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# The Bivalent Cu, Ni and Zn Complexes of Unsymmetrical ONO Tridentate Schiff Base Ligands Derived from 2-Aminobenzoic Acid: Antimicrobial and Molluscicidal Activity

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## Abstract

Bivalent Cu(II), Ni(II) and Zn(II) complexes were synthesized using the prepared tridentate Schiff base ligands derived from 2-aminobenzoic acid as the primary amine condensed with different substituted aldehydes (salicylic acid, O-vaniline, 2-carboxybenzaldehyde, 1-(3-formyl-4-hydroxyphenylazo)-4-methylbenzene and 1-(3-formyl-4-hydroxyphenylazo) benzene). The ligands and their corresponding complexes have been characterized using analytical, spectroscopic, conductivity and magnetic moment measurements. The analytical data showed the stoichiometry of the metals to ligands was (1:1). The conductivity data showed that all the complexes were non-electrolytes. The geometry of Cu(II) complexes were suggested to be distorted square planer. Ni(II) complexes are square-planar and Zn(II) complexes were tetrahedral. The antimicrobial investigation showed that the metal complexes had remarkable enhancement of activity with zinc complexes and were having highest susceptibility. The complex  $[ZnSL_4 \cdot H_2O]$  showed MIC value of 12.5  $\mu\text{g}/\text{disc}$  against *Candida albicans* and the complex  $[ZnSL_5 \cdot H_2O]$  indicated MIC value of 50  $\mu\text{g}/\text{disc}$  against *Streptococcus pneumoniae*. The molluscicidal activity tests against the land snail *Eobania vermiculata* showed that complexes were more toxic with LC 50 of 0.4 mg/L, while the LC 50 of the ligands was at 0.5 mg/L.

**Keywords:** Schiff base; Tridentate ligands; Metal complexes; Antimicrobial activity; Molluscicidal activity

presence of CH=N group that contain nitrogen atom as donor site is critical factor in the chemical properties, stability and biological activities of these ligands and their corresponding metal complexes [1,2]. Moreover, the presence of other donating groups like -OH, COOH or others adjacent to the azomethine group in the Schiff base ligands made them versatile multidendate ligands complexing with almost all metal ions and considered an additional factor of stability [3,4]. The rich coordination modes are considered important intermediates for the synthesis of different bioactive materials and for mimicking biological systems [5,6].

There are interesting research work on the compounds derived from substituted amino benzoic acids and their metal complexes [7-9]. They have significant prostaglandin H<sub>2</sub> Synthase peroxidase (PGHS-POX) inhibitory activity [7]. Diazotized anthranilic acid was used as reagent for the spectrophotometric determination of ethyl acetoacetate in aqueous solution [10]. Oxovanadium complexes of mixed ligands containing tridentate Schiff base ligands derived from anthranilic acid have been reported and found to be potent *in vitro* inhibitors against protein tyrosine phosphatase (PTP) [11]. Recently ternary Cu(II) complexes with fluorinated anthranilic acid derivatives reported to have *in vitro* cytotoxicity against A 549 (human pulmonary carcinoma cells), Jurkat (human T lymphocyte cell line) and HepG-2 (human liver hepatocellular carcinoma cells) [12]. These compounds exhibited antibacterial activity, consistent with the DNA binding affinities.

Anthranilic acid as a core precursor used to synthesize a number of anti-inflammatory agents and many researchers evaluated the anti-inflammatory activities of metal complexes containing Schiff base ligands derived from anthranilic acid [13-15].

Metal complexes with azo-azomethine dye ligands are prepared by the condensation of o-amino benzoic acid with 5-phenyl azo-salicylaldehyde and have been reported to have an enhanced activity compared to the corresponding organic ligands [16]. Copper(II) coordination polymers of nanostructure were synthesized from azo ligands (5-(4-carboxy

## Introduction

Schiff bases as ligands provides a foundation stone for designing new coordination compounds that have extensive applications in different fields. The availability of different types of primary amines and carbonyl compounds enabled the synthesis of Schiff bases with diverse structural features. The

phenyl azo) anthranilic acid) through sono chemical process. The study indicated that these coordination polymers might be suitable precursors for synthesis of nanoscale materials that produce different and interesting morphologies [17].

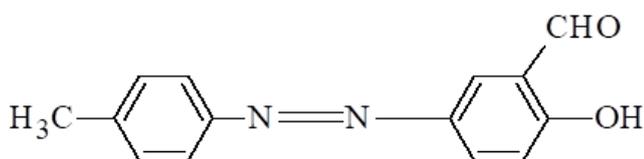
In view of the facts mentioned before, we encouraged and considered interesting to synthesize ligand compounds in a trial to develop a molecule combining different pharmacophore fragments in one structure, which may lead to interesting biologically active agents. The suggested ligands will be derived from 2-aminobenzoic acid as a primary amine and will be condensed with different aromatic aldehydes. The synthesized compounds will be investigated *in vitro* for their antibacterial and antifungal activities, in addition to the *in vivo* molluscicidal activity.

## Materials and Methods

### Chemicals

All the starting materials anthranilic acid (2-aminobenzoic acid), aldehydes, metal salts and solvents used in this research project were of analytical grade and were used in reactions as received without further purification. Azoaldehyde precursors were synthesized in our laboratory following well-known literature procedures and details are mentioned below (Figures 1 and 2).

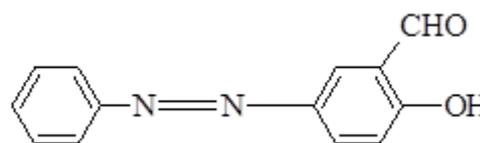
### Preparation of 1-(3-formyl-4-hydroxyphenylazo)-4-methylbenzene



**Figure 1** Structure of 1-(3-formyl-4-hydroxyphenylazo)-4-methylbenzene.

The procedure of preparation followed here is as reported [18]. Salicylaldehyde (2.44 g, 0.02 mol) was dissolved in water (30 mL) containing sodium hydroxide (0.80 g, 0.02 mol) and sodium carbonate (8.48 g, 0.08 mol) over a period of 30 min at temperature below 0°C. The resulting solution was added slowly to a diazonium solution of p-toluidine (2.14 g, 0.02 mol) in 26 mL hydrochloric acid/water solution (18 mL HCl and 8 mL Water) at a temperature of 0-5°C. The mixture was stirred for one hour at below 0°C and then allowed to warm slowly to room temperature. The precipitated product was collected by vacuum filtration and washed several times with 10% NaCl solution (~ 100 mL). The obtained reddish orange precipitated solid was dried in open air overnight and then at 45°C in oven. Yield obtained around 82%. Melting point measured is 143-145°C (reported 150°C [19], 142-144°C [20]).

### Synthesis of 1-(3-formyl-4-hydroxyphenylazo)benzene

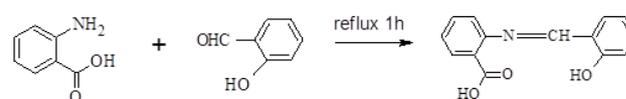


**Figure 2** Structure of 1-(3-formyl-4-hydroxyphenylazo)benzene.

The procedure of preparation followed here is as reported [18,21]. Salicylaldehyde (2.44 g, 0.02 mol.) was dissolved in water (30 mL) containing sodium hydroxide (0.80 g, 0.02 mol) and sodium carbonate (8.48 g, 0.08 mol) over a period of 30 min at temperature below 0°C. The resulting solution was added slowly to diazonium solution of aniline (1.86 g, 0.02 mol) in 26 mL hydrochloric acid/water solution (18 mL HCl and 8 mL water) at a temperature of 0-5°C with constant stirring. The mixture was stirred for one hour at temperature below 0°C and then allowed to warm slowly to room temperature. The precipitated product was collected by vacuum filtration and washed several times with 10% NaCl solution (~ 100 mL), then with distilled water and finally with ethanol. The obtained brownish orange precipitate solid was dried in open air overnight and then at 45°C in oven for nearly 5 hours. The product was recrystallized from hot ethanol. Yield obtained around 85%. Melting point measured is 114°C (reported 120°C) [19].

### Synthesis of schiff base ligands

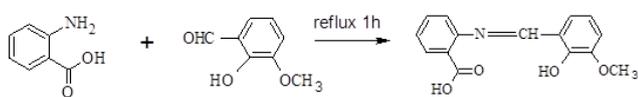
The organic Schiff base ligands generally were synthesized according to the standard reported methods as cited in literature [19,22,23]. They were prepared in (1:1) amine: aldehyde molar ratio. The calculated quantity of primary amine 2-aminobenzoic acid was dissolved in the proper solvent and mixed with the proper quantity of the aldehyde compound solution. The whole mixture was then refluxed with stirring for a proper time. The precipitated products were filtered, recrystallized and dried. The yields were calculated and melting points were measured and recorded for each prepared Schiff base ligand (**Scheme 1**). The details procedures for preparing different designed Schiff base are given in the following sub-sections.



**Scheme 1** Synthesis of schiff base ligands.

### Preparation of the Schiff base ligand 1-(2-carboxyphenylimino)-2-hydroxy-3-methoxybenzene (SL<sub>2</sub>)

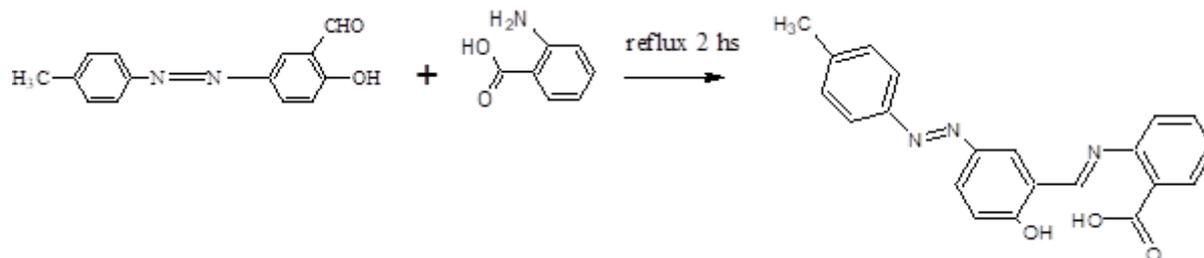
SL<sub>2</sub> ligand was prepared by adding (1.37 g, 0.01 mol) of 2-aminobenzoic acid dissolved in 25 mL hot ethanol to a solution of (1.52 g, 0.01 mol) 2-hydroxy-3-methoxy benzaldehyde in 25 mL hot ethanol taken in 100 mL round bottom flask. The mixture was refluxed with stirring for 1 hour. After cooling, the reddish orange precipitate produced was filtered and washed with cold ethanol and then with diethyl ether and air-dried. The product was finally recrystallized from ethanol to get pure solid product. Yield was about 80% and melting point was measured 97°C. The reaction for synthesis of SL<sub>2</sub> ligand is illustrated in the following **Scheme 2**.



**Scheme 2** Synthesis of 1-(2-carboxyphenylimino)-2-hydroxy-3-methoxybenzene.

### Preparation of the Schiff base ligand 1-(2-carboxyphenylimino)-2-hydroxy-3-methoxybenzene (SL<sub>3</sub>)

SL<sub>3</sub> ligand was prepared by adding (1.37 g, 0.01 mol) of 2-aminobenzoic acid dissolved in 25 mL hot ethanol to a solution of (1.5 g, 0.01 mol) 2-carboxy benzaldehyde in 25 mL hot ethanol. The mixture was refluxed with stirring for 1 hour.

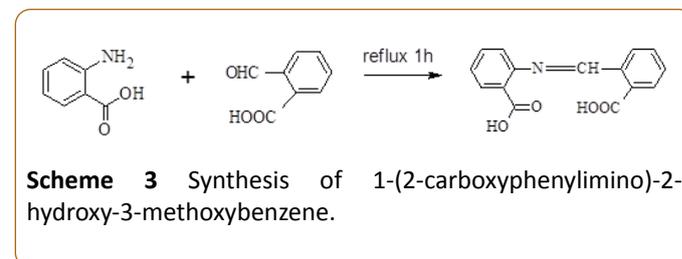


**Scheme 4** Synthesis of 1-(2-carboxyphenylimino)-1-(3-formyl-4-hydroxyphenylazo)-4-methylbenzene.

### Preparation of the Schiff base ligand 1-(2-carboxyphenylimino)-1-(3-formyl-4-hydroxyphenylazo) benzene (SL<sub>5</sub>)

SL<sub>5</sub> ligand was prepared by adding (1.37 g, 0.01 mol) of 2-aminobenzoic acid dissolved in 25 mL hot ethanol to a solution of (2.26 g, 0.01 mol) of the azoaldehyde [1-(3-formyl-4-hydroxyphenylazo)benzene] in 30 mL hot chloroform in 100

mL round bottom flask. The mixture was refluxed with stirring for 2 hours. After cooling, the dark orange precipitate produced was filtered and washed with ethanol, chloroform and finally with diethyl ether and was air-dried. The product was finally recrystallized from hot ethanol to get pure solid product, with yield was about 80% and melting point was measured 218°C. The reaction for synthesis of SL<sub>5</sub> ligand is illustrated in the following **Scheme 5**.

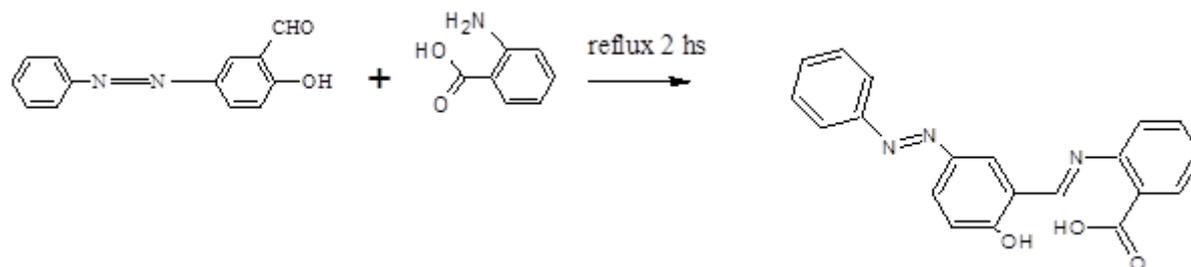


**Scheme 3** Synthesis of 1-(2-carboxyphenylimino)-2-hydroxy-3-methoxybenzene.

### Preparation of the Schiff base ligand 1-(2-carboxyphenylimino)-1-(3-formyl-4-hydroxyphenylazo)-4-methylbenzene (SL<sub>4</sub>)

SL<sub>4</sub> ligand was prepared by adding (1.37 g, 0.01 mol) of 2-aminobenzoic acid dissolved in 25 mL hot ethanol to a solution of (2.4 g, 0.01 mol) of the azoaldehyde [1-(3-formyl-4-hydroxyphenylazo)-4-methylbenzene] in 30 mL hot chloroform in 100 mL round bottom flask. The mixture was refluxed with stirring for 2 hours. After cooling the orange-brown precipitate produced was filtered and washed with ethanol, chloroform and finally with diethyl ether and was air-dried. The product was finally recrystallized from hot ethanol to get pure solid product with yield was about 70% and melting point was measured 288°C. The reaction for synthesis of SL<sub>4</sub> ligand is illustrated in the following **Scheme 4**.

mL round bottom flask. The mixture was refluxed with stirring for 2 hours. After cooling, the dark orange precipitate produced was filtered and washed with ethanol, chloroform and finally with diethyl ether and was air-dried. The product was finally recrystallized from hot ethanol to get pure solid product, with yield was about 80% and melting point was measured 218°C. The reaction for synthesis of SL<sub>5</sub> ligand is illustrated in the following **Scheme 5**.



**Scheme 5** Synthesis of 1-(2-carboxyphenylimino)-1-(3-formyl-4-hydroxyphenylazo) benzene.

### Template preparation of transition metal complexes

All targeted bivalent Cu, Ni and Zn metal complexes were prepared using same general reported procedure [2,19]. Calculated amount of the metals salt was dissolved in ethanol and mixed with the solution of the previously prepared Schiff bases (SL<sub>1</sub>-SL<sub>5</sub>) in 1:1 metal to ligand stoichiometric ratio in round bottom flask, and refluxed for 3 hours. Then the produced precipitated material was isolated as solid products, washed, recrystallized, dried and weighted. The yield, color, melting points were observed and noted.

### Instrumentation and physical measurements

In order to confirm the proposed structures of the prepared ligands and their corresponding metal complexes, different spectroscopic and analytical techniques were used. All the prepared organic ligands and their corresponding metal complexes were characterized by micro-elemental analysis using Thermo Fisher Scientific CHN/N/O analyzer (Leco Model VTF-900 CHN-S-O 932 version 1.3 x USA). The Mass spectral data was recorded on Thermo Scientific-LCQ fleet ion trap mass spectrometer using electrospray ionization (ESI) method.

IR spectra were recorded using Thermo Scientific Nicolet IS 50 FT-IR spectrometer in the range 400-4000 cm<sup>-1</sup> utilizing Attenuated Total Reflection (ATR) method for direct measurement of the IR spectrum for the powder solid samples.

For recording UV-visible spectra Evolution 300 UV-visible double beam Spectrophotometer was used and by dissolving the samples in DMSO solvent.

Nuclear Magnetic Resonance (<sup>1</sup>H NMR) spectra was obtained using Varian Mercury-400 BB (400 MHz) spectrometer operating at 400 MHz frequency for <sup>1</sup>H nuclei in CD<sub>3</sub>OD solutions using TMS (<sup>1</sup>H) as standard.

Magnetic susceptibility measurements was carried out for the metal complexes in order to support the geometry of the complexes and their magnetic behavior using Gouy's method apparatus balance (Holmarc's Magnetic Susceptibility, Gouy's Method Apparatus (Model No: HO-ED-EM-08)) at room temperature with Hg[Co(SCN)<sub>4</sub>] as calibrant.

In order to check the electrolytic or non-electrolytic nature of the synthesized complexes we used Hanna instrument HI 8633 N Multi-range conductivity meter to measure the molar conductivities of freshly prepared 1.0 × 10<sup>-3</sup> M solutions of the metal complexes in DMSO.

### Antimicrobial activity assessment

The microbial susceptibility of the ligands and their complexes were examined *in vitro* against bacterial and fungal strains. For antibacterial studies, compounds were tested against two Gram-positive *Staphylococcus aureus* (*S. aureus*) (ATCC 25923) and *Streptococcus pneumonia* (*S. pneumonia*) (ATCC 49619) and two Gram-negative *Escherichia coli* (*E. coli*) (ATCC 25922) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 27853) bacterial strains. For antifungal activity, the compounds were tested against the common fungal strain *Candida albicans* (*C. albicans*) (ATCC 10231). All the bacterial and fungal activity experiments were performed at the Department of Clinical Microbiology, Blood Bank Centre at Al-Baha city, KSA.

The assessment of the antibacterial and antifungal activities of the compounds were performed using agar diffusion assay method according to the Clinical and Laboratory Standards Institute (CLSI) susceptibility testing technique [24]. Muller Hinton Agar (MHA) was used as growth medium for bacterial strains and Sabouraud dextrose agar was used as culture medium for *C. albicans* fungus.

The stock solutions were prepared by dissolving 0.02 gm of each compound in 5 mL DMSO solvent that was used as negative control. Whatman sterilized 6 mm filter paper discs (Whatman antibiotic assay discs-model 2017-006 from Sigma-Aldrich) containing the tested compounds were placed on the agar surface in petri dishes. The petri dishes were put in an incubator at 35°C for 24 hours in case of bacteria and at 35°C for 48 hours for fungus. Zones of complete inhibition were measured (in mm) around the holes after the incubation period to monitor the fungal and bacterial susceptibilities. Each test was repeated in triplicate and the arithmetic mean values were recorded.

In addition, the minimum inhibitory concentration (MIC) values of the compounds showed highest activity against both bacterial and fungal strains were investigated. The metal complex [ZnSL<sub>5</sub>.H<sub>2</sub>O] was evaluated for MIC against *C. albicans*

as fungal strains and complexes and  $[ZnSL_6 \cdot H_2O]$  were tested for MIC against *S. pneumonia* as bacterial strains. Agar disc-dilution assay was used for MIC tests following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. Mueller-Hinton agar was used as growing medium. Decreasing concentrations were prepared in serial 2-fold dilution 100, 50, 25, 12.5, 6.25 and 3.125  $\mu\text{g}/\text{disc}$  for each tested compound were used for the MIC investigations.

Each sample was repeated three times and statistical analysis was performed with a limit was set at  $p < 0.05$  and data is presented as arithmetic mean.

### The molluscicidal activity against land snails

Some of the obtained ligands and their corresponding metal complexes were tested for their molluscicidal activity against one land snail *Eobania vermiculata* (*E. vermiculata*). It is the type of the genus *Eobania*. The Toxicity and lethal dose ( $LD_{50}$ ) was determined for the tested compounds in the Biology Department laboratories at Al Albayt University in Jordan. The snails were collected from local area Almafraq in Jordan and were reared for enough periods to acclimatize with laboratory conditions before assessment. The snails (10 snails) were maintained at nearly  $26^\circ\text{C}$  in plastic tanks ( $30 \times 15 \times 10$  cm) containing some sand and fresh water (bottle water) with neutral pH and fed with commercial fish food. The water in the tank was replaced every three days.

Molluscicidal activity evaluation of the tested compounds was performed according to WHO guidelines using immersion technique and using niclosamide as a control measure [25]. Four Schiff base ligands selected for this investigation, ( $SL_4$  and  $SL_5$ ) and the four corresponding metal complexes selected were  $[CuSL_4 \cdot H_2O]$ ,  $[NiSL_5 \cdot H_2O]$ . Primary stock solution was

prepared by dissolving 2 mg/ml niclosamide and 10 mg/ml of tested compounds in DMSO solvent. Final concentrations ranging from 0.02 mg/L to 0.2 mg/L for niclosamide and 0.1 mg/L to 0.5 mg/L for the tested compounds were used for the bioassays. The reared snails were immersed in mixed aqueous solution of the investigated compounds at the final concentrations for 24 hours without food. After exposure to the tested compounds the snails were transferred to fresh water without fed for another 24 hours as a recovery period to assess mortality. The mortality was determined after 24 hours and the 50% ( $LC_{50}$ ) lethal concentration was determined. Each molluscicidal experiment was repeated in duplicate for each compound and dose effect evaluation was done using Leitchfield and Wilcoxon method [26].

## Results and Discussion

### Synthesis of the ligand compounds and characterization

The targeted Schiff base ligands were successfully synthesized following the procedures mentioned in materials and methods section. In this investigation we used 2-aminobenzoic acid as a primary amine which was condensed with different aldehydes such as salicylaldehyde, o-vaniline, 2-carboxybenzaldehyde, 1-(3-formyl-4-hydroxyphenylazo)-4-methylbenzene and 5-phenylazosalicylaldehyde. All the organic ligands were isolated and characterized using different analytical techniques such as elemental analysis, mass spectra, UV-visible spectroscopy, IR spectra and NMR spectroscopy. The physical properties and elemental analysis of the synthesized ligands are presented in **Tables 1** and **2** respectively.

**Table 1** Physical properties of the prepared Schiff base ligands.

Ligand	Mol. Formula	Mol. Wt.	M.P $^\circ\text{C}$	Color	% of Yield	Mass spectra
SL <sub>1</sub>	C <sub>14</sub> H <sub>11</sub> NO <sub>3</sub>	241	205-206 $^\circ\text{C}$	Orange	85	242
SL <sub>2</sub>	C <sub>15</sub> H <sub>13</sub> NO <sub>4</sub>	271	96-98 $^\circ\text{C}$	Red	78	272
SL <sub>3</sub>	C <sub>15</sub> H <sub>11</sub> NO <sub>4</sub>	269	225 $^\circ\text{C}$	Beige	86	269.08
SL <sub>4</sub>	C <sub>21</sub> H <sub>16</sub> N <sub>3</sub> O <sub>4</sub>	374	288-290 $^\circ\text{C}$	Reddish brown	74	373.75
SL <sub>5</sub>	C <sub>20</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub>	345	218 $^\circ\text{C}$	Red	73	346

The prepared ligands were all colored solids with good yields and stable at room temperature. They were soluble in hot ethanol, chloroform and DMF.

**Table 2** Elemental analysis of the prepared Schiff base Ligands.

Ligand	Elemental Analysis			
	% C Calculated (Found)	% H Calculated (Found)	% N Calculated (Found)	% O Calculated (Found)
SL <sub>1</sub>	69.70 (69.74)	4.59 (4.22)	5.80 (5.62)	19.89 (19.94)
SL <sub>2</sub>	66.41 (66.25)	4.83 (4.90)	5.16 (5.38)	23.59 (23.40)

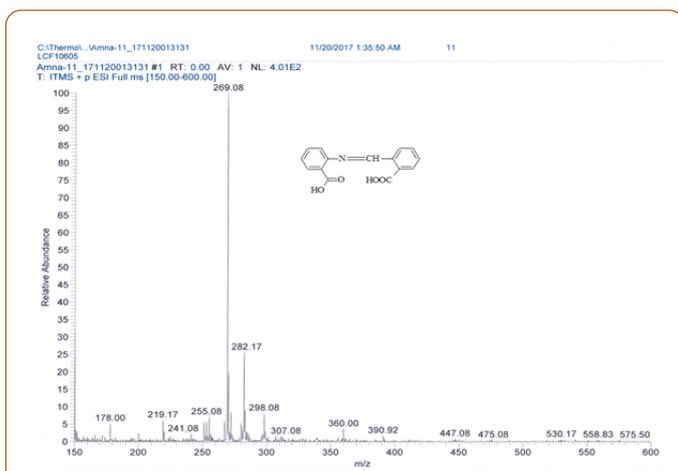
SL <sub>3</sub>	66.91 (66.82)	4.11 (4.35)	5.20 (5.42)	23.76 (23.54)
SL <sub>4</sub>	67.37 (67.24)	4.30 (4.52)	11.22 (11.48)	17.09 (16.76)
SL <sub>5</sub>	69.55 (70.02)	4.37 (4.63)	12.16 (12.15)	13.89 (13.62)

In order to confirm the structures and molecular weights of the desired ligands, mass spectrum was performed for the prepared ligands. The observed mass spectra for the synthesized Schiff base ligands are presented in **Table 1**. In addition, typical representative mass spectra of some synthesized Schiff bases are shown in **Figures 3** and **4**.

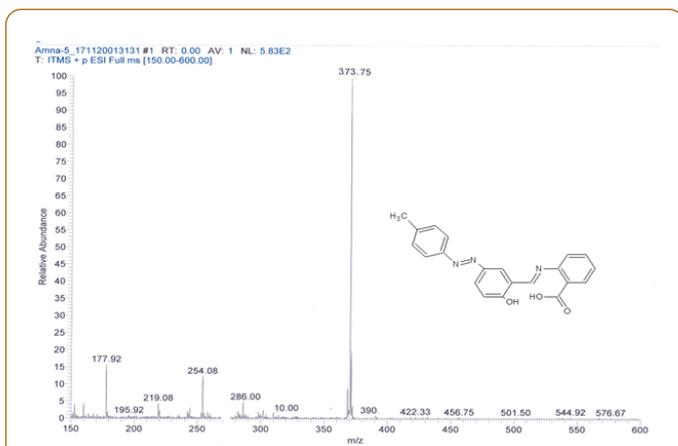
The mass spectra for all prepared Schiff bases were identical with their theoretical calculated molecular weights. This proves and confirms the proposed structures for the synthesized Schiff base ligands.

### Synthesis of metal complexes

The bivalent metals Cu(II), Ni(II) and Zn(II) were used to prepare metal complexes utilizing the synthesized tridentate Schiff base ligands molecules. The desired metal complexes were successfully synthesized following the standard reported template procedure as mentioned in the materials and methods section. The produced metal complexes were colored non-hygroscopic amorphous solids and in good yield. They were recrystallized from hot DMF solvent. All prepared complexes were stable at room temperature, insoluble in common organic solvents and only soluble in DMF and DMSO solvents. The molecular formulae, molecular weights, colors, and yields are presented in **Table 3**. All synthesized complexes decomposed without melting at temperatures higher than 300°C.



**Figure 3** Mass spectra for SL<sub>3</sub> (C<sub>15</sub>H<sub>11</sub>NO<sub>4</sub>) ligand.



**Figure 4** Mass spectra for SL<sub>5</sub> (C<sub>21</sub>H<sub>16</sub>N<sub>3</sub>O<sub>4</sub>) ligand.

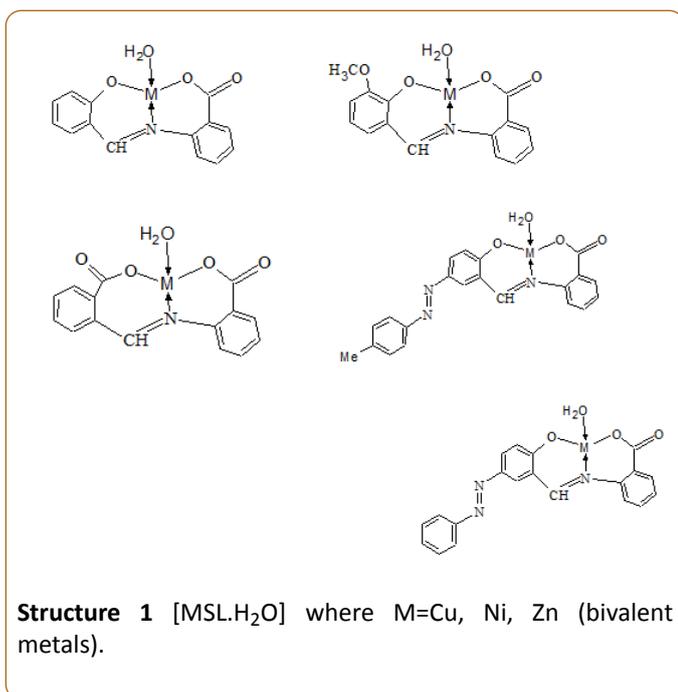
**Table 3** Physical properties of the Schiff base Complexes.

Metal complex	Mol. Formula	Mol. Weight	% Yield	Color
CuSL <sub>1</sub>	CuC <sub>14</sub> H <sub>10</sub> NO <sub>3</sub> .H <sub>2</sub> O	321.799	78	Green
NiSL <sub>1</sub>	NiC <sub>14</sub> H <sub>10</sub> NO <sub>3</sub> .H <sub>2</sub> O	316.946	76	Green
ZnSL <sub>1</sub>	ZnC <sub>14</sub> H <sub>10</sub> NO <sub>3</sub> .H <sub>2</sub> O	323.662	78	Light yellow
CuSL <sub>2</sub>	CuC <sub>15</sub> H <sub>10</sub> NO <sub>4</sub> .H <sub>2</sub> O	349.809	80	Green

NiSL <sub>2</sub>	NiC <sub>15</sub> H <sub>10</sub> NO <sub>4</sub> .H <sub>2</sub> O	344.956	79	Light green
ZnSL <sub>2</sub>	ZnC <sub>15</sub> H <sub>10</sub> NO <sub>4</sub> .H <sub>2</sub> O	351.672	78	Yellow
CuSL <sub>3</sub>	CuC <sub>15</sub> H <sub>9</sub> NO <sub>4</sub> .H <sub>2</sub> O	348.801	82	Green
NiSL <sub>3</sub>	NiC <sub>15</sub> H <sub>9</sub> NO <sub>4</sub> .H <sub>2</sub> O	343.948	80	Green
ZnSL <sub>3</sub>	ZnC <sub>15</sub> H <sub>9</sub> NO <sub>4</sub> .H <sub>2</sub> O	350.664	82	Beige
CuSL <sub>4</sub>	CuC <sub>21</sub> H <sub>14</sub> N <sub>3</sub> O <sub>4</sub> .H <sub>2</sub> O	453.921	72	Brown
NiSL <sub>4</sub>	NiC <sub>21</sub> H <sub>14</sub> N <sub>3</sub> O <sub>4</sub> .H <sub>2</sub> O	449.068	69	Light brown
ZnSL <sub>4</sub>	ZnC <sub>21</sub> H <sub>14</sub> N <sub>3</sub> O <sub>4</sub> .H <sub>2</sub> O	455.784	74	Brown
CuSL <sub>5</sub>	CuC <sub>20</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> .H <sub>2</sub> O	424.903	75	Brown
NiSL <sub>5</sub>	NiC <sub>20</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> .H <sub>2</sub> O	420.05	78	Light brown
ZnSL <sub>5</sub>	ZnC <sub>20</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> .H <sub>2</sub> O	426.766	73	Brown

The synthesized Cu(II), Ni(II) and Zn(II) complexes are depicted in Structure 1. The metal complexes were characterized using micro-elemental analysis, mass spectra, UV-visible, IR, NMR, Magnetic susceptibility and TGA analysis techniques to prove the proposed structures and geometries.

The elemental analysis (**Table 4**) is in agreement with the proposed structures of the complexes. In addition, the mass spectra of the synthesized complexes were carried out to determine and confirm the molecular weights. The observed mass spectra of NiSL1 (NiC<sub>14</sub>H<sub>10</sub>NO<sub>3</sub>.H<sub>2</sub>O) is 317.25 (calculated M.Wt=316.9), the observed peak for [ZnSL<sub>2</sub>] (ZnC<sub>15</sub>H<sub>10</sub>NO<sub>4</sub>.H<sub>2</sub>O) is 351.81 (calculated M.Wt=351.67) and for [ZnSL<sub>3</sub>] (ZnC<sub>15</sub>H<sub>9</sub>NO<sub>4</sub>.H<sub>2</sub>O) is 350.83 (calculated M.Wt=350.66). [NiSL<sub>4</sub>] (NiC<sub>21</sub>H<sub>14</sub>N<sub>3</sub>O<sub>4</sub>.H<sub>2</sub>O) the observed mass spectra was 449.12 (calculated M.Wt=449) for [ZnSL<sub>5</sub>] (ZnC<sub>20</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>.H<sub>2</sub>O) the observed mass spectra was 426.17 (theoretical calculated M.Wt=426.7). These mass spectrum observations confirm and support the proposed structure for these complexes. Typical representative mass spectra of some synthesized Schiff base complexes are shown in **Figures 5-7**.



**Table 4** Elemental analysis of the Schiff base Complexes.

Ligand	Elemental Analysis				
	% C Calculated (found)	% H Calculated (found)	% N Calculated (found)	% O Calculated (found)	% of metal Calculated (found)
CuSL <sub>1</sub>	52.25 (51.90)	3.75 (3.82)	4.35 (4.22)	19.88 (19.46)	19.77 (20.02)
NiSL <sub>1</sub>	53.05 (53.35)	3.81 (3.55)	4.42 (4.67)	20.19 (19.84)	18.53 (18.75)
ZnSL <sub>1</sub>	51.95 (51.66)	3.73 (3.68)	4.32 (4.68)	19.77 (19.70)	20.23 (20.06)
CuSL <sub>2</sub>	51.50 (51.21)	3.45 (3.33)	4.00 (4.16)	22.86 (22.94)	18.19 (17.89)

NiSL <sub>2</sub>	52.22 (52.00)	3.50 (3.61)	4.06 (4.53)	23.18 (23.24)	17.04 (17.62)
ZnSL <sub>2</sub>	51.23 (51.72)	3.43 (3.75)	3.98 (3.77)	22.74 (22.31)	18.62 (18.40)
CuSL <sub>3</sub>	51.60 (51.64)	3.17 (3.46)	4.01 (4.73)	22.93 (22.80)	18.29 (17.96)
NiSL <sub>3</sub>	52.38 (52.04)	3.22 (3.59)	4.07 (4.42)	23.25 (23.12)	17.08 (17.36)
ZnSL <sub>3</sub>	51.37 (51.04)	3.16 (3.22)	3.99 (4.29)	22.81 (22.57)	18.67 (18.94)
CuSL <sub>4</sub>	55.56 (55.69)	3.55 (3.82)	9.25 (9.01)	17.62 (17.22)	14.02 (13.88)
NiSL <sub>4</sub>	56.16 (56.01)	3.59 (3.78)	9.35 (9.12)	17.81 (17.84)	13.09 (13.37)
ZnSL <sub>4</sub>	55.34 (56.03)	3.53 (3.62)	9.21 (8.98)	17.55 (17.83)	14.37 (14.02)
CuSL <sub>5</sub>	56.49 (56.18)	3.55 (3.33)	9.88 (10.04)	15.06 (15.27)	15.02 (14.75)
NiSL <sub>5</sub>	57.19 (58.00)	3.59 (3.82)	10.00 (9.86)	15.23 (14.92)	13.99 (14.22)
ZnSL <sub>5</sub>	56.24 (56.80)	3.54 (3.50)	9.84 (10.04)	14.99 (15.16)	15.39 (15.04)

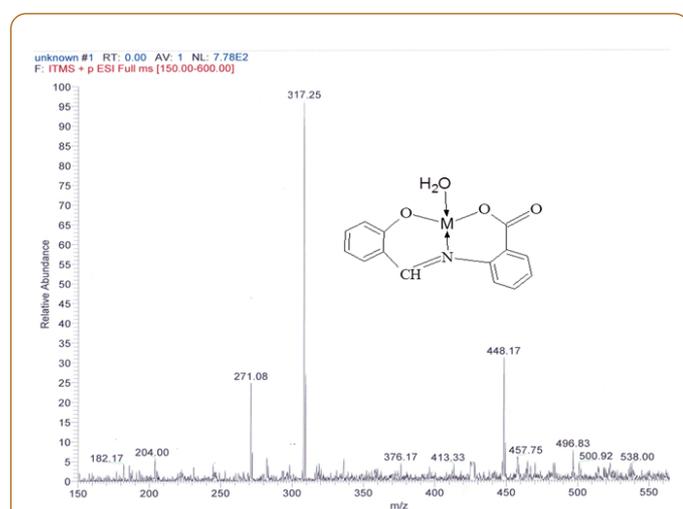


Figure 5 Mass spectra for NiSL<sub>1</sub> (NiC<sub>14</sub>H<sub>10</sub>NO<sub>3</sub>·H<sub>2</sub>O) complex.

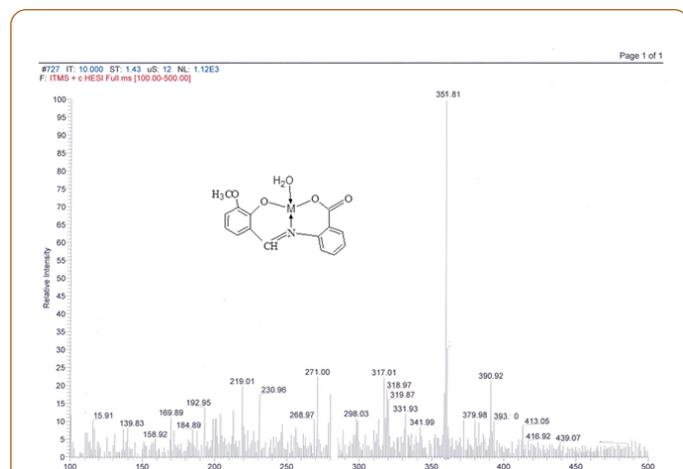


Figure 6 Mass spectra for ZnSL<sub>2</sub> (ZnC<sub>15</sub>H<sub>10</sub>NO<sub>4</sub>·H<sub>2</sub>O) complex.

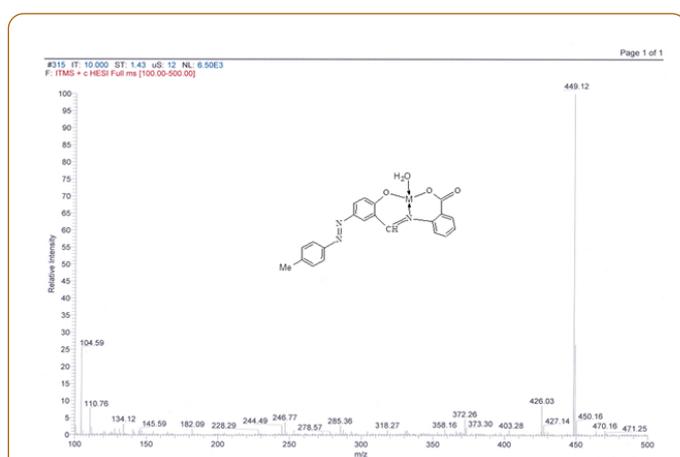


Figure 7 Mass spectra for NiSL<sub>4</sub> (NiC<sub>21</sub>H<sub>14</sub>N<sub>3</sub>O<sub>4</sub>·H<sub>2</sub>O) complex.

## UV-visible spectra and magnetic susceptibility measurements

The UV-visible spectral data are used to study the geometry of the prepared metal complexes. The UV-vis absorption spectra were recorded in DMSO solution ( $10^{-3}$ ) for the Schiff base ligands and their Cu(II), Ni(II) and Zn(II) complexes in the range of 200-800 nm at room temperature. The electronic spectra of the synthesized tridentate ligands obtained by the condensation of the different aldehydes with 2-aminobenzoic acid are nearly similar. Upon coordination with metal ions, the absorption bands of the corresponding metal complexes were observed somewhat shifted to longer wavelength with low frequency (red shift) (i.e., positive solvatochromism).

The bands at around 340-365 nm were observed in the UV-vis spectra of the Ligands SL<sub>1</sub>, SL<sub>2</sub>, and SL<sub>3</sub> which can be assigned to  $n \rightarrow \pi^*$  transitions of the azomethine group [27]. Some bands in the spectra of the free ligands were shown at lower energy in the range of 260-280 nm may be attributed to  $n \rightarrow \pi^*$  transitions due to conjugation of  $\pi$  bond in the benzene ring [28]. While the bands appeared at higher energies are

attributed to  $\pi \rightarrow \pi^*$  of the benzene ring and CH=N group. These bands were shifted (red shift) in the spectra of the corresponding metal complexes with some changes in frequencies and intensities indicating the coordination of the ligands to the metal atoms and the participation of nitrogen atom of the CH=N group and the oxygen atom of the phenolic group in bonding with metal atom Centre [29].

The UV-visible absorption spectra of the ligands containing azo and azomethine groups i.e., (SL<sub>4</sub> and SL<sub>5</sub>) show important bands. The first broad band appeared at 360-365 nm that can be attributed to  $\pi \rightarrow \pi^*$  transitions within the ligand molecule involving the  $\pi$  electrons of the azomethine and azo groups [30,31]. Since the  $\pi \rightarrow \pi^*$  transitions of imine and azo linkages appear in close proximity, the two bands may overlap in each other, thus the broad absorption in the region 360-365 nm may be assigned to  $\pi \rightarrow \pi^*$  transitions in C=H and N=N groups [32]. The second band appeared at 370-375 nm attributed to  $n \rightarrow \pi^*$  transitions of azo-aromatic chromosphere [30,31,33].

Typical UV-vis absorption spectrum in DMSO for some Schiff base ligands is shown in Figures 8 and 9.

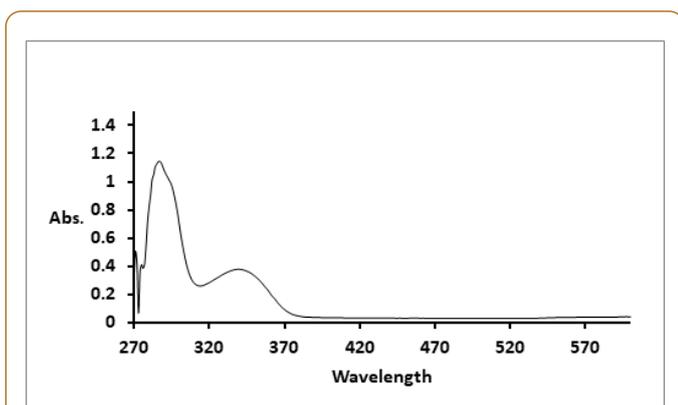


Figure 8 UV-visible spectra for the ligand SL<sub>2</sub>.

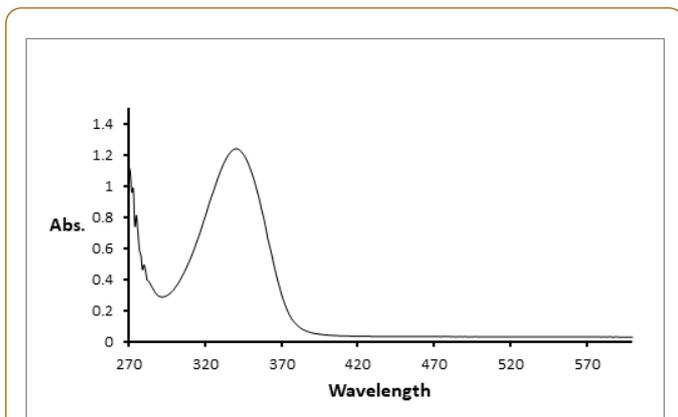


Figure 9 UV-visible spectra for the ligand SL<sub>5</sub>.

The spectra of some Cu(II), Ni(II) and Zn(II) complexes compared to those of the free ligands show that the bands appear at lower intensities and shifted to longer wavelength (bathochromic effect) due to coordination with metal ions [33,34]. The weak band shoulder appeared in the range of

440-465 nm in the spectra of complexes may be assigned to metal-ligand charge transfer (MLCT) transitions that expected for complexes of metals with low oxidation state [27]. In the transition metal complexes due to coordination with ligands, there is a change in electron distribution between the metal and the ligand resulting in charge transfer bands.

In addition, two bands appearing at 380-395 nm and 420-460 nm may be assigned to d-d transition of the single d electron of the metal ion suggesting the four coordinated geometry for the metal complexes [34].

The important representative UV-vis spectra of some prepared complexes are shown in Table 5. When compared with that of ligands we noticed the  $n \rightarrow \pi^*$  absorption bands of the organic ligands (due to azomethine) shifted to longer wave length with lower intensity on coordination with metal atoms confirming the coordination of the nitrogen atom of the azomethine group [27].

The Ni(II) complexes of the tridentate ligands showed bands at 465-480, 540-550 and 725-730 nm corresponding to the energy transitions  ${}^1A_{1g} \rightarrow {}^1E_g$ ,  ${}^1A_{1g} \rightarrow {}^1A_{2g}$ , and  ${}^1A_{1g} \rightarrow {}^1B_{1g}$  respectively. Moreover the magnetic moment measurements  $\mu_{\text{eff}}$  showed diamagnetic nature of the nickel complexes of the tridentate ligands which is a characteristic of the metals with d<sup>8</sup> configuration. These observations and the non-electrolytic nature of these complexes suggested square planar geometry for the Ni(II) complexes [2,35].

The Cu(II) complexes of the tridentate Schiff base ligands showed broad bands at around 740-750 nm corresponding to  ${}^2B_{1g} \rightarrow {}^2A_{1g}$  transitions. In addition, the magnetic moment measurement values 1.83-1.85 BM which are less than 1.90 and are closed to the spin only magnetic moment value for the d<sup>9</sup> Cu(II) supporting the mononuclear nature of these complexes [36]. These observations suggested a distorted square-planar geometry around the central copper atom [37].

While, the Zn(II) complexes showed UV-visible bands at nearly 570-585 nm and the magnetic moment observations showed diamagnetic nature of zinc complexes. Moreover, the molar conductance measurements exhibited low values indicated non-electrolytic nature of the Zn(II) complexes obtained from the tridentate ligands. These observations along with analytical data presumed tetrahedral geometry for zinc complexes, which is a common geometry for metals with d<sup>10</sup> configuration [38].

### FT-IR spectra

Important and key infrared spectral data for the representative ligands and their complexes are presented in Figures 10-12. Most of the IR spectra for ligands and that for complexes are nearly similar. FT-IR spectral analysis was performed in order to locate the coordination sites that may connect the ligands with the metal ions in chelation process. It is well known that the bands position and peak intensities are expected to be amended on coordination.

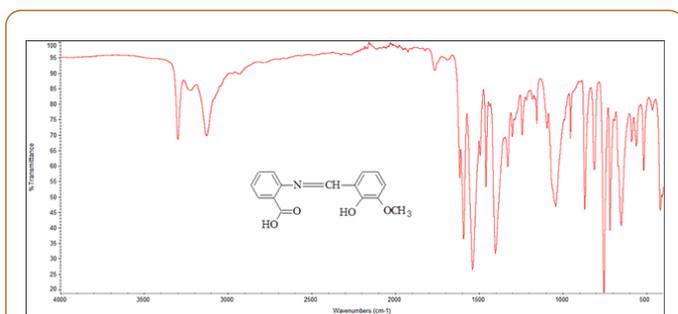


Figure 10 IR spectra for the ligand SL<sub>2</sub>.

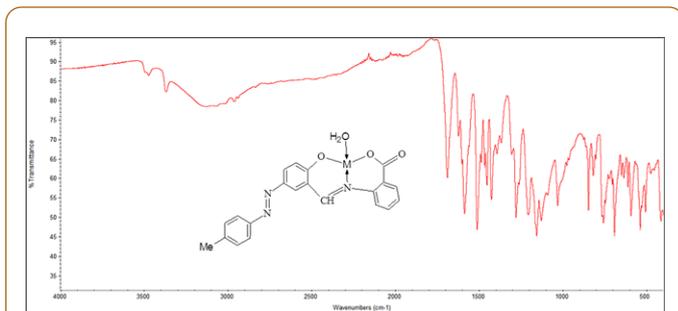


Figure 11 IR spectra for the Nickel complex [NiSL<sub>4</sub>·H<sub>2</sub>O].

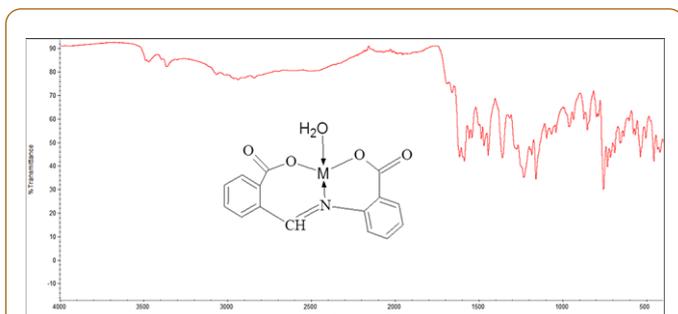


Figure 12 IR spectra for the copper complex [CuSL<sub>3</sub>·H<sub>2</sub>O].

The bonding of ligands to metals was investigated through the comparison of the IR spectral bands of the ligands and the bands of their corresponding metal complexes. In the FT-IR spectrum of organic Schiff bases a strong band appeared in the range of 1580-1615 cm<sup>-1</sup> assigned to the stretching vibrations of the azomethine (C=N) linkage band [16]. In the spectra of the metal complexes this band was shifted in all metal complexes to lower frequency by nearly -5 to -16 cm<sup>-1</sup> due to donation of the electrons from N atom of the C=N group to the metal atom during the process of coordination [2,23]. This proves the coordination of the azomethine group through nitrogen atom to the metal ion [16,23,39]. The bands appeared in the range of (1550-1560) and (1390-1430) cm<sup>-1</sup> of the free Schiff base ligands may be assigned to asymmetric and symmetric νCOOH stretching vibrations [40,41]. These bands were shifted slightly to lower frequencies by nearly -10 to -20 cm<sup>-1</sup> range that may attributed to the involvement of the oxygen atom of the carboxylic group in bonding formation with metal atom in complexes. The large difference in the

asymmetric νCOOH and symmetric νCOOH bands indicate monodentate binding nature of the carboxylic group in the metal complexes [41].

Also, in the spectra of the ligands there is a band at 2900-2965 cm<sup>-1</sup> of the stretching vibrations of the OH of the carboxylic group was absent in the spectra of the metal complexes indicating the formation of M-O bond and losing the proton of the COOH group [16]. The IR spectra of the tridentate ligands having phenolic OH group showed a broad band at the range of 3360-3475 cm<sup>-1</sup>. These bands were disappeared in the IR spectra of the corresponding metal complexes indicating the participation of oxygen atom of the phenolic group in bonding with metal atom. Moreover, new bands in the IR spectra of the metal complexes appeared in the range of 515-550 or (470-520) cm<sup>-1</sup> may assigned to (ν<sub>M-O</sub>) band vibrations and 410-460 cm<sup>-1</sup> may be assigned to (ν<sub>M-N</sub>) band vibrations [23,41,42]. These bands support the formation of M-O and M-N bonds respectively on complexation with metal atoms [23]. These bands were not observed in the spectra of the ligands, which proves the coordination of the metal ions with phenolic oxygen (OH) and azomethine nitrogen of CH=N group.

Another band was observed in the FT-IR spectrum of the organic ligands SL<sub>5</sub> and SL<sub>6</sub> at 1480-1496 cm<sup>-1</sup> that may assigned to the stretching vibration of the azo chromophore ν(N=N) group [39]. This band does not show any shift in the spectra of the metal complexes indicates that azo group does not participate in coordination [43]. In the spectra of metal complexes a broad band in the range of 3000-3500 cm<sup>-1</sup> may be assigned to the coordinated water molecule.

Therefore the IR spectra reveal that the ligands act as tridentate molecules and coordinated to the metal ions via phenolic oxygen, carboxylic oxygen and azomethine nitrogen atoms. These IR spectral data are in accord to the proposed structures of the ligands and their corresponding complexes.

### <sup>1</sup>H NMR spectroscopy

The <sup>1</sup>H NMR spectra of the prepared ligands carried out for some compounds and chemical shifts for signals were observed. A representative <sup>1</sup>H NMR spectra of the tridentate ligands is shown in **Figure 13**. In the <sup>1</sup>H NMR spectrum a signal at δ 10.27 ppm assigned to the phenolic -OH protons [2,44]. All ligands exhibit a characteristic singlet signal at range of δ 8.47-8.90 ppm is assignable to carboxylic (COOH) group [45,46]. The singlet due to COOH group was not shown in the spectrum of the corresponding metal complexes supporting the involvement of carboxylic group of the anthranilic acid in coordination through the displacement of COOH proton [45]. The sharp singlet appeared in the spectrum of the ligands appeared in the range around 7.21-7.65 is due to the azomethine protons (CH=N) and confirms its formation [2,33,34]. The phenyl multiple of the ligands was shown at a range of 6.49-6.92 [2]. The spectrum of the ligands SL<sub>2</sub> showed a signal at δ 3.846 ppm may attributed to methoxy (-OCH<sub>3</sub>) group [44].

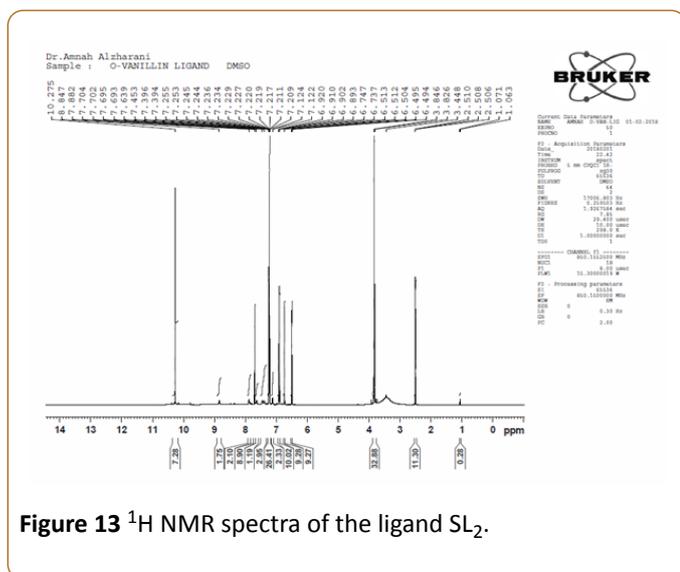


Figure 13  $^1\text{H}$  NMR spectra of the ligand  $\text{SL}_2$ .

### Molar conductivity of metal complexes

Some of the prepared Cu(II), Ni(II) and Zn(II) complexes were examined for their conductivity nature in order to establish the charge of the metal complexes. The molar conductance of the soluble metal complexes was measured in DMSO ( $10^{-3}$  M) solution at room temperature  $23.8^\circ\text{C}$ . The molar conductivity measurements (Table 5) falls in the region  $1.29\text{--}3.29\text{ S.cm}^2.\text{mol}^{-1}$  indicating very small conductivity values, suggesting that all metal complexes prepared are non-electrolytic in nature [16]. These observations indicate that the synthesized metal complexes are neutral (non-ionic) and no charged ions present outside the coordination sphere of the metal complexes [23].

Table 5 Important UV-vis spectral data and magnetic moment for some metal complexes.

Compound	Absorption bands ( $\lambda$ in nm)	Magnetic Moment $\mu_{\text{eff}}$ (B.M)	Molar Conductance $\text{S cm}^2 \text{mol}^{-1}$
CuSL <sub>1</sub>	395, 430, 745	1.83	1.28
ZnSL <sub>1</sub>	465, 585	diamagnetic	2.20
ZnSL <sub>2</sub>	425, 570	diamagnetic	2.69
CuSL <sub>3</sub>	380, 465, 745	1.84	0.96
ZnSL <sub>3</sub>	324, 450, 580	diamagnetic	2.06
CuSL <sub>4</sub>	328, 455, 750	1.85	1.95
NiSL <sub>4</sub>	480, 550, 730	diamagnetic	3.29
CuSL <sub>5</sub>	380, 480, 740	1.84	1.76
NiSL <sub>5</sub>	465, 540, 725	diamagnetic	2.96
ZnSL <sub>5</sub>	455, 585	diamagnetic	2.55

### Biological assessment investigation

Antibacterial and antifungal activities: The Schiff base ligands and their copper(II), Nickel(II) and Zinc(II) complexes synthesized in this investigation were examined *in vitro* for antibacterial susceptibility against two Gram-positive bacterial strains *Streptococcus aureus* (ATCC 25923) and *Streptococcus pneumoniae* (ATCC 49619). In addition, the antibacterial activity was tested against the two Gram-negative bacterial strains *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC 27853). Moreover,

the compounds were evaluated for antifungal activity against the common fungal strain *C. albicans* (ATCC10231). The assessments of the antibacterial and antifungal activities of the compounds were performed using agar disc-diffusion assay. The bacterial and fungal susceptibilities were estimated by measuring the zones of complete inhibition (in mm) around the holes after the incubation period. Each experiment was repeated twice and the arithmetic mean values of the duplicate measurements were recorded (Table 6).

Table 6 Antibacterial and antifungal activity of the ligands and their Cu(II), Ni(II) and Zn(II) complexes.

Compound	Zone of inhibition (mm)				
	Gram-positive bacteria		Gram-negative bacteria		Fungus
	<i>S. pneumoniae</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>
SL <sub>1</sub>	6.8	9.0	R	R	9.6

SL <sub>2</sub>	7.3	8.2	R	R	9.2
SL <sub>3</sub>	11.6	10.2	R	R	11.3
SL <sub>4</sub>	11.8	11.0	8.2	8.8	13.4
SL <sub>5</sub>	13.4	12.0	6.7	6.6	12.8
CuSL <sub>1</sub> .H <sub>2</sub> O	22	16	R	R	16.2
NiSL <sub>1</sub> .H <sub>2</sub> O	17.0	12.4	R	R	17.8
ZnSL <sub>1</sub> .H <sub>2</sub> O	28	30	R	R	26.6
CuSL <sub>3</sub> .H <sub>2</sub> O	12.6	11.3	R	R	14.5
NiSL <sub>3</sub> .H <sub>2</sub> O	14	12.0	R	R	16.5
ZnSL <sub>3</sub> .H <sub>2</sub> O	16.2	13.5	R	R	18.9
CuSL <sub>4</sub> .H <sub>2</sub> O	20	15.6	9.6	10.2	18.2
NiSL <sub>4</sub> .H <sub>2</sub> O	16.4	13.2	9.6	9.8	16.6
ZnSL <sub>4</sub> .H <sub>2</sub> O	22	20.2	13.2	9.6	30.4
CuSL <sub>5</sub> .H <sub>2</sub> O	8	9	7.5	8.6	11.4
NiSL <sub>5</sub> .H <sub>2</sub> O	10	11	8.2	9.0	10.8
ZnSL <sub>5</sub> .H <sub>2</sub> O	30	18.8	11.0	12.5	27.6

The tested organic ligands (SL<sub>1</sub> and SL<sub>2</sub>) showed inactivity against the Gram-positive bacteria with zones of inhibitions < 10 mm), While the ligands (SL<sub>3</sub>, SL<sub>4</sub> and SL<sub>5</sub>) showed weak activity with zones of inhibition between (11.6-14.8 mm) against both Gram-positive bacterial strains *Streptococcus pneumoniae* and *Streptococcus aureus*. The weakly active ligands have azo (N=N) group and COOH group that may enhanced the activity of these ligands a little more than other ligands. The examined ligand compounds did not show any effect against the Gram-negative bacteria, which showed resistance to these compounds. Antifungal test for the prepared ligand compounds exhibit week influence on the growth of the fungal strain *Candida albicans* with a diameter of inhibition zones range in between 9.2-12.8 mm.

The metal complexes showed remarkable enhancement in the activity against the species *Streptococcus pneumoniae* and *Streptococcus aureus* of Gram-positive bacterial strains under the same conditions. The metal complexes were showed to be more toxic than the parent ligands with inhibition zones range of (12.6-30 mm) against *Streptococcus pneumoniae* and (9-30 mm) against *Streptococcus aureus* bacterial strains. Similarly on chelation, the antifungal activity was increased compared to the parent organic ligands and the inhibition zones were in the range of (10.8-30.4 mm) against *C. albicans* fungal strains. This antimicrobial enhancement responsibility might be attributed to the influence of the bivalent metal ion of the prepared coordination compounds. This also can be interpreted on the ground of Tweedy's chelation theory and the effect of the metal ion on the normal cell processes [22,27]. Chelation considerably reduces the polarity of the metal ion due to the partial sharing of the positive charge with the donor atoms of the ligands that result in delocalization of  $\pi$ -electrons with in the entire chelate ring system formed

during coordination. This may enhance the lipophilic character of the central metal atom, increases the liposolubility of the metal chelates and enhances the penetration through the lipid layers of the microbial cell membranes [23,47]. Moreover, the mode of action of the compounds may involve the formation of a hydrogen bond through the azomethine nitrogen atom (CH=N) with the cell constituents causing in interference with the normal cell process [42,48].

Generally, the metal coordination compounds deactivate different enzymes at the cellular level that have an essential role in different metabolic pathways of these microorganisms. Moreover, the solubility, conductivity and dipole moment factors which affect the presence of metal ions may enhance the antibacterial and antifungal activities of the coordination compounds compared to the free organic ligands.

Observations also showed that among all the bacterial strains, *Streptococcus pneumoniae* was the most susceptible to metal complexes compared to *Streptococcus aureus*, the Gram-positive bacteria. This may be attributed to the difference in cell wall structure [42]. It is also observed that the metal complexes in general have better activity against the fungus *C. albicans* compared to the antibacterial activity. The difference in the effectiveness of different complexes against different organisms (i.e., fungus and bacteria) depends either on the impermeability of the cells of the microbes or the difference in ribosomes of the microbial cells [42,48].

The results observed indicated that the Zn(II) metal complexes show higher antibacterial and antifungal activity compared with other analogous Cu(II) and Ni(II) complexes. Cu(II) and Ni(II) complexes generally have nearly similar activities with slight differences. The activity of Zn(II) complexes may be due to their higher stability constant [48].

Since the complexes have different donating atoms and some other groups attached with the ligands and containing metals with the same oxidation state (+2), therefore the more effective factors that affect the antimicrobial activity are nature of the central metal atom and the substituents and moieties that present in the ligands.

The observed antifungal test results for the complex  $[\text{ZnSL}_4 \cdot \text{H}_2\text{O}]$  which showed 30.4 mm zone of inhibition against *C. albicans* fungus and the antibacterial test that showed the complex  $[\text{ZnSL}_5 \cdot \text{H}_2\text{O}]$  exhibit zones of inhibition of 30 mm against the bacterial strains, *S. pneumoniae*. These observations encourage us to investigate the minimum inhibitory concentration (MIC) to quantify the lowest concentration of the tested chemicals, which prevent visible growth of the selected bacteria or fungus.

The MIC investigation was carried out by screening the selected complexes against *S. pneumonia* bacterial strains and *C. albicans* as fungal strains. Agar disc-dilution assay was used for MIC tests following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. Mueller-Hinton agar was used as growing medium. Solutions of 100, 50, 25, 12.5, 6.25 and 3.125  $\mu\text{g}/\text{disc}$  were used for the MIC investigations. The highest potency exhibited by the complex  $[\text{ZnSL}_4 \cdot \text{H}_2\text{O}]$  was shown against *C. albicans* fungal strains with MIC value of 12.5  $\mu\text{g}/\text{disc}$ . While notable susceptibility was indicated for *Streptococcus pneumonia* bacterial strains with MIC value of 50  $\mu\text{g}/\text{disc}$  for the complex  $[\text{ZnSL}_5 \cdot \text{H}_2\text{O}]$ .

The strong antibacterial and antifungal activities of these particular complexes may be attributed to the zinc metal ion and presence of some active moieties like the azo (N=N) group along with the azomethine group in the ligands. Some photographs for the antibacterial and fungal tests are shown in Figures 14 and 15.

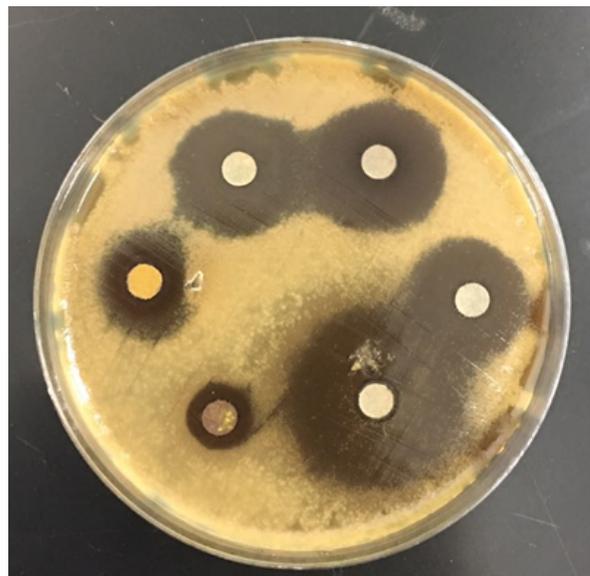


Figure 14 Antifungal Test.

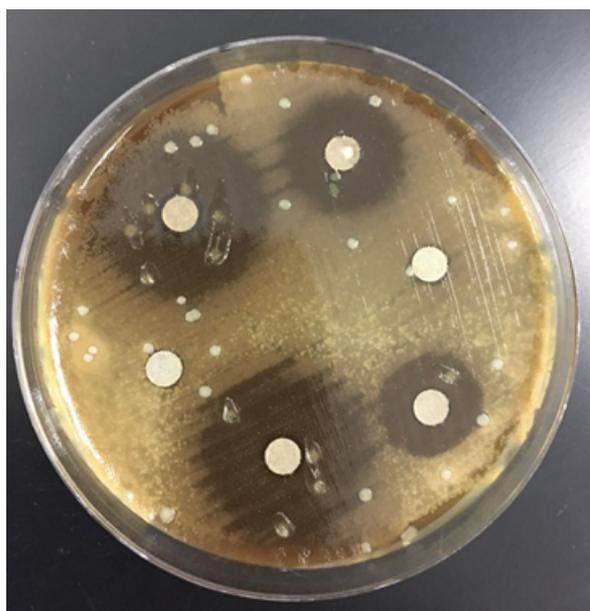


Figure 15 Antibacterial Test.

**Molluscicidal activity:** Some of the obtained ligands and their corresponding metal complexes were tested for their molluscicidal activity against one land snail *Eobania vermiculata* (*E. vermiculata*). The Toxicity and lethal dose ( $\text{LD}_{50}$ ) was determined for the tested compounds according to WHO guidelines using immersion technique and using niclosamide as positive control [25]. The mortality of the tested snails was determined after 24 hours of exposure in working solution with different concentrations of 0.1, 0.2, 0.3, 0.4, 0.5 mg/L of the tested compounds. Each molluscicidal experiment was repeated in duplicate (due to limited sources

of experiments) for each compound and dose effect evaluation was done using Leitchfield and Wilcoxon method [26]. The number of mortalities and 50% death ( $LC_{50}$ ) values are shown in **Table 7**.

**Table 7** Molluscicidal activity of the selected ligands and complexes against *E. vermiculata* snails by the immersion method (mg/l).

Compounds	No. of mortalities				
	0.1 mg/L	0.2 mg/L	0.3 mg/L	0.4 mg/L	0.5 mg/L
SL <sub>1</sub>	--	--	--	2	5
SL <sub>2</sub>	--	--	--	2	5
SL <sub>3</sub>	--	--	--	3	7
SL <sub>4</sub>	--	--	2	3	6
SL <sub>5</sub>	--	--	1	3	6
[CuSL <sub>3</sub> .H <sub>2</sub> O]	--	--	3	6	8
[NiSL <sub>4</sub> .H <sub>2</sub> O]	--	--	3	6	7
[CuSL <sub>4</sub> .H <sub>2</sub> O]	--	--	4	5	8
[ZnSL <sub>5</sub> .H <sub>2</sub> O]	--	--	3	5	9

Four Schiff base ligands selected for this investigation, two of which are bidentate (SL<sub>7</sub>, and SL<sub>9</sub>) and two are tridentate (SL<sub>4</sub> and SL<sub>5</sub>) and the four corresponding metal complexes selected were [CuSL<sub>4</sub>.H<sub>2</sub>O], [NiSL<sub>5</sub>.H<sub>2</sub>O], [Zn(SL<sub>7</sub>)<sub>2</sub>] and [Zn(SL<sub>9</sub>)<sub>2</sub>].

The observations presented in **Table 7** indicate that the ligands and their complexes did not show any activity at concentrations below 0.3 mg/L. The complexes showed good activity at concentration of 0.4 mg/L and the complex [ZnSL<sub>5</sub>.H<sub>2</sub>O] showed highest activity that can be interpreted might be due to the presence of N=N moiety in these compounds. It is also noted that the complexes are more active than their parent ligands which may be explained on the basis of the presence of metal ion that may enhance the molluscicidal activity. The  $LC_{50}$  of the complexes was at concentration of 0.4 mg/L, while the  $LC_{50}$  of the ligands was at higher concentrations of 0.5 mg/L.

## Conclusion

In the present work we synthesized some tridentate Schiff base ligands from 2-amino benzoic acid as primary amine which was condensed with different substituted aromatic aldehydes. These ligands were used to synthesize Cu(II), Ni(II) and Zn(II) complexes. The ligands and their complexes were characterized using different spectroscopic, physical and physicochemical techniques. The results showed that the tridentate ligands formed distorted square planer complexes with Cu ions, square planar with Ni atoms and tetrahedral with Zn atoms with 1:1 (M:L) reaction ratio. These ligands are coordinated with one phenolic oxygen, one nitrogen of the azomethine group and oxygen atom of the OH carboxylic group. The proposed structures of the prepared copper and nickel and zinc complexes were consistent with the chemical, physical, spectroscopic data and thermal analysis. The synthesized ligands and their corresponding complexes were

examined for their antibacterial and antifungal susceptibility. Some of the tested organic ligands (SL<sub>1</sub>, and SL<sub>2</sub>) showed inactivity against the Gram-positive bacteria with zones of inhibitions (<10 mm), While the ligands (SL<sub>3</sub>, SL<sub>4</sub> and SL<sub>5</sub>) showed weak activity with zones of inhibition between (11.6-14.8 mm) against both gram positive bacterial strains *Streptococcus pneumonia* and *S. aureus*. The examined ligand compounds did not show any effect against the Gram-negative bacteria, which showed resistance to these compounds.

The antifungal tests for the prepared ligand compounds exhibit week influence on the growth of the fungal strain *C. albicans* with zone of inhibition range between 9.2-12.8 mm.

The metal complexes showed remarkable enhancement in the activity against the species *Streptococcus pneumonia* and *Streptococcus aureus* of Gram-positive bacterial strains under the same conditions. The inhibition zones range of (12.6-30 mm) against *Streptococcus pneumoniae* and (10.2-30 mm) against *Streptococcus aureus* bacterial strains. Similarly on chelation, the antifungal activity was increased compared to the parent organic ligands and the inhibition zones were in the range of (10.8-30.4 mm) against *C. albicans* fungal strains.

The results was observed and indicated that the Zn(II) metal complexes show higher antibacterial and antifungal activity compared with other analogous Cu(II) and Ni(II) complexes. The observed antifungal test results for the complex [ZnSL<sub>4</sub>.H<sub>2</sub>O] which showed 30.4 mm zone of inhibition against *C. albicans* fungus and the antibacterial test that showed the complex [ZnSL<sub>5</sub>.H<sub>2</sub>O] exhibit zones of inhibition of 30 mm against the bacterial strains *S. pneumoniae*.

The MIC investigation was carried out by screening selected complexes that have highest activity against *S. pneumonia* bacterial strains and *C. albicans* as fungal strains. The highest potency exhibited by the complex [ZnSL<sub>4</sub>.H<sub>2</sub>O] was shown against *C. albicans* fungal strains with MIC value of 12.5 µg/

disc. While a notable susceptibility was indicated for *Streptococcus pneumoniae* bacterial strains with MIC value of 50 µg/disc for the complex [ZnSL<sub>5</sub>.H<sub>2</sub>O].

The obtained ligands and some of their corresponding metal complexes were tested for their molluscicidal activity against the land snail *E. vermiculata*. The observation indicated that the ligands and their complexes did not show any activity at concentrations below 0.3 mg/L. The complexes showed good activity at concentration of 0.4 mg/L and the complex [ZnSL<sub>5</sub>.H<sub>2</sub>O] showed highest activity. It is also noted that the complexes were more active than their parent ligands which may be explained on the basis of the presence of metal ion that may enhance the molluscicidal activity. The LC<sub>50</sub> of the complexes was at concentration of 0.4 mg/L, while the LC<sub>50</sub> of the ligands was at higher concentrations of 0.5 mg/L.

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