	3429, 977, 703	3153	1618	603	432	343
$[ZnLHCl_2]$	3452	-	3172	1606	520	424	347

stretch of M–O [22] and showed a stretching of the group M–N of the generated complexes in the limited area 432–420 cm^{-1}. Furthermore, M–Cl was observed in the region of 347–314 cm^{-1}. This confirms the ligand association to the metal through O, N, and Cl atoms. Lattice water in the Ni-complex appears in the range of 3429, 977, and 703 cm^{-1}. All the infrared spectra are shown in Fig. S4–S7.

Molar Conductivity Measurements

Table 5 shows the measured molar conductivities of M^{+2} = Co, Ni, and Zn complexes in DMF solutions at 25 °C. This indicates that none of the complexes were electrolytes, as all complexes contain Cl ions inside the sphere [23].

UV-vis Spectra of Ligands and Their Complexes

At room temperature, the electronic spectra of ligand and complexes have been recorded in DMF solution within the range of 200–1100 nm. The obtained data are presented in Table 5 and Fig. S8–S11. High-intensity absorption peaks can be observed in the UV-vis spectra of the Schiff base ligand at 243, 328, and 409 nm caused by the charge transfer (CT) transitions of carbonyl and azomethine, and also the π–π* and n–π* transitions [24]. The complexes' spectra showed shifts in the positions of absorption bands, which suggested that the OH and HC=N functional groups were coordinating the metal ions [25]. The Co(II) complex in DMF revealed bands at about 410, 430, and 980 nm that were attributed to CT, $^4T_{1g} \rightarrow {}^4T_{1g(P)}$, and $^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$, respectively. The magnetic moment of 4.56 BM for the Co(II) complex's observed electronic spectrum indicates the possibility that high-spin octahedral geometry would appear [26]. The nickel complex spectrum appeared in the *d-d* region in three bands (976, 674, and 436 nm). These bands related to $^4A_{2g} \rightarrow {}^4T_{2g(F)}$, $^4A_{2g} \rightarrow {}^4T_{1g(F)}$, and $^4A_{2g} \rightarrow {}^4T_{1g(P)}$ with magnetic moment value equal to 3.94 BM. This information gives octahedral geometry for the Ni-complex. The electronic spectra of the d^{10} for the Zn(II) complex exhibited a charge transfer band at 471 and 441 nm [27], giving an octahedral shape around the Zn complex. All synthesized complexes are non-electrolyte complexes, according to the molar conductivities.

Characterization of Schiff Bases Ligand and Their Complexes by Thermal Studies

Table 6 illustrates the thermal data of the ligand and its complexes that are noted. It was observed that the ligand in Fig. S12 showed three stages of breakdown from its TGA curve. The disintegration of the $C_{15}H_{14}N_3O_2$ molecule in the first stage resulted in an estimated mass loss of 53.38% (calculated 53.33%) in the range of 50 to 390 °C and an approximate mass loss of 14.91% (calculated 14.52%) in the range of 390 to 610 °C owing to the breakup of the $NO+2H_2$ molecule. In the final stage, from 610–980 °C, the calculation of mass loss of 12.41.8% (calculated 12.50%), due to the loss of a C_2H molecule with complete decomposition, takes residue $C_{13}H_4O$.

Table 5. Electronic spectra for Schiff base ligand and complexes

Compound	λ_{max} (nm)	Assignments	μ_{eff} (B.M)	Molar conductivity (Ohm^{-1} cm^2 mol^{-1})	Geometry
Ligand (LH)	243	π→π*	-	-	-
	328	n→π*			
	409	CT			
$[CoLHCl_2]$	980	$^4T_{1g} \rightarrow {}^4T_{2g(F)}$	4.56	15.6	Octahedral
	430	$^4T_{1g(p)} \rightarrow {}^4T_{1g}$			
	410	CT			
$[NiLHCl_2]\cdot 3H_2O$	976	$^4A_{2g} \rightarrow {}^4T_{2g(F)}$	3.94	19.0	Octahedral
	674	$^4A_{2g} \rightarrow {}^4T_{1g(F)}$			
	436	$^4A_{2g} \rightarrow {}^4T_{1g(P)}$			
	324	n→π*			
$[ZnLHCl_2]$	471	CT	Diamagnetic	6.5	Octahedral
	441	CT			

Table 6. The pyrolysis processes of compounds

Compounds	Decomposition	Weight loss (%)			Reaction
		T_{max} (°C)	Calc.	Found	
LH ($C_{30}H_{22}N_4O_4$)	Step 1	50–390	53.33	53.38	$-C_{15}H_{14}N_3O_2$
	Step 2	390–610	14.52	14.91	$-(NO + 2H_2)$
	Step 3	610–980	12.50	12.41	$-C_2H$
	Residue				$C_{13}H_4O$
$[CoLHCl_2]$	Step 1	67–320	17.00	17.46	$-(2Cl + 2H_2O)$
	Step 2	320–422	33.52	33.62	$-(C_{11}H_{14} + NO)$
	Step 3	422–755	15.47	15.23	$-(N_2H_2 + 2C)$
	Step 4	755–840	5.08	5.08	$-NH$
	Step 5	840–970	4.65	4.87	$-CH$
	Residue				$CoO + 16C$
$[NiLHCl_2].3H_2O$	Step 1	58–200	22.44	22.22	$-(3H_2O + 2Cl + CH + NH_2)$
	Step 2	200–290	9.78	9.77	$-C_4H_4$
	Step 3	290–493	36.90	37.50	$-(C_6H_{11} + N_2H_2 + NO_3)$
	Step 4	493–977	0.65	0.43	$-2H$
	Residue				$NiO + 19C$
[ZnLHCl2]	Step 1	100–410	0.31	0.33	$-H_2$
	Step 2	410–620	9.97	10.10	$-(C_2H_4 + Cl)$
	Step 3	620–730	14.87	14.97	$-(Cl + NO_2 + 2H_2)$
	Step 4	730–920	3.26	3.21	$-NH_2$
	Step 5	980	28.51	28.78	$-(N_2O + C_7H_8)$
	Residue				$ZnO + C_2H_4 + 19C$

Table 7. Total antioxidant of the ligand and complexes

Sample	50 µg/mL		100 µg/mL		150 µg/mL	
	Absorbance	Conc.	Absorbance	Conc.	Absorbance	Conc.
$[CoLHCl_2]$	0.091	13.82	0.059	8.90	0.057	8.56
$[NiLHCl_2]\cdot 3H_2O$	0.080	12.04	0.091	8.26	0.043	6.51
$[ZnLHCl_2]$	0.097	14.62	0.062	9.39	0.051	7.73
LH	0.085	12.80	0.061	9.17	0.034	5.08
Vitamin C	0.133	20.11	0.124	18.75	0.144	17.31

The TGA curves of M = Co^{+2}, Ni^{+2}, and Zn^{+2} showed four to five decomposition steps in Fig. S12–S15 [28]. Thermal analyses of the metal complexes indicated that the division or loss happens over multiple steps and stages, which means good thermal stability. For example, the first step's loss is water at 50–100 °C, indicating that the water is outside the coordination range. Each step involves losing a portion of their weight and releasing certain substances from the prepared compounds. Because the ions that formed the complex had different characteristics, they broke down in various temperature ranges. After the dissociation process is finished, the remaining material could be a component of the complex's metal oxide [29-30].

Phosphomolybdate Assay

In the present study, we applied a phosphomolybdate assay to estimate the reduction activities of ligands and their complexes in different concentrations of 50, 100, and 150 µg/mL, as appears in Table 7 and shown in Fig. S16. The results in 50 µg/mL Co-complex gives the highest absorbance, which means more reduction ability than other complexes, while 100 µg/mL of the MP Ni-complex has a higher absorbance

Table 8. Antibacterial and antifungal activity of the ligand and complexes

	Candida albicans	*E. coli*	*S. aureus*	*Bacillus*	*Pseudomonas*
LH	14	10	10	10	12
Tetracycline	12	10	12	12	12
Co-complex	16	12	14	13	14
Ni-complex	18	13	15	10	14
Zn-complex	20	20	23	26	25
DMF (solvent)	0	0	0	0	0

value, and in 150 µg/mL Zn-complex is the best [31]. Compared to this result with ascorbic acid, The declining graph of the TAC assay showed that all three complexes and ligands have low effects against the phosphomolybdenum cation radicals compared with standard vitamin C.

Microbiological Investigation

The ligand's antifungal and antibacterial properties and related complexes were tested *in vitro* against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *Bacillus*) bacteria, as shown in Table 8 and Fig. S17. The result showed that Zn-complexes give higher activity from all complexes against *C. albicans* and all bacteria, which is also more than tetracycline activity, which refers to complexation-enhancing biological activity [32].

■ CONCLUSION

This work led to the effective creation of new Schiff base complexes containing Co(II), Ni(II), and Zn(II). A tetradentate ligand attaches to the central ion *via* the oxygen of hydroxy naphthaldehyde and azo-methine nitrogen—a hexadentate form of the complexes completed with chloride ion. Analysis of the spectral data from this experiment indicated that all compounds generated had a 1:1 L:M ratio, which is congruent with a mononuclear structure. Spectral and elemental investigations, in addition to magnetic moment and conductivity, revealed that all complexes in the DMF solution were non-electrolytes for octahedral structures. All compounds' antibacterial and antifungal activities against 4 bacterial and fungal organisms gave effective inhibition zones, while the Zn complex had higher effects on the bacteria. Total antioxidant activity for all ligands and their complexes shows good activity, which means the complexation enhances the activity of the metal complex.

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■ CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

■ AUTHOR CONTRIBUTIONS

Aseel Hikmat Abd Al-Ameer came up with the concept, carried out the experiment, produced the text, and carried out the analysis. Naser Shaalan was the project's supervisor. The findings were considered, and the final paper received approval from all authors.

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