

# Synthesis, Characterization, Antibacterial and Antioxidant Studies of Isoniazid-based Schiff Base Ligands and Their Metal Complexes

## Abstract

The condensation of aromatic aldehyde with amine results in the formation of Schiff's bases, which are considered to be one of the most significant groups of ligands. Schiff's base metal complexes have received a lot of attention because of their high biological activity. Such include antimicrobial, anticancer, antibacterial, and antifungal activities. The current research is focused on the synthesis and characterization of two Schiff's base ligands obtained from the reaction of isoniazid with 4-(N,N-dimethylamino)benzaldehyde (L1) and 5-nitro-2-furfuraldehyde (L2), respectively and their metal complexes with  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ni}^{2+}$  ions. FT-IR, Uv-Vis, conductivity, and magnetic susceptibility ( $\mu_{\text{eff}}$ ) measurements were used to describe the ligands (L1 and L2) and their complexes. The square planar geometry of  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  complexes, as well as the tetrahedral geometry of  $\text{Zn}^{2+}$  complexes, are supported by the Uv-Vis spectra and magnetic moments data. Their antibacterial effectiveness against various pathogenic microorganisms was evaluated in the presence of standard Kanamycin-30. The  $\text{CuL2}$  complex, among all the metal complexes derived from the L2 ligand, exhibited better antibacterial activity against *S. aureus* than that of Kanamycin-30. When compared to the BHT antioxidant, all  $\text{Cu}^{2+}$  complexes showed excellent efficacy.

**Key words:** Schiff base, Transition metals, Isoniazid, Kanamycin-30, Antioxidant

## 1. Introduction

Schiff bases are regarded as an exceptionally significant family of organic compounds with multiple uses in several biological fields [1-4]. Furthermore, Schiff base is an appealing family of ligands that has played an important role in the advancement of coordination chemistry. Schiff bases and their complexes have excellent applications in several disciplines, such as antibacterial, antifungal, anti-oxidative, anti-inflammatory, antitumor, anticancer, anti-HIV, chemical sensor, therapeutic, optical materials, analytical, and electrochemistry [5-8].

Schiff bases, which are formed via the reaction of isoniazid with organic aldehydes or ketones, are an important family of chelating ligands, and their metal complexes with transition metal ions are of particular interest owing to their importance in biological, industrial, chemical, and pharmaceutical applications. Isoniazid is a medicine with the established therapeutic value that is used to treat a broad range of bacterial infections,

including TB, and leprosy [9]. Schiff bases generated from the reaction of pyridine ring containing aldehydes with isoniazid demonstrate stronger antitubercular efficacy than isoniazid [10]. The origin of biological properties of isoniazid-based Schiff bases and their metal complexes is due to the present of (-CO-NH-N=C) unit in their structures. The chelating compounds of aryl hydrazones (such as semi-carbazones, thiosemicarbazones etc.) have been shown to function as enzyme inhibitors and are valuable owing to their pharmacological applications [11-13]. It is well known that metal complexes obtained from isoniazid-based Schiff bases exhibit greater antibacterial activity than isoniazid as well as free ligands. The anti-oxidant action of the Schiff base is further improved when it is coordinated with transition metal ions [14].

Nowadays, multidrug-resistant tuberculosis, also known as MDRTB, is a well-known illness. This kind of tuberculosis is caused by an organism that is resistant to both isoniazid and rifampin. These two medications are well-known owing to their widespread usage in the treatment of tuberculosis patients. Therefore, to search a novel drug based on isoniazid is an important research task.

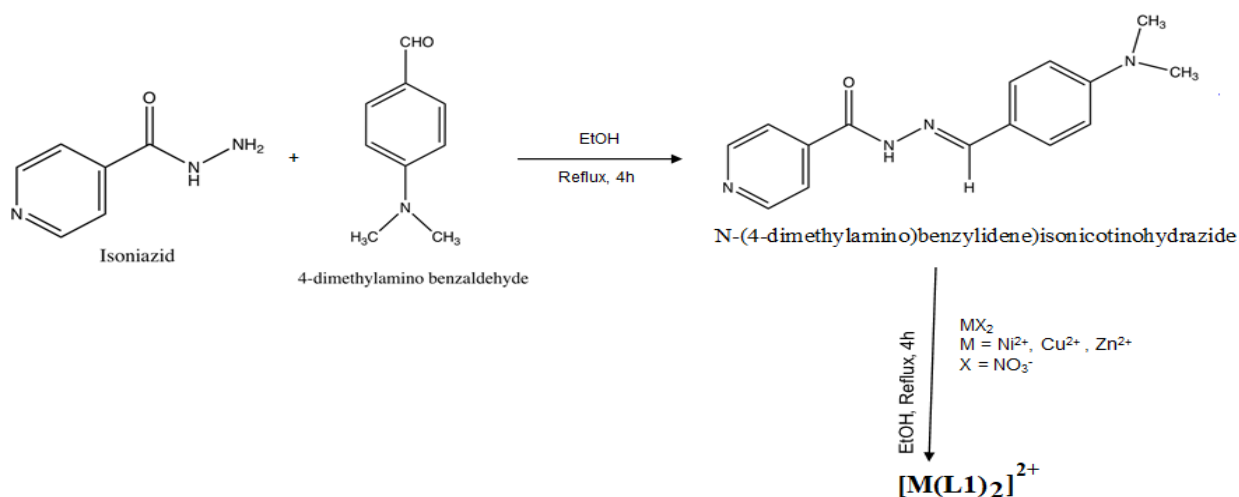
In continuation of our work on isoniazid-derived Schiff bases and metal complexes, we will present here the synthesis, characterization, antibacterial, and antioxidant activities of two isoniazid-based Schiff bases and their metal complexes with some divalent transition metal ions.

## **2. Experimental Methods**

All required chemicals with 99.9% purity were purchased from Merck and Loba chemicals. A METTLER PM 200 electronic balance was used to complete the weighing process. All produced metal complexes' melting or decomposition temperatures were measured using an electrothermal melting point equipment type o.AZ6512. The IR spectra of the produced compounds were acquired using a KBr disc on an FTIR-8400, SHIMADZU, Japan. The complexes' conductivities were measured in DMSO using a Horiba conductivity meter B173 with a set cell constant. The Uv-Vis spectra of the ligand and its complexes were measured using a THERMOELECTRON NICOLET evolution 300 UV-Visible spectrophotometer in DMSO solution ( $1 \times 10^{-5}$  M). Sherwood Scientific Magnetic Susceptibility Balance was used in order to determine the values of all complexes' magnetic moments. Thin Layer Chromatography (TLC) was used to assess the ligand's purity and its metal complexes.

### 2.1. a. Synthesis of N-(4-Dimethylamino)benzylidene)isonicotinohydrazide Schiff base Ligand (L1)

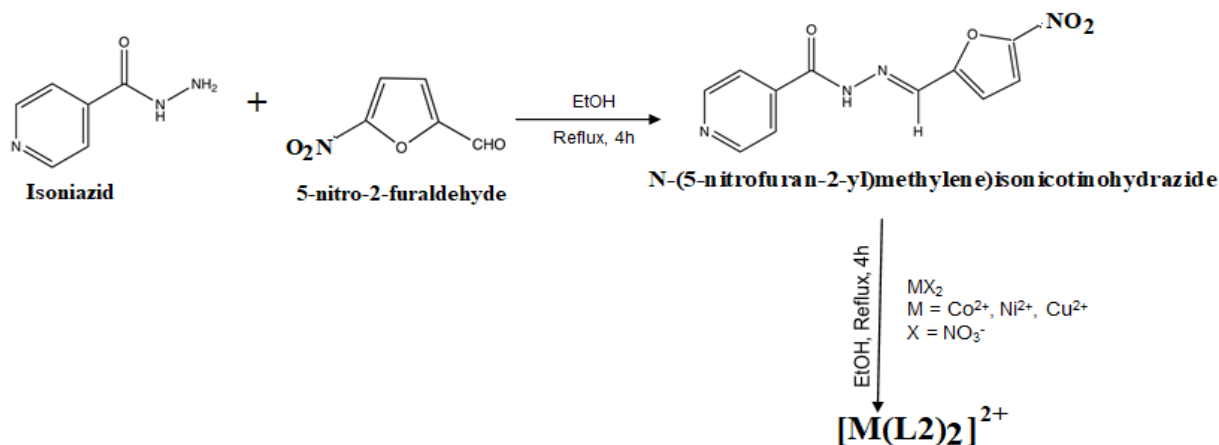
N-(4-dimethylamino)benzylidene)isonicotinohydrazide, Schiff base Ligand, L1 was synthesized by the conventional condensation reaction (Scheme 1) of 4-(N,N-dimethylamino)benzaldehyde with isoniazid (INH) in an equimolar ratio (i.e., 1:1). An ethanolic solution of INH (1.37 g, 10.00 mmol) was taken in a round bottom flask, and then ethanolic solution of 4-(N, N-dimethylamino)benzaldehyde (1.49 g, 10.00 mmol) was added to it with continuous stirring. As a catalyst, little amount of glacial acetic acid (2-3 drops) was added dropwise to this mixture. The resultant mixture was allowed to reflux for about 4 hours. TLC monitored the purity of the obtained product throughout the whole reaction. A yellow precipitate of the ligand was produced, which was then filtered and washed several times with cold  $\text{CH}_3\text{CH}_2\text{OH}$  solution before being dried in a desiccator over anhydrous  $\text{CaCl}_2$ .



Scheme 1: Synthesis of the Schiff base ligand, L1 and its metal complexes

### 2.1. b. Synthesis of N-(5-nitrofuranyl)methylene)isonicotinohydrazide Schiff base Ligand (L2)

N-(5-nitrofuranyl)methylene)isonicotinohydrazide, Schiff base (L2) was prepared according to the above procedure (scheme 2) from the equimolar ratio of isoniazid and 5-nitro-2-furfuraldehyde. A pale-yellow precipitate was obtained.



Scheme 2: Synthesis of the Schiff base ligand, L2 and its metal complexes

## 2.2. General Procedure for the Synthesis of Metal Complexes with L1 and L2 ligands

15 mL warm ethanolic solutions (1 mmol) of nitrate salts of metal ( $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Cu}^{2+}$ ) were slowly added to the warm ethanolic solution (15 mL) of Schiff base ligand L1 (2 mmol) in a reflux set. The resulting mixture was refluxed for about 4 h. After cooling, the obtained precipitates were filtered and washed with cold  $\text{C}_2\text{H}_5\text{OH}$  solution and dried under a vacuum on anhydrous  $\text{CaCl}_2$ .

Metal complexes of  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Cu}^{2+}$  with L2 ligand have synthesized by following the above procedure. Each complex has a distinct color, is soluble in DMF and DMSO but insoluble in common polar solvents.

## 2.3 Antibacterial Studies

Antimicrobial activity of the L1 and L2 ligands and their metal complexes with  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Zn}^{2+}$  ions were performed against *S. aureus*, and *E. coli* in DMSO by disc diffusion approach [15-16]. All pathogenic bacteria under this study were collected from the Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh.

## 2.4 Antioxidant Studies

The DPPH free radical scavenging technique was used to calculate antioxidant activity. The inhibition percentage, which was associated with the compounds' radical scavenging activity, was calculated by using the following formula [17]:

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Absorbance of the blank} - \text{Absorbance of the tested sample}}{\text{Absorbance of the blank}} \times 100$$

Calculation of IC<sub>50</sub> values: In order to get the IC<sub>50</sub> value, a linear regression was performed between the percentage inhibition and log concentration. Higher antioxidant activity is indicated by a lower IC<sub>50</sub> value [18].

### 3. Results and Discussion

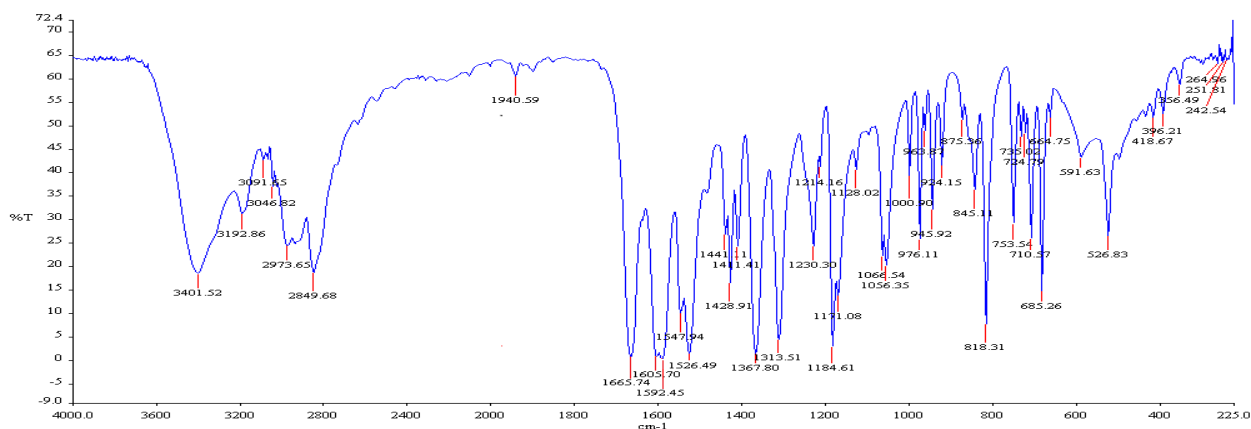
The physical properties of the L1 and L2 and their metal complexes have shown in **Table 1**. The molar conductance values of the complexes lie in the range of 121-128 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup> demonstrating their 1:2 electrolytic character [19-20]. The magnetic moments of the CuL1 and CuL2 complexes were found to be in the range of 1.87-1.90 B.M, confirming the square planar shape of the Cu<sup>2+</sup> complexes. On the other hand, these values for the obtained NiL1 and NiL2 complexes were determined to be 0.41 BM and 0.42 BM, respectively, which supports the diamagnetic character of the Ni<sup>2+</sup> complexes. All synthesized Zn<sup>2+</sup> complexes were also diamagnetic due to the d<sup>10</sup> electronic configuration of the Zn<sup>2+</sup> ions [21-24].

**Table 1: Physical properties of the ligands (L1 and L2) and their metal complex**

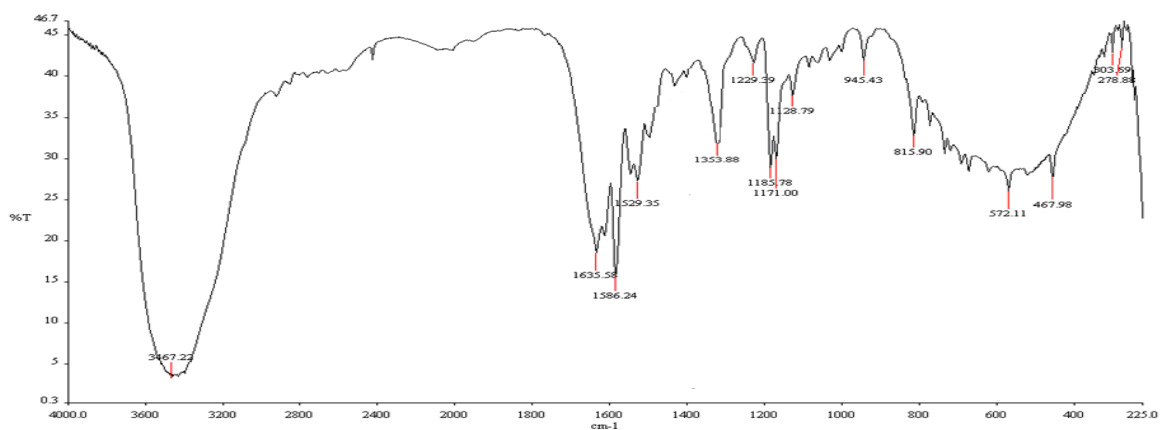
Ligand/Complexes (Symbol)	Appearance and body color	M.W	% Yield	M.P/De (°C)	Molar Conductivity (ohm <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup> )	μ <sub>eff</sub> (B.M)
C <sub>15</sub> H <sub>16</sub> N <sub>4</sub> O (L1)	Yellow powder	268.30	82	205		
C <sub>11</sub> H <sub>8</sub> N <sub>4</sub> O <sub>4</sub> (L2)	Pale Yellow powder	260.20	78	185		
[Ni(L1) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> (NiL1)	Deep Red	719.50	71	>300	121	Dia
[Ni(L2) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> (NiL2)	Red	703.30	70	269	128	Dia
[Cu(L1) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> (CuL1)	Brown	724.10	73	>300	125	1.90
[Cu(L2) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> (CuL2)	Brown	707.90	74	271	121	1.87
[Zn(L1) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> (ZnL1)	White	726.00	60	>300	128	Dia
[Zn(L2) <sub>2</sub> ](NO <sub>3</sub> ) <sub>2</sub> (ZnL2)	White	709.80	71	262	123	Dia

### 3.1 IR Spectral Studies

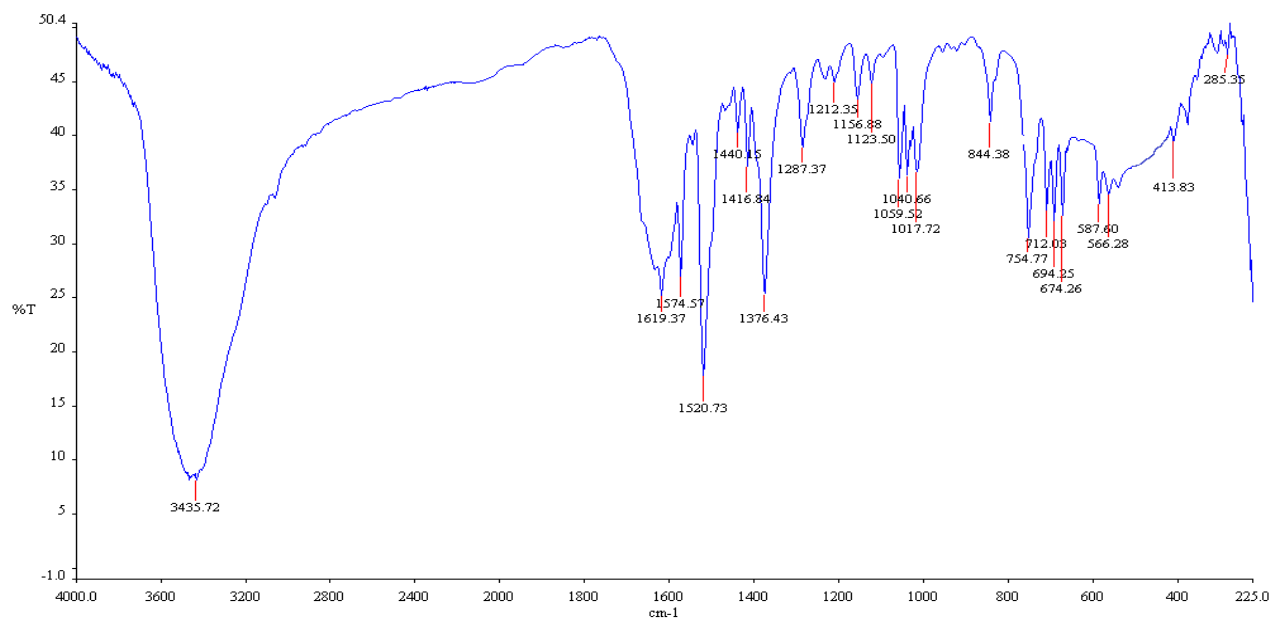
The bands at  $(1605) \text{ cm}^{-1}$  and  $(1620) \text{ cm}^{-1}$  in the IR spectra, which are caused by the stretching vibration of the azomethine groups (C=N) of the L1 and L2 ligands, were shifted to a lower frequency in the spectra of the  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ni}^{2+}$  complexes. This proved that the azomethine group was involved in the coordination through its nitrogen atom (N) [25]. All metal complexes obtained from L1 and L2 ligands display  $\nu(\text{C}=\text{O})$  band at  $1619\text{-}1660 \text{ cm}^{-1}$ , which is lower than the free ligands value. This clearly suggests the coordination of the carbonyl group with the metal ions through its oxygen atom. New bands around  $(523\text{-}595) \text{ cm}^{-1}$  and  $(413\text{-}497) \text{ cm}^{-1}$  detected in the low-frequency area for all complexes (generated from the L1 and L2 ligands) correspond to the frequency of (M-O) and (M-N), respectively [26-27]. All of the IR spectra data for the two ligands (L1 and L2) and their metal complexes are shown in **Table 2** and **Figures 1-8**.



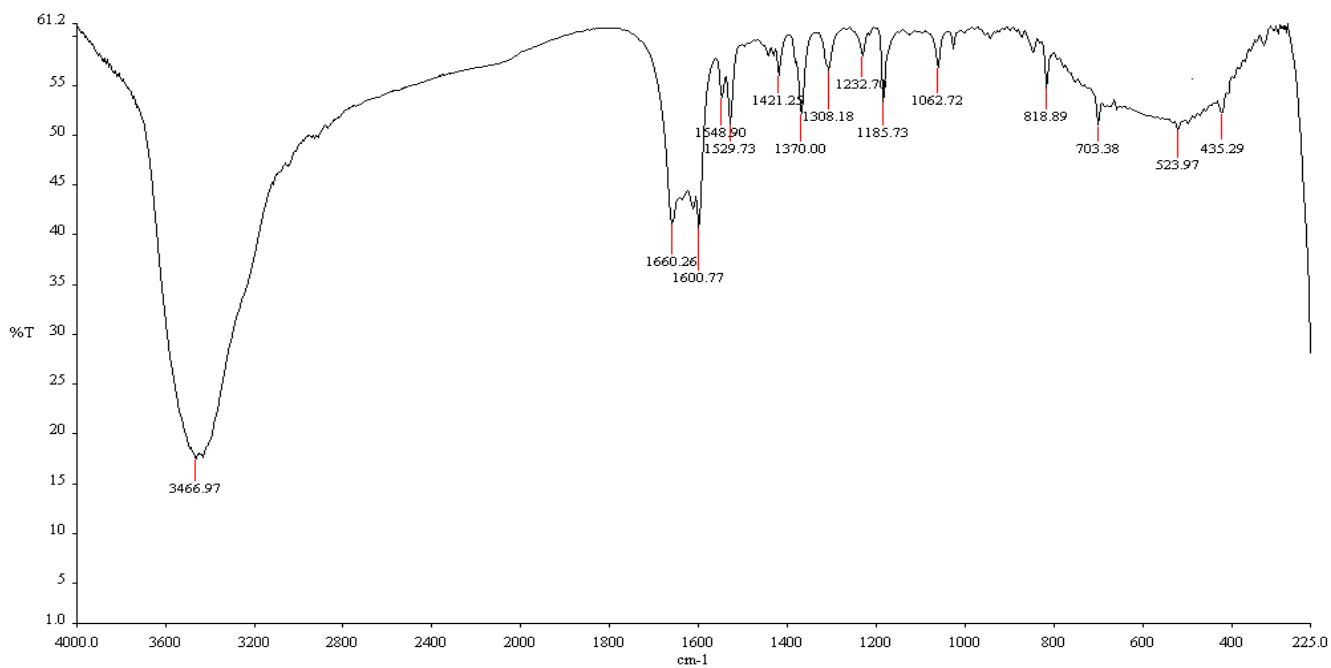
**Figure 1:** IR spectrum of L1



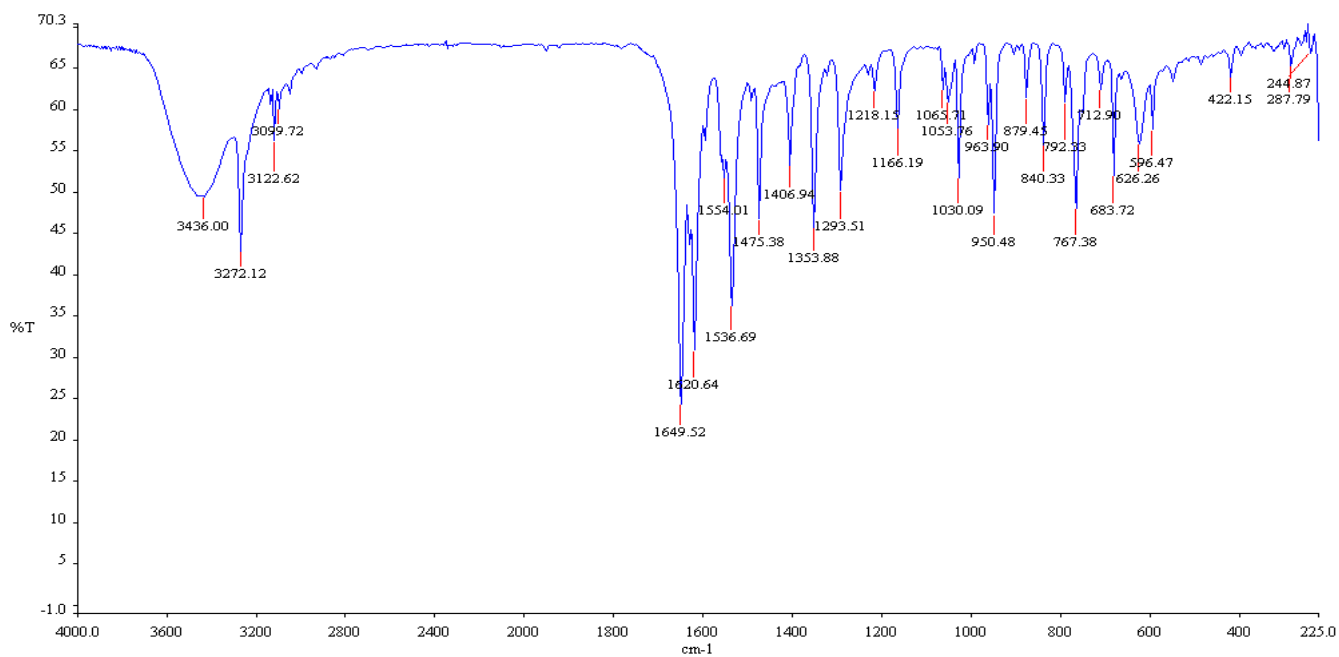
**Figure 2: IR spectrum of NiL1**



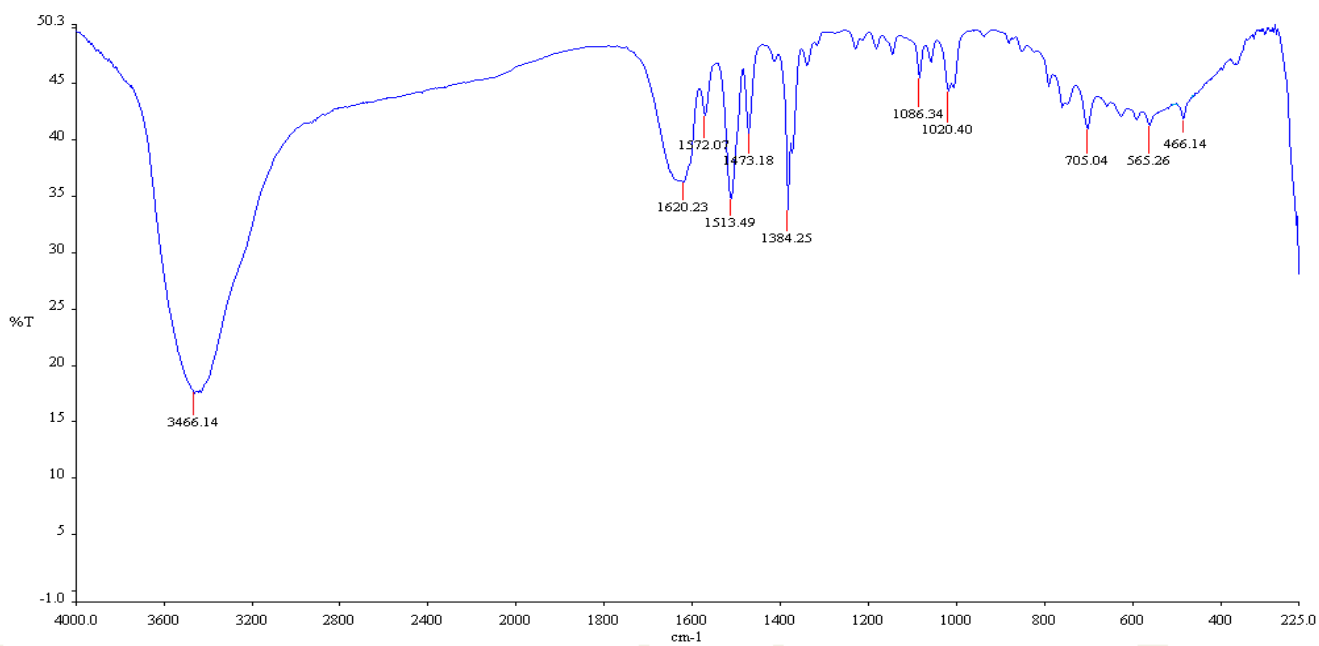
**Figure 3: IR spectrum of CuL1**



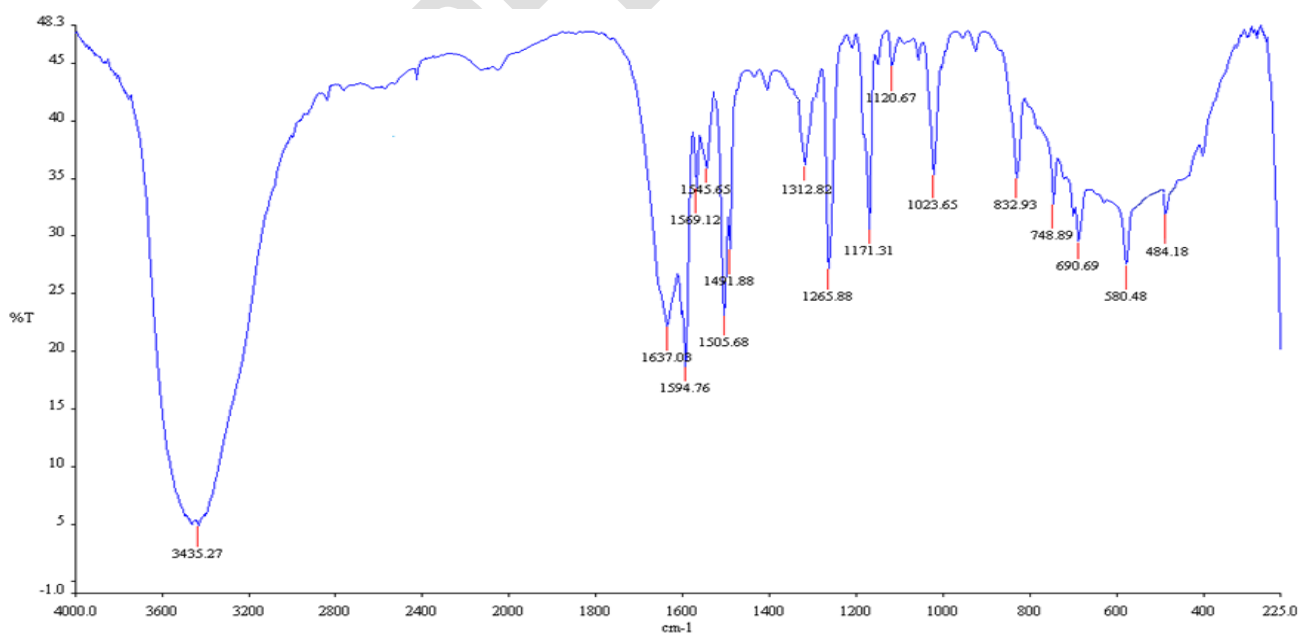
**Figure 4: IR spectrum of ZnL1**



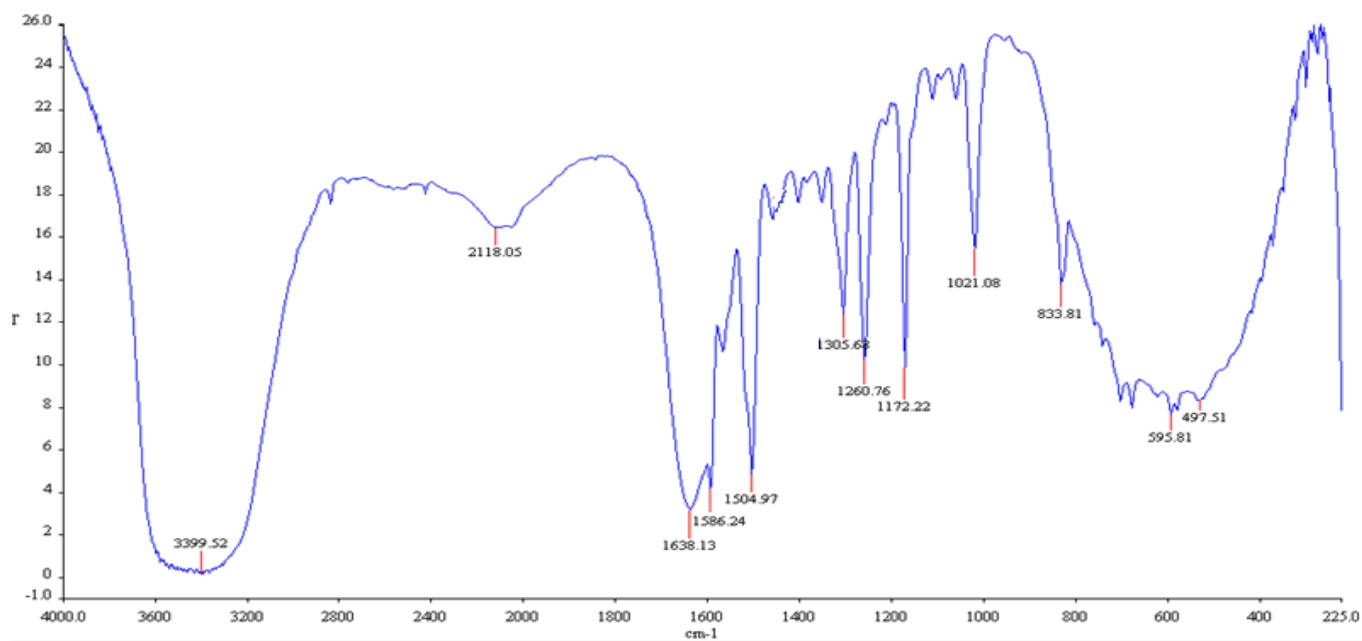
**Figure 5: IR spectrum of L2**



**Figure 6:** IR spectrum of NiL2



**Figure 7:** IR spectrum of CuL2



**Figure 8:** IR spectrum of ZnL2

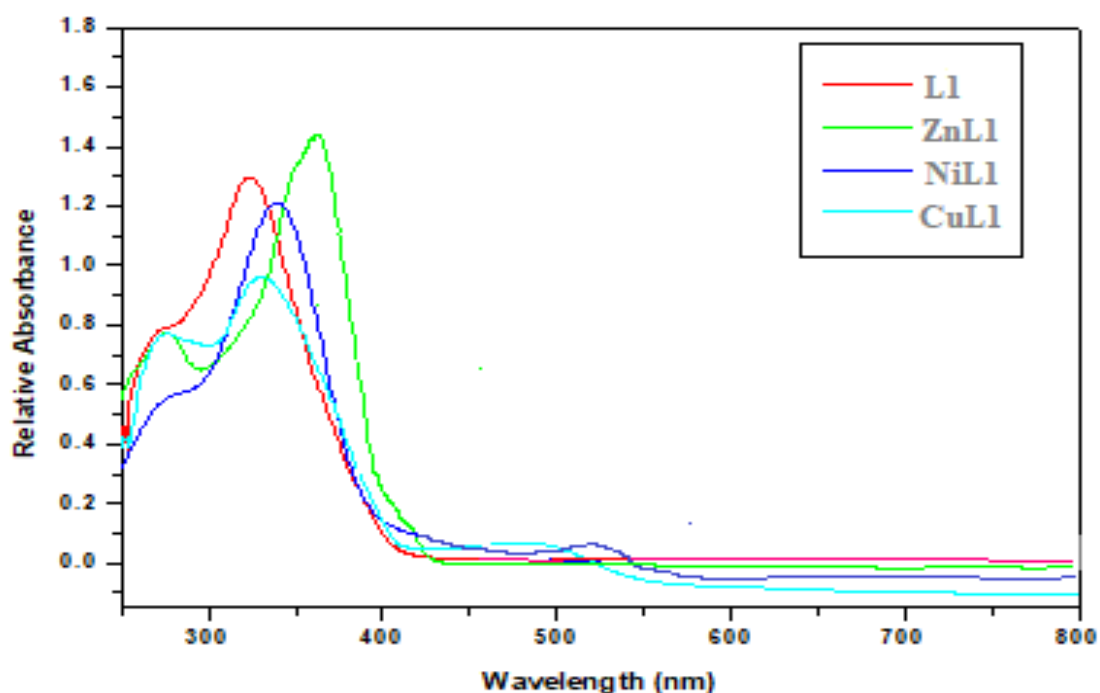
**Table 2:** Key Infrared bands ( $\text{cm}^{-1}$ ) of the ligands (L1 and L2) and their metal complexes

Ligand/ Complexes	$\nu(\text{NH})$	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{M}-\text{O})$	$\nu(\text{M}-\text{N})$
<b>L1</b>	<b>3192</b>	<b>1665</b>	<b>1605</b>	--	--
<b>L2</b>	<b>3272</b>	<b>1649</b>	<b>1620</b>		
<b>NiL1</b>	<b>3467</b>	<b>1635</b>	<b>1586</b>	<b>572</b>	<b>467</b>
<b>NiL2</b>	<b>3466</b>	<b>1620</b>	<b>1572</b>	<b>565</b>	<b>466</b>
<b>CuL1</b>	<b>3435</b>	<b>1619</b>	<b>1574</b>	<b>566</b>	<b>413</b>
<b>CuL2</b>	<b>3435</b>	<b>1637</b>	<b>1594</b>	<b>580</b>	<b>484</b>

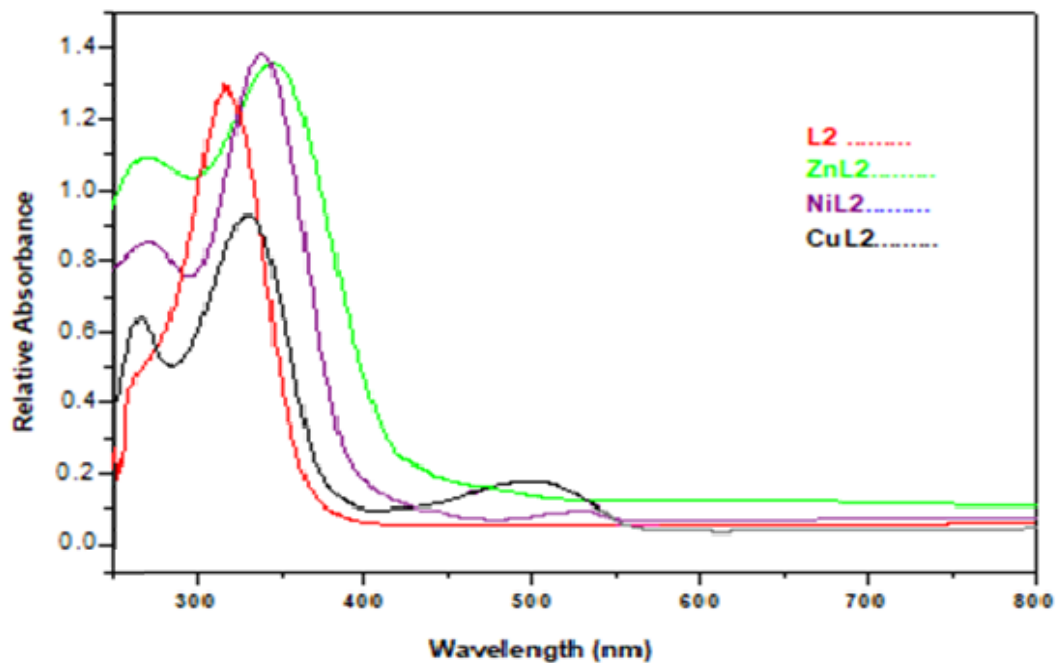
<b>ZnL1</b>	<b>3466</b>	<b>1660</b>	<b>1600</b>	<b>523</b>	<b>435</b>
<b>ZnL2</b>	<b>3399</b>	<b>1638</b>	<b>1586</b>	<b>595</b>	<b>497</b>

### 3.2 Electronic Spectra

The L1 ligand showed two distinct bands at 274 and 323 nm, which were for  $\pi-\pi^*$  and  $n-\pi^*$  transitions, respectively. Like L1 ligand, all complexes showed these two transitions. The band due to  $n-\pi^*$  transition was observed at a longer wavelength which could be due to the involvement of the azomethine group in chelation. The CuL1 and NiL1 complexes exhibit bands at 490 nm and 525 nm. The band located at 490 nm for the CuL1 complex is due to the  ${}^2B_{1g} \rightarrow {}^2E_g$  transition whereas the band positioned at 525 nm for the NiL1 complex is for the  ${}^1A_{1g} \rightarrow {}^1A_{2g}$  transition [28-29]. The magnetic susceptibility together with UV-Vis spectra supports the square planar geometry around the CuL1 and NiL1 complexes [21-22]. On the other, the ZnL1 showed a CT band at 374 nm. Like L1, the L2 ligand also exhibits two bands at 266 nm and 330 nm, which are due to  $\pi-\pi^*$  and  $n-\pi^*$  transitions. All complexes derived from the L2 ligand also showed the same transitions as the L1 derived metal complexes. But the charge transfer (CT) band near at 374 nm was not observed in the case of the ZnL2 complex. However, all characterizations mainly FT-IR, UV-Vis and magnetic susceptibility measurements support the tetrahedral environment of the  $Zn^{2+}$  complexes [23-24]. All bands with proper assignments have shown in **Table 3** and **Figures 9-10**.



**Figure 9:** Uv-Vis spectra of L1 and its metal complexes



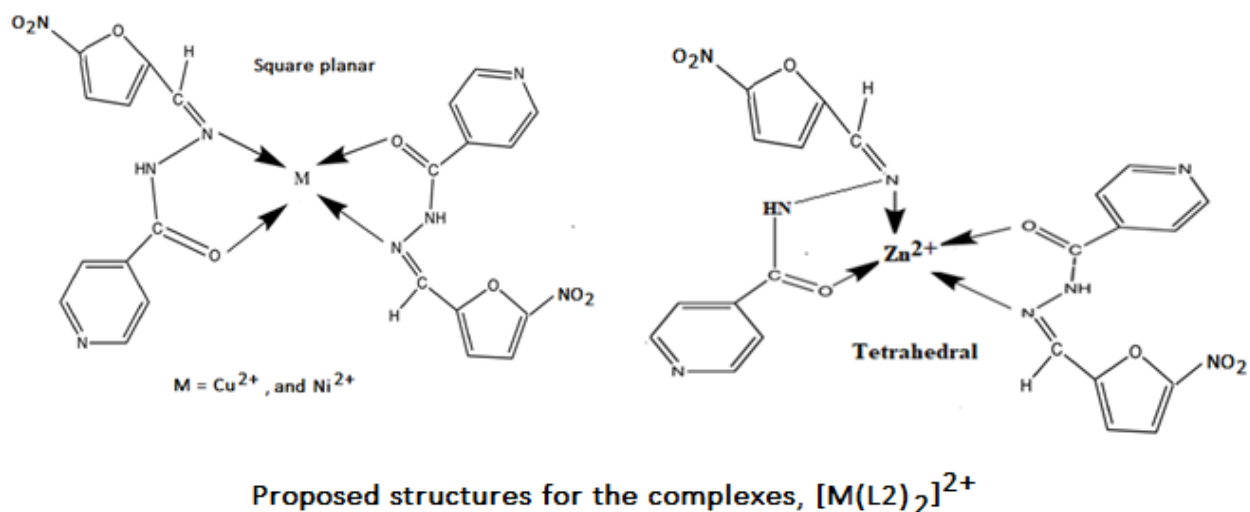
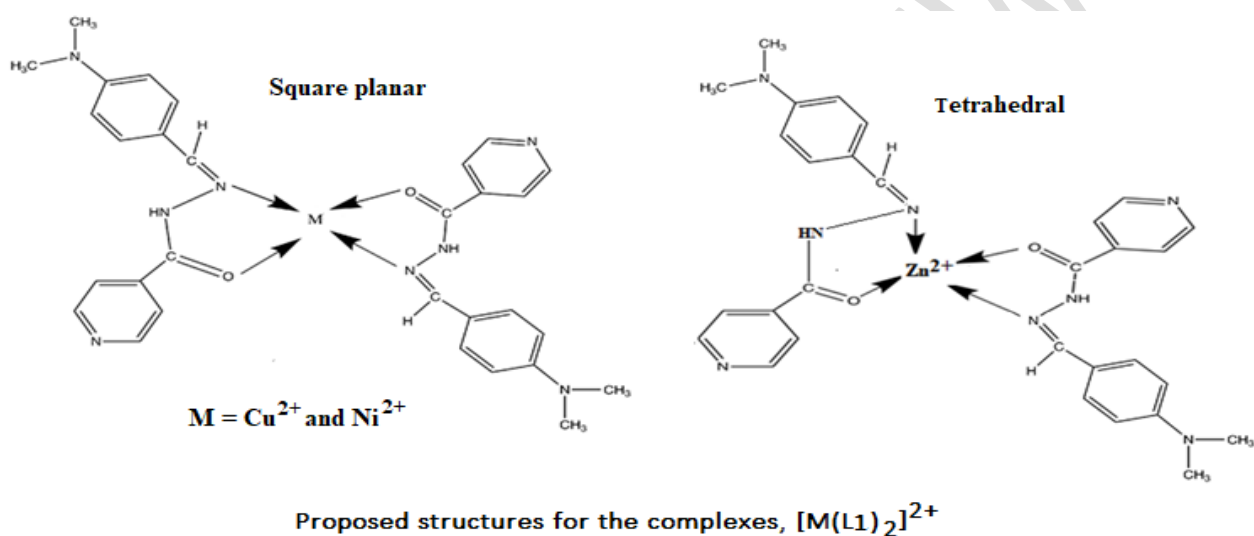
**Figure 10:** Uv-Vis spectra of L2 and its metal complexes

**Table 3:** Key UV bands (nm) of ligands (L1 and L2) and their metal complexes

Ligand/ Complexes	Band Position (nm)	Assignment	$\mu_{\text{eff}}$ (BM)	Geometry
L1	274 323	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$		
L2	266 330	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$		
NiL1	280 340 525	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ $1A_{1g} \rightarrow 1A_{2g}$	Dia	Square planar
NiL2	271 338 535	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ $1A_{1g} \rightarrow 1A_{2g}$	Dia	Square planar
CuL1	276 330 490	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ $^2B_{1g} \rightarrow ^2E_g$	1.90	Square planar
CuL2	270 338	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	1.87	Square planar

	<b>500</b>	${}^2B_{1g} \rightarrow {}^2E_g$		
<b>ZnL1</b>	<b>276</b> <b>353</b> <b>374</b>	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ CT	<b>Dia</b>	<b>Tetrahedral</b>
<b>ZnL2</b>	<b>270</b> <b>345</b>	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	<b>Dia</b>	<b>Tetrahedral</b>

**Proposed Structure:** On the basis of the above characterizations, the following structures can be proposed for the produced metal complexes:



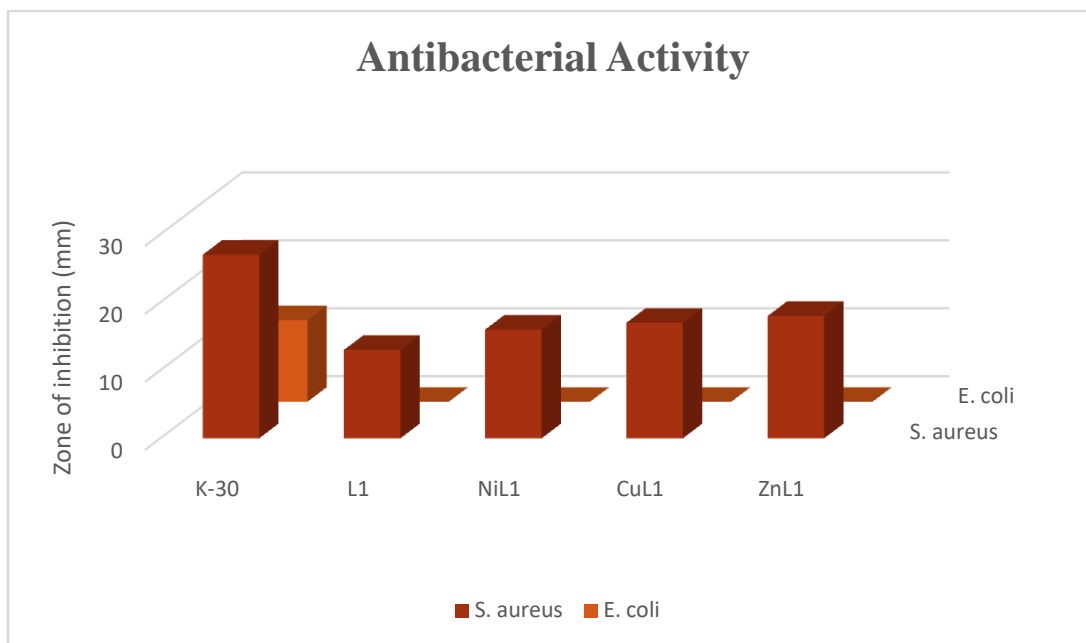
### 3.3. Antibacterial Activity

The antibacterial activity of the two ligands (L1 and L2) and their metal complexes against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) was investigated. The diameter of the inhibitory zone was measured in millimeters, and the results of their antibacterial activity have presented in **Table 4**. The ligand L1 and its metal complexes did not show any zone of inhibition against *E. coli* (**Figure 11**) whereas the L2 and its metal complexes showed good activity against *E. coli* and the following trend is observed: Kanamycin-30 > CuL2 > ZnL2 > NiL2 > L2 (**Figure 12**). On the other hand, both ligands and their metal complexes showed good activity against *S. aureus*. The ZnL1 complex among all metal complexes synthesized from the L1 ligand, showed the highest antibacterial activity against *S. aureus*. In the case of the L2 derived metal complexes, the CuL2 complex exhibited better antibacterial activity against *S. aureus* than that of Kanamycin-30. The following trend is observed: CuL2 > Kanamycin-30 > NiL2 > ZnL2 > L2. Several ideas have been proposed to explain the biological activity of different transition metal coordination complexes. Increased antibacterial activity of metal (II) complexes may be a result of the metal ion's influence on the normal condition of the bacterial cell process. Several studies have revealed that the structural components with extra (C=N) bonds to nitrogen and oxygen donor systems decrease enzyme activity because of metal coordination. Chelation increases the lipophilicity of the central metal ion, hence enhancing the hydrophobicity and liposolubility of the complex. This makes it more likely that the complex substance will penetrate the lipid layers of the cell membrane of the microorganisms. As soon as the complexes enter the cell, they begin to obstruct the metal-binding sites that are present in the enzymes of the bacteria. In turn, this disrupts the cell's respiration process by inhibiting protein synthesis, which limits the organism's growth [30-34].

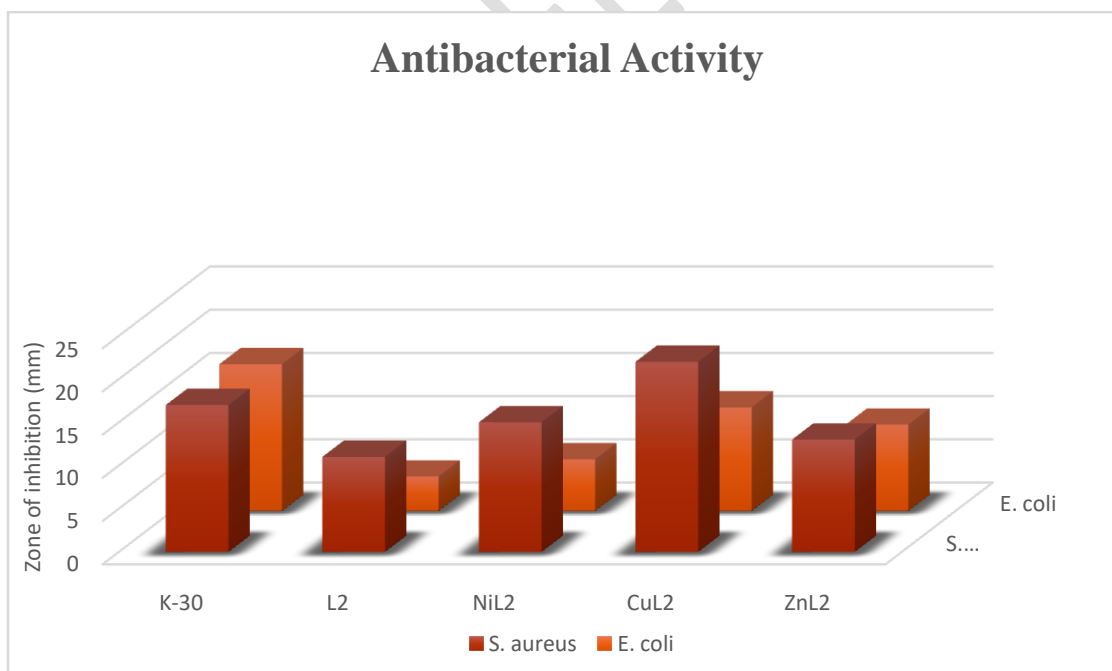
**Table 4: Antibacterial activities of the two ligands (L1 and L2) and their metal complexes**

Diameter of Zone of Inhibition (mm) of tested compounds (100µg/disc)		
Compounds	Gram Negative	Gram Positive
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Kanamycin (30 µg/disc)	12	27
<b>Ligand (L1)</b>	-ve	13
NiL1	-ve	16
CuL1	-ve	17
ZnL1	-ve	18
<b>Ligand (L2)</b>	04	11
NiL2	06	15
CuL2	12	22

ZnL2	10	13
Kanamycin (30 µg/disc)	17	17



**Figure 11:** Graphical representation of antibacterial activity of ligand L1 and its metal complexes against mentioned bacteria with standard Kanamycin-30 (K-30).



**Figure 12:** Graphical representation of antibacterial activity of ligand L2 and its metal complexes against mentioned bacteria with standard Kanamycin-30 (K-30).

### 3.4 Antioxidant activity

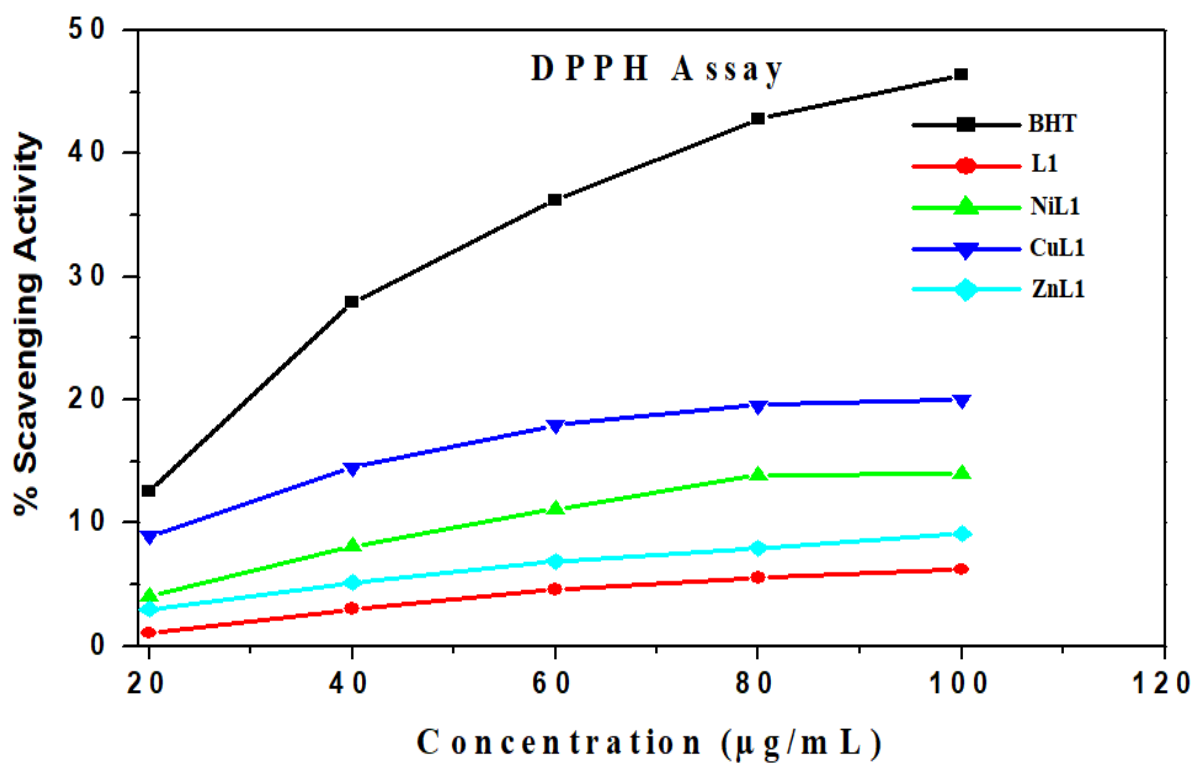
The antioxidant properties of L1 and its metal ion complexes were evaluated using the free radical molecule 1, 1-Diphenyl-1-picryl hydrazyl (DPPH). **Table 5** displays the percentage of DPPH radical scavenging activity of L1, metal complexes, as well as BHT (butylated hydroxytoluene) as a reference. According to the findings, all of the metal complexes showed moderate DPPH radical scavenging activity (**Figures 13-14**). The order can be expressed as BHT > CuL1 > NiL1 > ZnL1 > L1. Same trend was also observed in the case of the metal complexes derived from the L2 ligand (**Table 6**). Both cases the Cu<sup>2+</sup> complexes have higher antioxidant activity than other synthesized complexes. DPPH scavenging activity of the metal complexes obtained from the ligand (L1 or L2) is much greater than that of the free ligand (L1 or L2), suggesting that complexes are good free radical scavengers and antioxidants than free ligand (L1 or L2) but lower when compared to standard BHT. The radical scavenging activity of the standard BHT, as well as the metal complexes, was shown to increase in a dose-dependent way. The chelation of the ligands with transition metal ions resulted in a considerable enhancement in the antioxidant capabilities of the ligands. The oxidizing potentials (OP) of the complexes are associated with the presence of chemicals that exert their effects by breaking the chain of free radicals via the contribution of hydrogen atoms [31, 35]. So, the outcomes of this study imply that the CuL1 and CuL2 complexes may be employed to treat pathological illnesses induced by oxidative stress. The IC<sub>50</sub> value of all mentioned compounds in combination with standard BHT has been calculated and shown in **Tables 5-6**.

**Table 5: % of Scavenging activity of BHT, ligand L1, and its metal complexes**

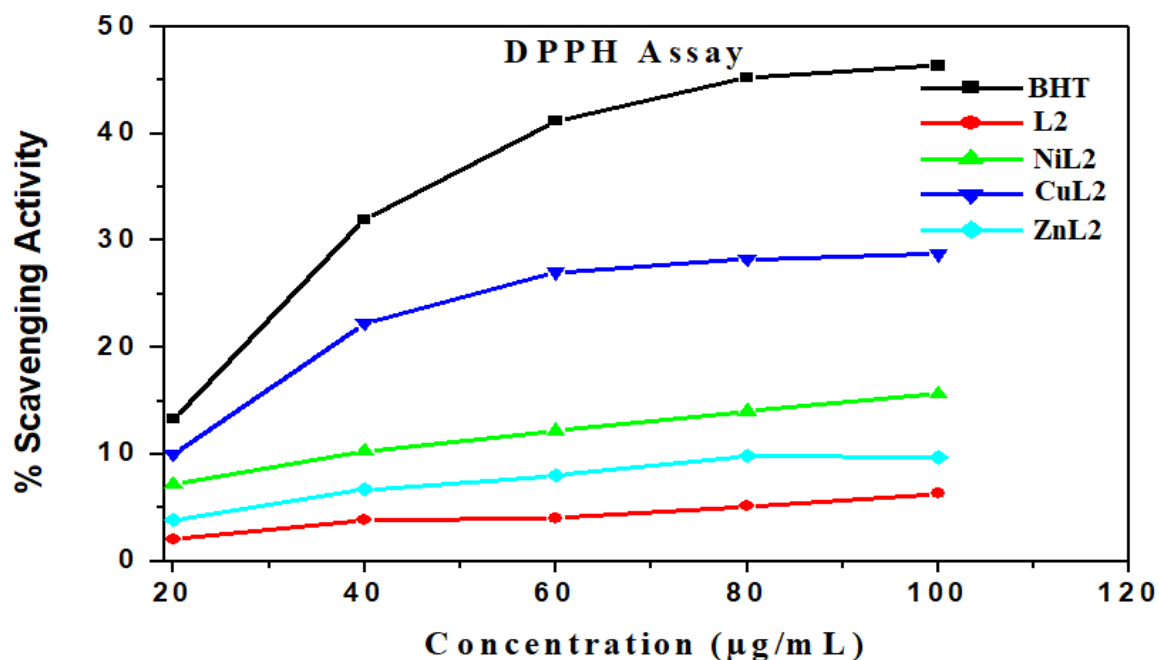
Conc. (µg/mL)	% BHT	% L1	% NiL1	% CuL1	% ZnL1
20	12.54	1.04	4.03	8.96	3.01
40	27.88	2.99	8.11	14.54	5.15
60	36.21	4.61	11.14	17.93	6.89
80	42.76	5.54	13.89	19.56	7.95
100	46.38	6.21	14.04	20.00	9.11
IC <sub>50</sub> Value:	100.81	771.92	368.20	309.46	641.04

**Table 6: % of Scavenging activity of BHT, ligand L2, and its metal complexes**

Conc. (µg/mL)	% BHT	% L2	% NiL2	% CuL2	% ZnL2
20	13.29	2.03	7.21	10.02	3.80
40	31.97	3.88	10.29	22.25	6.71
60	41.20	4.03	12.23	27.03	8.00
80	45.23	5.16	14.03	28.26	9.86
100	46.36	6.29	15.65	28.75	9.73
IC <sub>50</sub> Value:	96.25	993.10	429.72	182.98	624.27



**Figure. 13:** DPPH radical scavenging activity of the L1 and its metal ions complexes at different concentrations (20, 40, 60, 80, and 100  $\mu\text{g/mL}$ ) with standard BHT.



**Figure. 14:** DPPH radical scavenging activity of the L2 and its metal ions complexes at different concentrations (20, 40, 60, 80, and 100  $\mu\text{g/mL}$ ) with standard BHT.

#### 4. Conclusion

Isoniazid based two bidentate Schiff base ligands (L1 and L2) and their divalent metal complexes were successfully synthesized and also characterized. The L1 and L2 ligands coordinated to the central metal (II) ions through carbonyl oxygen (O) and azomethine nitrogen (N) donor atoms. No inhibition zone was observed in the case of the L1 ligand and its metal complexes against *E. coli* (Gram negative). But all complexes showed good antibacterial activity against *S. aureus*. The ZnL1 complex among all metal complexes synthesized from the L1 ligand, showed the highest antibacterial activity against *S. aureus*.

On the other hand, the CuL2 complex exhibited better antibacterial activity against *S. aureus* than that of Kanamycin-30. For the both ligands, the Cu<sup>2+</sup> complexes exhibited the highest antioxidant efficacy. Before considering copper complexes as potentially useful antibacterial treatments for people, there has to be sufficient research conducted on the toxicity of these compounds to humans.

## 5. References

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