International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 30-07-2026, Accepted: 31-08-2026, Published: 15-09-2026
Volume 10, Issue 3, 2026, Page No. 48-57

Synthesis, characterization, antibacterial and antioxidant activities of some new mixed-ligand metal complexes of a Schiff base ligand with organic acids

Md. Masuqul Haque*, Savin Shayok Dipro, Titash Ray, Hafizur Rahman, Md. Kudrat-E-Zahan, Md. Nazrul Islam, Md. Yeamin Reza
Department of Chemistry, University of Rajshahi, Rajshahi, Bangladesh
Corresponding Author: Md. Masuqul Haque
DOI: https://doi.org/10.66856/chemistry.2026.10.3.10058

Abstract
Mixed ligand complexes of Cu(II), Zn(II) and Cd(II) have been prepared from isoniazid derived Schiff base and dicarboxylic acids. The complexes were characterized by conductivity, magnetic susceptibility, FT-IR and UV–vis spectroscopic studies. All complexes were nonelectrolytes. The Cu(II) complexes have magnetic moments in the range 1.74-1.85 BM confirming their paramagnetic nature while the Zn(II) and Cd(II) complexes were diamagnetic in nature as expected for their d^{10} configurations. The FT-IR data indicated the coordination of the Schiff base through the carbonyl oxygen and azomethine nitrogen atoms while the dicarboxylate ligands coordinated through the oxygen atoms of the carboxylate groups. The Cu(II) complex was suggested to be square-planar, while the Zn(II)/Cd(II) complexes were proposed to be tetrahedral. The complexes showed moderate antibacterial and DPPH radical scavenging activities. Among them, [Cd(L-1) (suc)] exhibited the highest antibacterial activity with inhibition zones of 19-20 mm against Pseudomonas sp. and Escherichia coli. The Cu(II) complexes exhibited better antioxidant activity than the respective Zn(II) and Cd(II) complexes. The results suggest the possible biological activity of the synthesized mixed-ligand complexes.

Keywords: Schiff base, isoniazid, mixed-ligand complexes, antibacterial activity, antioxidant activity

Introduction

Continuous progress has resulted in significant advances in the study of the bonding processes between transition metal ions and a variety of ligands in bioinorganic and medicinal chemistry [1]. Schiff bases are chelating ligands of particular interest in the coordination chemistry of both main group elements and transition metals [2]. They are stable over a wide range of oxidation and reduction conditions and the imine groups (-show both hard and soft Lewis's base characteristics [3-6]. Isoniazid (isonicotinyl hydrazide) is a drug of choice for the prevention and treatment of latent and active tuberculosis [7]. It forms metal chelates with a lot of divalent ions [8]. Transition metal ions such as Mn^{2+}, Fe^{2+}, $Cu^{[2+}$ and Zn^{2+} are important for human body [9-10]. Interaction of the carbonyl groups of biologically active organic dicarboxylic acids include oxalic acid and phthalic acid [10-11] with the metal ions can result in the formation of chelate complexes with various types of geometrical structures [12]. Mixed-ligand transition-metal complexes have recently attracted renewed interest because of their ability to catalyze a wide variety of chemical reactions such as oxidation, reduction, oxidative cleavage, and hydroformylation [13]. This has aroused new interest as such complexes catalyze a broad spectrum of organic reactions [14]. This is because they can cause the decomposition of hydrogen peroxide [15-17]. Due to its interesting coordination behavior with many oxidation states, redox properties and geometry [18–19], Cu(II) has been extensively studied in coordination chemistry. It is known to play an essential role in many biological processes [20]. Similarly, zinc (Zn) is an important trace element in the body due to its great biological effects [21]. It is present in many body tissues including skin, bone, liver, muscles and brain [22] This element ranks as the most common transition metal in the brain after iron [23]. The current focus of interest is the interactions of zinc with another molecule [24]. Zinc is found in the active sites of enzymes catalyzing the hydrolysis of compounds by water and proteins binding to the DNA [25-26]. Cadmium (II) complexes have been shown to exhibit antibacterial activity against the pathogenic fungus Candida albicans, gram-positive bacteria such as Staphylococcus aureus and Enterococcus faecalis as well as gram-negative bacteria including Pseudomonas aeruginosa and Escherichia coli [27]. H. F. Abd El-Halim *et al*. recently reported a Schiff base ligand prepared from 2-aminophenol and quinoline-2-carboxaldehyde. Mixed ligand divalent transition metal complexes of this Schiff base have been synthesized by the incorporation of 1,10-phenanthroline as second ligand. All the synthesized compounds were screened for anticancer, antibacterial and antifungal activities. The best anticancer activity was exhibited by the Cu^{2+} complex among the tested compounds. In contrast, the free ligand showed very strong antifungal activity, which was higher than that of any synthesized compounds [28].

Based on these facts, we report a large number of new mixed ligand complexes of divalent metal ions with isoniazid derived Schiff base (L-1) as the primary ligand and dicarboxylic acids such as oxalic acid, malonic acid and succinic acid as the secondary ligands (L-2). The Schiff base ligand and mixed ligand complexes were characterized by Fourier transform infrared spectroscopy (FT-IR), ultraviolet–visible spectroscopy (UV-Vis), molar conductance and magnetic susceptibility measurements. In addition, the antibacterial and the antioxidant properties of the Schiff base and its mixed ligand complexes were also investigated in this study.

Experimental

The chemicals and solvents used were of analytical grade (≥99.9%) and procured from Merck and Loba Chemie and

used without further purification. The melting points or decomposition temperatures were determined with an electrothermal melting point apparatus. The molar conductivity was determined at room temperature by a conductivity meter (WPACM35) with a platinumized dip-cell electrode. The magnetic susceptibility of the complexes was determined using a SHERWOOD SCIENTIFIC magnetic susceptibility balance. The electronic absorption spectra were recorded at the Department of Chemistry, University of Rajshahi using a SHIMADZU UV-1200 double-beam spectrophotometer. FT-IR spectra were taken at the Central Science Laboratory, University of Rajshahi, using a SHIMADZU FTIR-8400 spectrometer with the KBr pellet technique in the wave number range 4000-400 cm^{-1}.

1. Synthesis of N-Isonicotinamido-furfuraldimine Schiff base as primary ligand, L-1

In a round bottom flask, 10 mmol (1.37 g) of isoniazid (INH) and 15 ml of ethanol (C_2H_5OH) were taken and INH was properly dissolved by stirring the mixture magnetically. Then 10 mmol (0.82 mL) of furfural was dropped in it. The mixture was refluxed for ca. 4 hours. The mixture was cooled slowly to room temperature and allowed to stand overnight to give the Schiff base (SB) ligand (L-1). The final product was obtained by filtration and repeated washing with ethanol. The white crystalline product was finally dried overnight in a vacuum desiccator with anhydrous $CaCl_2$. Yield: 84%, Chemical Formula: $C_{11}H_9N_3O_2$; Molecular Weight: 215 2; Colour: white; Melting point: 190-191℃.

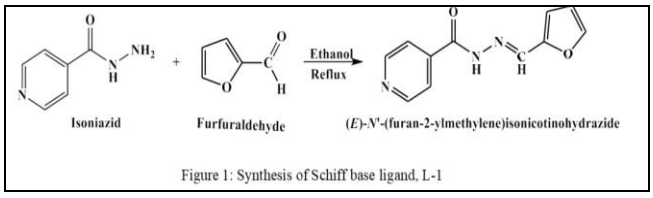


Figure 1: Synthesis of Schiff base ligand, L-1

2. Synthesis of Mixed Ligand Complexes

The mixed-ligand complexes of Cu^{2+} were prepared by dissolving the first ligand, L-1 (0.215 g, 1 mmol) in 10 mL of hot methanol (CH_3OH). This was then mixed thoroughly with 10 mL of a hot methanolic solution of respective metal (II) nitrate (1 mmol; $Cu(NO_3)_2 \cdot xH_2O$, 0.255 g). It was stirred for about an hour. Then, as a second ligand (L-2), about 5 mL of a warm methanolic solution of 1 mmol of the appropriate dicarboxylic acid (in this research work oxalic acid, succinic acid or malonic acid was used) was added dropwise to the flask to obtain the mixed-ligand complexes of Cu^{2+} (these being referred to as S-1, S-2, and S-3, respectively). The mixture was then heated under reflux for another three hours. The colored precipitate was filtered, washed thoroughly with cold methanol and dried in vacuo over anhydrous $CaCl_2$. The mixed ligand complexes of Zn^{2+} and Cd^{2+} were also prepared by the same method.

3. Antibacterial Activity

The antibacterial activity of the Schiff base ligand (L-1) and its mixed ligand metal complexes with organic acids was evaluated by the well-known disc diffusion method [29-30]. In this study, Escherichia coli (E. coli) and Pseudomonas sp. were the bacterial strains used. Kanamycin (K-30) was used as a standard antibacterial agent for comparison. The experiments were carried out in the Microbiology Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.

4. Antioxidant Properties

The antioxidant activities of complexes were studied by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging test [31]. The method measures the antioxidant's ability to reduce the DPPH radical. The antioxidant can donate a hydrogen atom to the DPPH radical in methanol and is converted into its non-radical form DPPH-H. The solution is deep violet, which shows that the DPPH radical has a strong absorption maximum around 520 nm [32]. This absorption band disappears after quenching the radical. The color change is visible and can be measured by a UV-visible spectrophotometer at 517 nm. The percentage of DPPH scavenging was calculated as:

DPPH scavenging activity = $(Ao - A_{sample})/Ao \times 100$

Where A_o is the absorbance of the control and A_{sample} is the absorbance of the sample to be tested. The IC_{50} value (concentration of the sample for 50% inhibition) was calculated by linear regression analysis of percentage inhibition against logarithm of concentration, with the lower the IC_{50} value, the higher the antioxidant activity.

Results and Discussion

The complexes [M(L-1) (L-2)] were produced through the interaction of Cu(II), Zn(II), and Cd(II) with ligand L-1 and organic dicarboxylic acids (L-2). These complexes exhibit distinct coloration, remain stable at ambient temperature, are insoluble in typical polar solvents but dissolve in DMSO and DMF, and have well-defined melting points. A concentration of 10^{-3} M in DMSO was used to determine the molar conductivity, and the temperature used was the usual room temperature. This non-electrolytic property of the complexes is demonstrated by the decreased conductance value, which gives evidence for this characteristic [33]. Table 1 presents the analytical and physical properties of the obtained complexes. The effective magnetic moments (μ_{eff}) of Cu(II) complexes are in the range between 1.74 and 1.85 BM, indicating the paramagnetic nature of Cu(II) complexes. On the other hand, low values (below 0.5 B.M) of μ_{eff} for Zn(II) and Cd(II) complexes correspond to their $d^{[10]}$ electronic configurations and are diamagnetic in nature [33].

Table 1: Analytical and Physical Properties Data of L-1 and its Mixed Ligand Metal Complexes with L-2

Symbol of compound	Compound	Melting point/℃	Color	Solubility DMSO & DMF	Molar conductance Ohm^{-1} cm^2mol^{-1}	μeff in B.M
L-1	C11H9N3O2	190-191	White	(+) ve		
S-1	Cu(C11H9N3O2)(oxa)]	>300	Light green lemon	(+) ve	5	1.83
S-2	[Cu(C11H9N3O2)(suc)]	267-268	Lemon green	(+) ve	7	1.85
S-3	[Cu(C11H9N3O2)(mal)]	262-263	Light green	(+) ve	4	1.74
S-4	[Zn(C11H9N3O2)(suc)]	>300	White	(+) ve	5	Dia
S-5	[Zn(C11H9N3O2)(oxa)]	>300	White	(+) ve	3	Dia

S-6	[Cd(C11H9N3O2)(mal)]	257-258	Fade Yellow	(+) ve	4	Dia
S-7	[Cd(C11H9N3O2)(suc)]	>300	White	(+) ve	6	Dia
S-8	[Cd(C11H9N3O2)(oxa)]	>300	Fade Yellow	(+) ve	7	Dia

1. Analysis of FT-IR Spectra

The FT-IR spectra of the Schiff base ligand L-1 and its mixed-ligand complexes of Cu^{2+}, Zn^{2+}, and Cd^{2+} with various dicarboxylic acids are presented in Figures 2, 3, and 4, respectively. The stretching vibrations of the carbonyl group, *v*(C=O), and the carbon–nitrogen bond, *v*(C=N), cause the individual peaks in the infrared (IR) spectra of the ligand, L-1 (Table 2). These peaks occur at 1665.5 cm^{-1} and 1591.6 cm^{-1}, respectively [34]. After synthesizing the Cu(II) complexes, the peak at 1665.5 cm^{-1}, corresponding to the v(C=O) stretching vibration in the Schiff base ligand, shifts to 1608.7–1606.5 cm^{-1}. This frequency shift shows that the carbonyl oxygen atom coordinates to the metal ions, as illustrated in Fig 2. Peaks in the infrared spectra of the complexes between 652.2 and 657.5 cm^{-1} are attributed to M-O bond stretching vibrations [35]. For the Schiff base, the azomethine peak at 1591.6 cm^{-1} shifts to lower frequencies, ranging from 1550.0 to 1572.5 cm^{-1}, in all complex spectra. This change further confirms that the azomethine nitrogen atom coordinates with the metal ions [36]. Extra bands appearing between 557.4 and 556.0 cm^{-1} in the complexes' infrared spectra are linked to v(M-N) vibrations. The strong C=O absorption band of the carboxylic acid group in free oxalic acid appears at 1692.5 cm^{-1} and 1441.8 cm^{-1} [37]. All complexes show characteristic bands at 1608.7–1606.5 cm^{-1} and 1383.8–1375.4 cm^{-1}, assigned to the asymmetric and symmetric stretching vibrations of the coordinated carboxylate group.

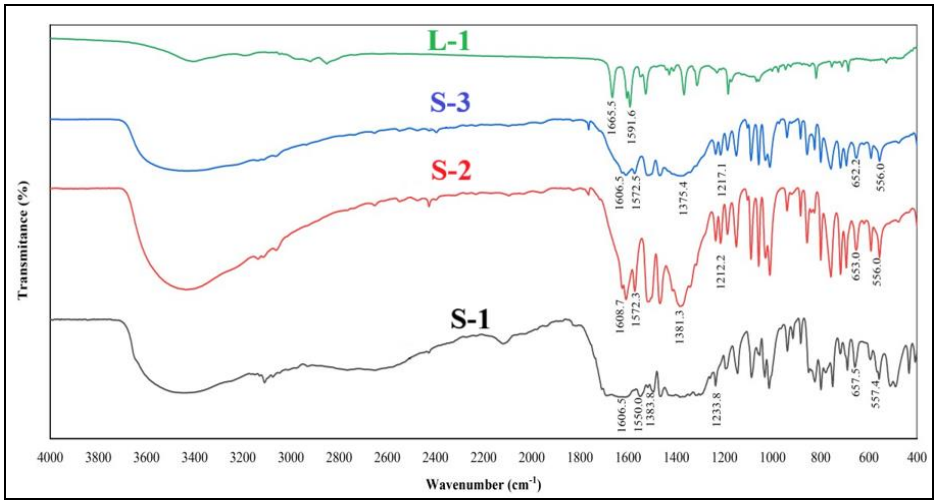


Fig 2: FT-IR Spectrum of Ligand, L-1 and Its Mixed Ligand Complexes of Cu^{2+} with L-2 (Oxalic acid, Succinic acid, and Malonic acid)

Decon showed that the difference in amplitude between Δv = $v_{asy.}$ (COO−) - $v_{sym.}$ (COO−) vibrations distinguishes ligand coordination modes, monodentate or bidentate [38]. The observed difference ranges from 222.7 to 231.1 cm^{-1}, indicating each carboxylate group (–COOH) coordinates in a monodentate way [38]. This suggests dicarboxylic acids act as bidentate ligands by coordinating to metal canters through two separate monodentate carboxylate groups.

The C-O bond in the complexes is indicated by a band between 1176.2 and 1151.7 cm^{-1} [38]. Overall, the IR data indicate the ligand coordinates to the metal centers through oxygen and nitrogen atoms.

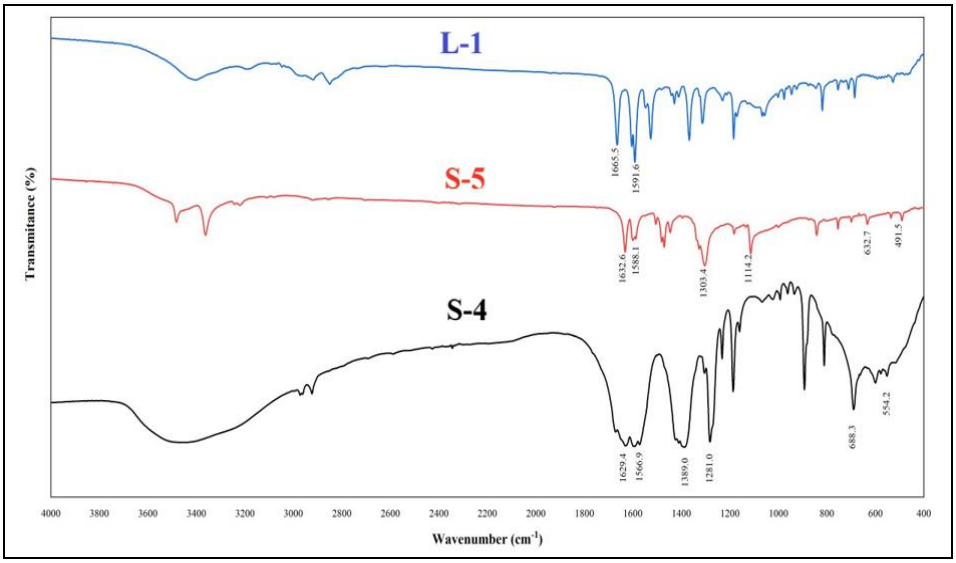


Fig 3: FT-IR Spectrum of Ligand, L-1 and Its Mixed Ligand Complexes of Zn^{2+} with L-2 (Oxalic acid and Succinic acid)

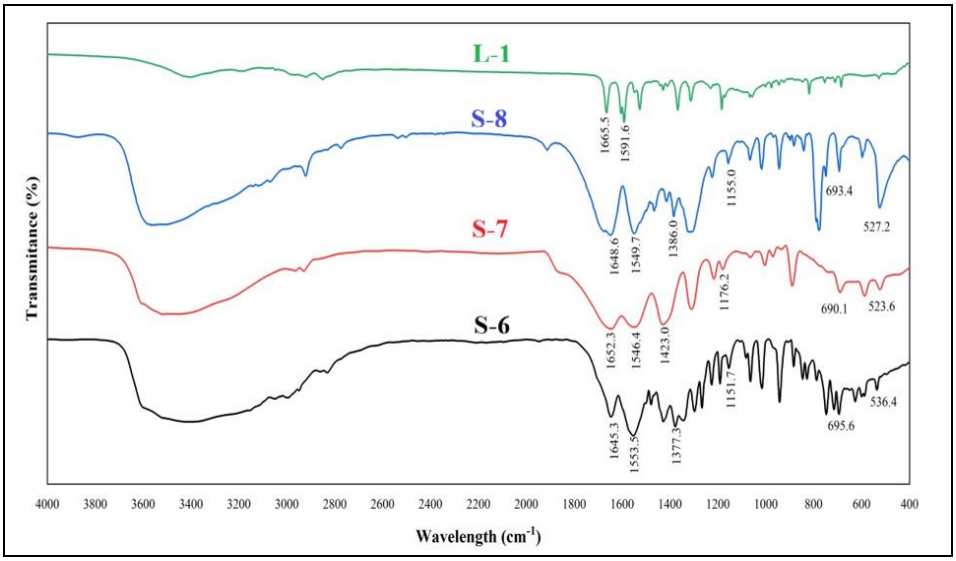


Fig 4: FT-IR Spectrum of Ligand, L-1 and Its Mixed Ligand Complexes of Cd $^{2+}$ with L-2 (Oxalic acid, Succinic acid, and Malonic acid)

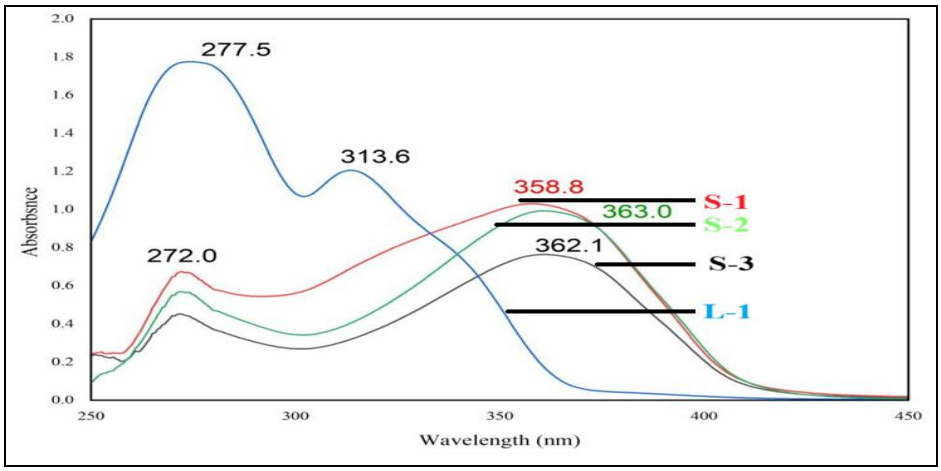


Fig 5: UV–Visible Spectra of Ligand L-1 and Its Cu(II) Mixed-Ligand Complexes with L-2 (Oxalic, Malonic, and Succinic Acids)

Table 2: Key Infrared Bands (cm $^{[-1]}$) of L-1 and its Mixed Ligand Metal Complexes with L-2

Compound	*ν*(C=O)	*ν*(C=N)	*ν*(O-H)	*ν*(C-O)	*ν*(COO)sym.	*ν*(COO)asy.	Δ*ν*	*ν*(M-N)	*ν*(M-O)
L-1	1665.5	1591.6	3404.2	-	-	-	-	-	-
S-1	1606.5	1550.0	3492.4	1233.8	1606.5	1383.8	222.7	557.4	657.5
S-2	1608.7	1572.3	3439.5	1212.2	1608.7	1381.3	227.4	556.0	653.0
S-3	1606.5	1572.5	3566.6	1217.1	1606.5	1375.4	231.1	556.0	652.2
S-4	1606.5	1550.0	3463.7	1281.0	1606.5	1389.0	217.5	557.4	657.5
S-5	1608.7	1572.3	3483.2	1114.2	1608.7	1303.4	303.1	556.0	653.0
S-6	1645.3	1549.7	3492.4	1151.7	1645.3	1377.3	268.0	695.6	536.4
S-7	1652.3	1546.4	3439.5	1176.2	1652.3	1423.0	262.6	690.1	523.6
S-8	1648.6	1553.5	3566.6	1155.0	1648.6	1386.0	231.1	693.4	527.2

2. UV-Visible spectra

The Uv-Visible spectra of the L-1 and its mixed ligand metal complexes of Cu $^{2+}$(symbolized as S-1, S-2. And S-3) have shown in Fig 5. The UV-Visible spectrum of the ligand exhibits two distinct bands: one at 277.5 nm and another at 313.6 nm. The band at 277.5 nm corresponds to the π→π* transition, whereas the 313.6 nm band is attributed to the n→n* transition [39].

Charge transfer transitions were observed in all complexes, resulting from either ligand-to-metal charge transfer (LMCT) or metal-to-ligand charge transfer (MLCT). In the electronic spectra of the complexes measured in DMSO solution, an absorption band in the region 358.8–363.0 nm was detected, as indicated in Table 3. This absorption band is associated with charge transfer processes between the ligand and the metal [40]. The complexes exhibiting both π→π* and metal-to-ligand charge-transfer bands typically favors square-planar geometry [39-40]. The absorption band at 272.0 nm in the UV region is likely due to the π→π transition, as shown in Fig 5. On the other hand, the electronic spectra of the mixed-ligand complexes containing Zn^{2+} and Cd^{2+}, presented in the Figures 6 and 7, showed typical absorption bands in the UV region. The absorption bands appearing at longer wavelengths are due to ligand-to-metal charge-transfer (LMCT) and/or metal-to-ligand charge-transfer (MLCT) transitions [40]. The strong

absorption bands in the shorter-wavelength part of the UV region are primarily due to π→π* transitions of the ligand chromophores. As Zn^{2+} and Cd^{2+} are d^{10} metal ions, d–d transitions are not expected. The spectral characteristics observed, in conjunction with the coordination behaviour of the ligands, agree with the tendency of these metal ions to prefer a tetrahedral coordination geometry [37].

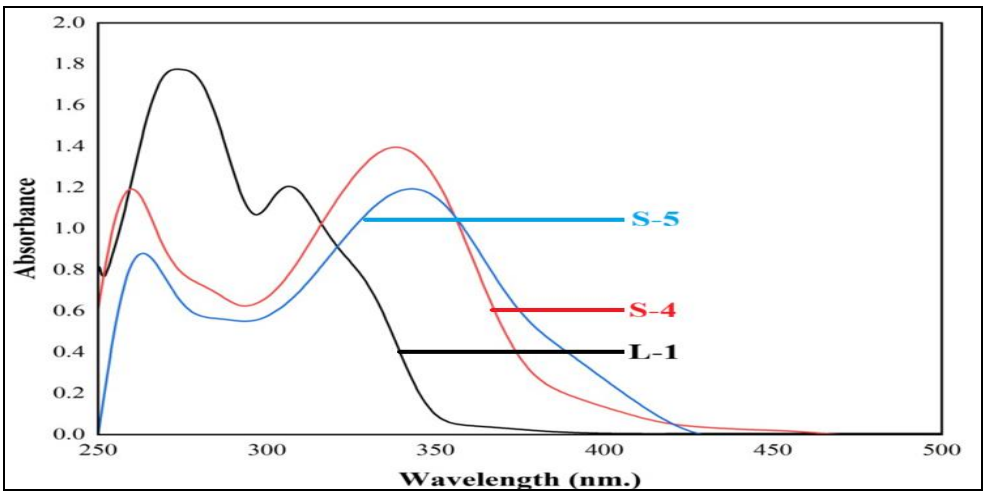


Fig 6: UV–Visible Spectra of Ligand L-1 and Its Zn(II) Mixed-Ligand Complexes with Oxalic Acid and Succinic Acid

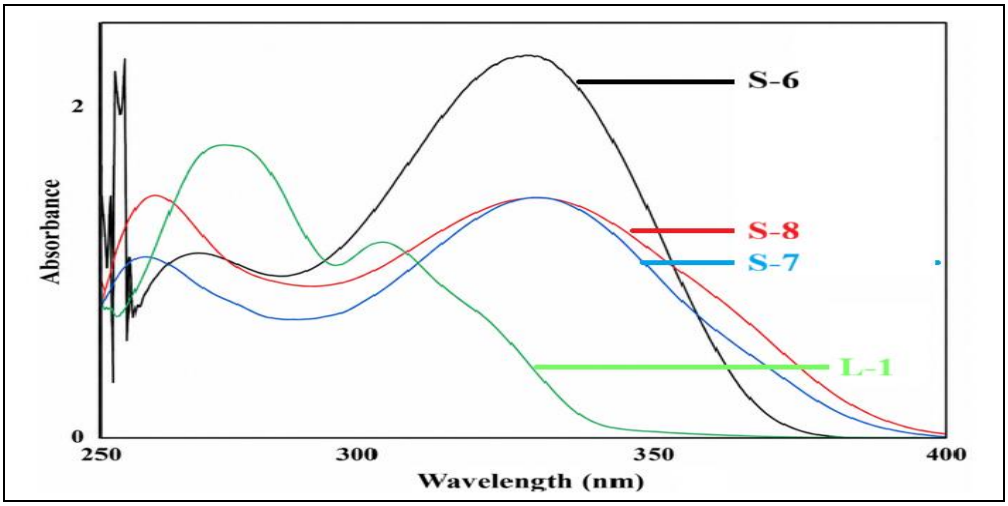
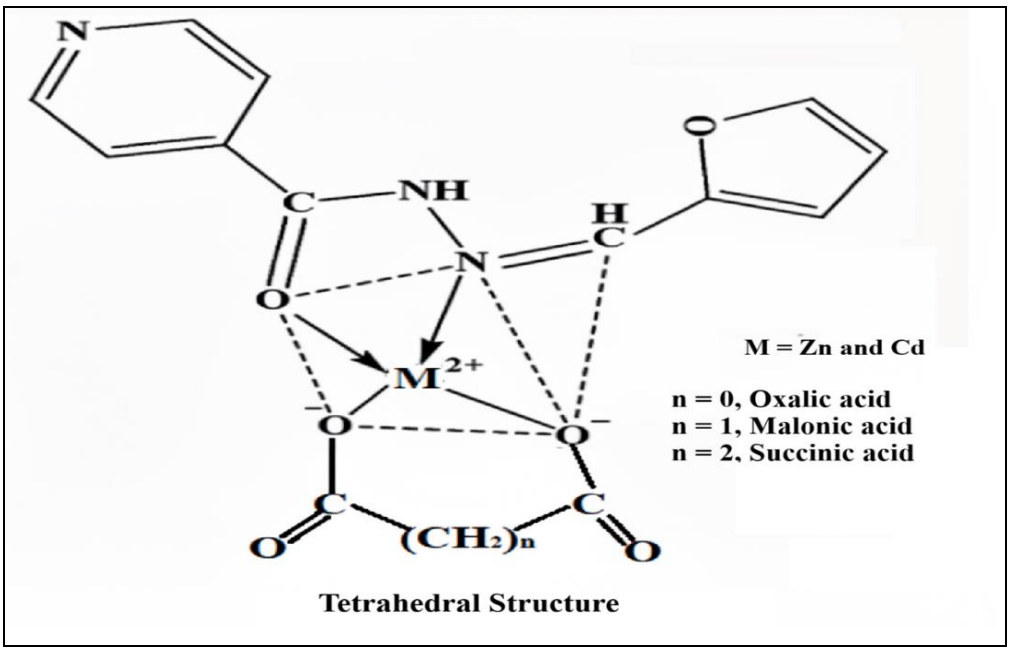
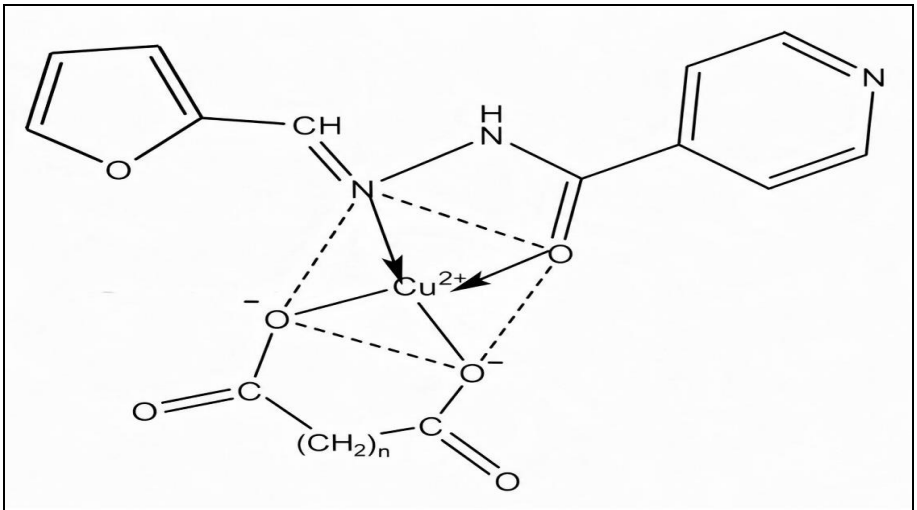


Fig 7: UV–Visible Spectra of Ligand L-1 and Its Cd(II) Mixed-Ligand Complexes with L-2 (Oxalic, Malonic, and Succinic Acids)





Square planar structures of Cu(II) complexes

Based on the characterization data presented above, the following structures are proposed for the synthesized complexes:

The characteristic FT-IR absorption bands of L-1 and its mixed-ligand complexes of Cu(II), Zn(II), and Cd(II) with L-2 are presented in Table 3. The changes in band positions and intensities observed upon complex formation were used to identify the probable coordination sites of the ligand and to confirm metal–ligand interactions.

Antibacterial Activity of L-1 and Synthesized Mixed Ligand Metal Complexes

Antibacterial activity of the Schiff base ligand L-1 and its mixed-ligand complexes with L-2 (Oxalic acid, Succinic acid and Malonic acid) against *Escherichia coli* and *Pseudomonas sp.* at a concentration of 100 µg/10 µL in DMF was studied and has shown in Photo 1. The inhibitory zone values were measured in diameter (mm), and the antibacterial activity results are listed in Table 4. The Schiff base ligand shows a very small inhibition zone against the selected bacterial strains. Moreover, among ligand L-1 and its mixed ligand complexes with L-2, [Cd(L-1)(suc)] exhibited the highest antibacterial activity, with zones of inhibition ranging from 19 to 20 mm against *Pseudomonas* sp. and *Escherichia coli*. Overtone's concept and Tweedy's chelation theory can explain the greater activity of the complexes [41].

The significant antibacterial activity of metal complexes is because only lipid-soluble materials are effective, making lipophilicity a main control factor in antibacterial activity. On chelation, due to overlap and the partial sharing of the positive charge of the metal ion with the orbitals of the donor groups of the ligands, the polarity of metal ions is further reduced. The metal ions are therefore easily adsorbed on the surface of the cell wall of the organisms. This interferes with respiration within the cells and prevents protein production. Consequently, this restricts the proliferation of more organisms. In addition, the methyl group substituent plays a significant role in enhancing the lipophilic nature of the metal complexes, which contributes to their remarkable antibacterial activity. Other factors, such as solubility, coordinating sites, complex geometry, steric hindrance, concentration, and hydrophobicity, also significantly affect antibacterial potency [41-44].

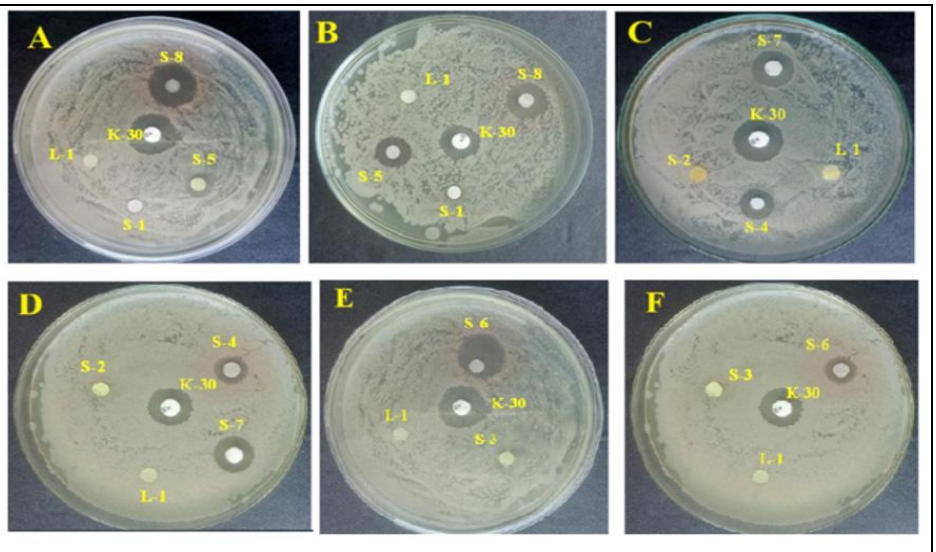


Photo 1: Photographic representation of zone of inhibition of L-1 and its mixed ligand metal complexes against Pseudomonus sp. (A, C, and E) and Escherichia coli (B, D, and F)

Table 4: Antibacterial Activities of L-1 and Its Metal Complexes with L-2 (Oxalic Acid, Succinic Acid and Malonic Acid)

Diameter of Zone of Inhibition (mm) of tested compounds (100µg/disc)			
Compounds	Symbol of Compound	Gram Negative	
		Escherichia coli	*Pseudomonas* sp.
Kanamycin (30 µg/disc)	K-30	18	24
Ligand (L-1)	L-1	3	3
[Cu(L-1)(oxa)]	S-1	6	8
[Cd(L-1)(oxa)]	S-8	15	19
[Zn(L-1)(oxa)]	S-5	13	15
[Cu(L-1)(suc)]	S-2	6	7
[Cd(L-1)(suc)]	S-7	16	20
[Zn(L-1)(suc)]	S-4	13	17
[Cu(L-1)(mal)]	S-3	7	8
[Cd(L-1)(mal)]	S-6	15	19

5. Antioxidant properties of L-1 and Synthesized Mixed Ligand Metal Complexes

The antioxidant activity of the produced ligands L-1 and its mixed ligand complexes with L-2 (including oxalic acid, succinic acid, and malonic acid) was examined using the free radical compound 2,2-diphenyl-1-picryl hydrazyl (DPPH) and BHT (butylated hydroxytoluene) as a reference standard. The antioxidant activity was assessed at different concentrations (20, 40, 60, 80, and 100 µg/mL). When DPPH is dissolved in DMF, it exhibits a violet color which then changes to a pale red color [32]. The DPPH radical-scavenging activities (%) and IC_{50} values of BHT, the ligands, and their mixed-ligand complexes containing L-2 are presented in Table 4. These results are also depicted in Fig 8. The findings indicate that all of the metal complexes exhibited a modest level of DPPH radical scavenging activity in comparison to the Schiff base ligands, which was comparable to that of BHT. In comparison to the standard BHT, the antioxidant activity of Cu(II) complexes was found to be significantly higher than that of Cd(II) and Zn(II) complexes. This was the case across all of the compounds that were examined. It is possible to attribute the difference in the antioxidant activity of the Schiff base metal complexes to the coordination environment as well as the redox characteristics of the complexes. An axial ligation, the size of the chelate ring, and the degree of unsaturation in the chelate ring are some of the factors that, in general, determine the redox characteristics of the metal complexes [45-47]. When compared to other complexes that have been created, the strong antioxidant activity of Cu (II) complexes can be attributed to the high reducing capacity of Cu^{2+} as well as its proton donation characteristic, which allows Cu^{2+} to function as a superoxide scavenging center [48]. The lower activity of Zn (II) and Cd (II) can be attributed to the fact that Zn (II) ion is not classified as a transition metal, which consequently prevents it from engaging in electron-transfer processes [48].

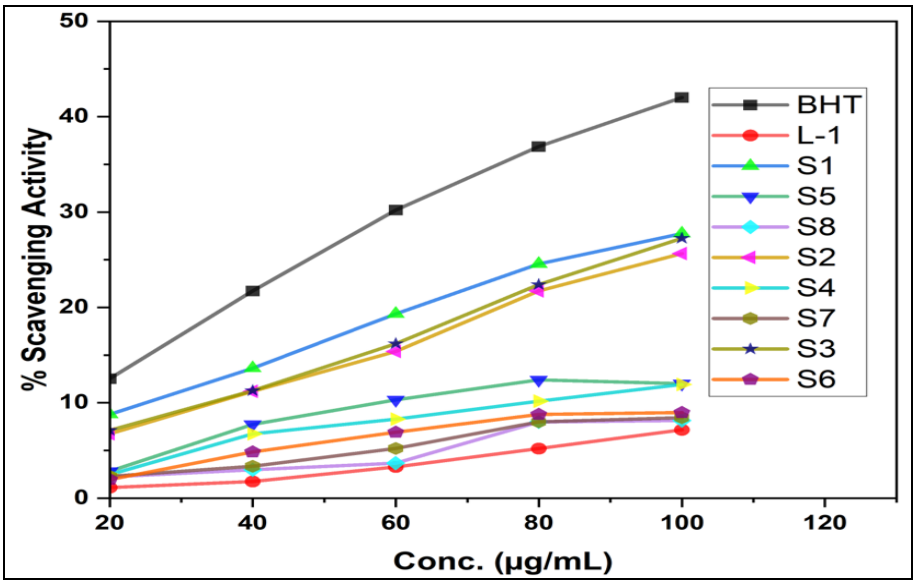


Fig 8: Concentration-dependent DPPH scavenging activity of L-1 and its mixed-ligand complexes with L-2 (oxalic acid, malonic acid, and succinic acid)

Table 4: Percentage scavenging activity and IC_{50} values of BHT, ligand L-1, and its mixed-ligand complexes with L-2 (oxalic acid, malonic acid, and succinic acid)

Conc. (µg/mL)	% BHT	% L-1	% S1	% S5	% S8	% S2	% S4	% S7	% S3	% S6
20	12.54	1.12	8.79	2.83	2.23	6.75	2.47	2.29	7.12	1.95
40	21.73	1.75	13.62	7.74	2.98	11.2	6.75	3.35	11.28	4.85
60	30.21	3.28	19.32	10.32	3.68	15.4	8.29	5.22	16.2	6.92
80	36.87	5.2	24.56	12.41	7.99	21.73	10.17	8.01	22.4	8.78
100	42.01	7.17	27.74	12.01	8.15	25.65	11.93	8.45	27.25	8.99
IC_{50}	118	655	188	416	594	200	437	585	189	545

Conclusion

The current work suggests that the combination of an isoniazid-based Schiff base (L-1) with dicarboxylic acids (L-2) is a good strategy for the development of physiologically active mixed ligand metal complexes. The structural properties as well as biological responses of the complexes rely on the nature of the metal ion and ligand environment. The observed antibacterial and antioxidant effects, notably for the Cd(II) and Cu(II) complexes, suggest that metal complexation may enhance or change the biological characteristics of the parent Schiff base. These results show the potential of mixed-ligand coordination chemistry as a helpful method for the synthesis of novel biologically important metal compounds. More studies on detailed structural and mechanistic factors are required to understand their method of action and biological potential.

Acknowledgements

Authors would like to acknowledge the infrastructure and financial support of the Faculty of Science, Rajshahi University, Bangladesh. The authors are also thankful to Department of Chemistry, Rajshahi University, Bangladesh for providing laboratory facilities to conduct this works.

Conflicts of interest

The authors declared no potential conflicts of the interest with respect to the research and authorship of this article.

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