

Preparation, Characterization and Biological Evaluation of β -Lactam Derived from 6-Amino Penicillanic Acid and Salicyldehyde

Rasha Hasan Jasim^{1*}, Mohammed Hamid Said² and Batool Qusai Ali¹

¹Department of Chemistry, Faculty of Education for Girls, University of Kufa, Iraq

²College of Pharmacy, University of Babylon, Iraq

Abstract

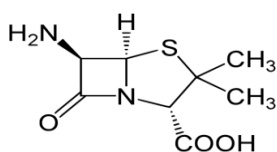
The present report is concerned with the synthesis novel Schiff base ligand by condense 6-Amino Penicillanic acid with Salicyldehyde. The ligand was characterized by ¹H-NMR, ¹³C-NMR, mass spectrometry, UV-Vis and FT-IR studies. The ligand have got more than one moiety which are capable of chelation, therefore complexes of Co(II), Ni(II), Cu(II), and Zn(II) were prepared. These complexes were characterized by UV-Vis, FT-IR, magnetic susceptibility and Molar conductivity studies. All these techniques indicate 1:2 stoichiometry of synthesized complexes. In all the complexes, Schiff base ligand acts as a tridentate ligand. Finally, the ligand and complexes have been screened for their antibacterial activity against four types of bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus facialis* and *Proteus mirabilis*) then it isolated from different classes of ulcerative infections while L showed to be tridentate ligand with single negative charge through three active groups, L has been associated with metal ions to form the claw complexes to get octahedral shape. The prepared ligand and its complexes illustrated a good inhibitory ability towards the three varieties bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*), while the four complexes failed to be suggested in treatment of ulcers that caused by *Streptococcus facialis*. So, the prepared compounds could be good alternatives to the common drugs which are used in treatment of ulcers.

Keywords: β -Lactam; Schiff base; 6-Amino penicillanic; Antibacterial; Antibiotic

Introduction

Developments in the field of coordination chemistry, which is closely bound up with syntheses and development organic and biological molecules as chelating ligand [1,2]. These molecules have been very extensive in recent years. A study of biomolecules ligand complex formation is interested to medical and pharmaceutical fields, because of its high biological activity against bacteria, fungi and tumors, in addition to its high stability as coordination compounds and solubility in the general solvents [3].

Schiff bases ligands have often been used as chelating ligands in the field of coordination chemistry. Chelating ligands containing N, S and O donor atoms show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions. These ligands can be classified by the number of site that gives electrons to the ion metallic (single claw Bidentate, tridentate, Tetradentate, etc.,) [4-9], it is known that the existence of metal ion bonded to biologically active compounds may enhance their activities Schiff base ligands with nitrogen donor atoms of the azomethine group and another donor atoms of the Schiff base ligand such as oxygen of carboxylic group afforded stable complexes with various transition metals. Beta-Lactam considered important item of antibiotics that contain a (C=O) group in β -Lactam ring in structural [10]. 6-Amino Penicillanic acid is considered as important compound in the varieties of β -Lactam types and traded in the first of antibiotics [11].



6-Aminopenicillanic acid

Experimental

Chemical

All chemicals used were reagent grade (Sigma Aldrich, B.D.H, C.D.H. and S.L. company) including 6-Amino Penicillanic acid, Salicyldehyde, CuCl₂·2H₂O, CoCl₂·6H₂O, NiCl₂·6H₂O, ZnCl₂, absolute ethanol, Pyridine, and DMSO.

Instrumentation

The proton ¹H and carbon ¹³C Nuclear Magnetic Resonance (NMR) spectra were recorded in DMSO were recorded on a Bruker Advance II 400 Spectrometer at room temperature the chemical shifts are reported in ppm relative to TMS (tri methyl saline). FT-IR spectra were recorded on 8400 FTIR Shimadzu spectrometer in DMSO-d₆ solvent. UV-Visible spectra were recorded on Shimadzu 1800 series spectrometer for ligand and complexes in DMSO-d₆ solvent. The Magnetic Moment Measurement was using Auto Magnetic Susceptibility Balance Sherwood scientific, The Molar Conductance Measurement in DMSO solvent using Digital conductivity meter alpha -800, addition to mass spectrum.

Preparation of Schiff base ligand

Ligand was prepared by dissolving 6-Aminopenicillanic acid (2.16

*Corresponding author: Rasha Hasan Jasim, Department of Chemistry, Faculty of Education for Girls, University of Kufa, Iraq, Tel: +9647816244470; E-mail: dr.rashahussainee@yahoo.com

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g, 0.01 mole) in 15 ml absolute ethanol (basic medium) and then add (1.22 g, 0.01 mole) of Salicylaldehyde the result mixture was refluxed for 14 hrs, following up its reaction using thin layer chromatograph, after filtered, dried and returned recrystallized from absolute ethanol the reaction follows illustrates this.

Preparation of complexes

For the preparation of complexes, a ligand (0.700 g, 0.002 mole) solution was mixed with 0.001 mole of (0.170 g, 0.2379 g, 0.2377 g, and 0.136 g) of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, and ZnCl_2 respectively in 15 ml of absolute ethanol. The mixture was refluxed for 4 h on a water bath, then cooling to room temperature, the colored complexes got precipitated slowly, which was filtered, washed repeatedly with distilled water and ethanol. Now, the complexes were dried over anhydrous calcium chloride in desiccators. Table 1 show chemical and physical properties of free ligand and its complexes.

Cases of study

Using cotton swab, samples were collected from focus of different ulcer types for patients suffered from ulcer as a complication for numerous diseases, so they were treated at Al Sadder Medical City in The Al Najaf Al Ashraf Government, Iraq. The phenotypic diagnosis was done according to the Morphological Characteristics after cultivation of these specimens on the MacConkey Agar, Manetol Salt Agar, Brain Heart Infusion Agar, and *Pseudomonas Agar* (For fluorescing).

Microscopic examination

In order to classify the isolated bacteria from the ulcer specimens the microscopic examination was done at magnification power $\times 1000$, Gram dye was used to classify the isolated bacteria into positive and negative response to Gram dye.

Biochemical tests

Catalase, Oxidase, Indole, Methyl Red, Simmons (Citrate consumption), and Gelatin Lysis Tests were applied to diagnose the isolated bacteria.

Biological activity

Disk Diffusion Method was applied for evaluation of the inhibitor ability of the original ligand and its complexes in the biological activity of the most active plantation of the isolated bacteria (*Staphylococcus aureus*, *pseudomonas aeruginosa*, *Streptococcus facials* and *Proteus mirabilis*).

Results and Discussion

Infrared spectra

The FT-IR of prepared Schiff bases were show absorption band at 3259 cm^{-1} due to $\nu(\text{OH})$ of carboxylate group this band shifted to lower frequency in the spectra of all complexes, the bands at 1629 cm^{-1} and 1720 cm^{-1} were assigned to stretching vibration of $\nu(-\text{CH}=\text{N}-)$ azomethene and $\nu(\text{C}=\text{O})$ lactam groups respectively on the complexation these bands have been shifted to higher frequency in the spectra of all complexes. The spectra of complexes recorded a new band in the range of $594\text{--}440\text{ cm}^{-1}$ for coordination with metal, the coordination occurred through the nitrogen atom of azomethene and two oxygen atom of $(\text{C}=\text{O})$ lactam and (OH) of carboxylate group. The IR frequencies of free ligand and complexes are reported in Table 2. Figures 1 and 2 show a spectra of free ligand and copper complex [12,13].

Electronic spectra

The electronic spectrum of the ligand and complexes have been measured in DMSO solution between 200-1100 nm at room temperature. The UV-Visible spectra of ligand include absorption peaks at wave lengths 288 nm, 270 nm which are assigned to $\pi-\pi^*$ transition of aromatic ring and $n-\pi^*$ transition for nonbonding pair of electron of nitrogen azomethene group, respectively. In the UV-Vis spectrum of complexes exhibits two peaks, due to the (C.T) [14-16]. The UV-Vis data of ligand and complexes are reported in Table 3 and Uv-Vis spectrum of the Schiff base ligand (Figure 3).

Mass spectrum

The mass spectrum of free ligand is shown in Figure 4. The spectrum manifestation a peak represents the parent ion (p+1) centered at 307. The general pattern of fragmentation for Schiff base ligand are summarized in Scheme 1 including four proposed pathways for breaking ligand [17,18].

$^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of the schiff base ligand

$^1\text{H-NMR}$ spectrum of Schiff base ligand in DMSO-d_6 appearance of different peaks (Figure 5). A signal at (δ 8.6) ppm was assigned for singlet due one proton of $-\text{CH}=\text{N}$ linkage in the ligand. The signal obtained in range (δ 7.2-7.8), other signals of $^1\text{H-NMR}$ for ligand have been explained in Table 4. The $^{13}\text{C-NMR}$ spectrum of Schiff base ligand also recorded in DMSO-d_6 (Figure 6 and Table 5). The $^{13}\text{C-NMR}$ spectrum of ligand showed the azomethene carbon peak at (δ 153) ppm. The carboxylic ($\text{C}=\text{O}$) group peak showed at (δ 167) ppm and the aromatic carbon peak are observed at (δ 124, 130, 124, 130, 116, 168) ppm [19,20].

Magnetic susceptibility and molar conductivity for coordination complexes

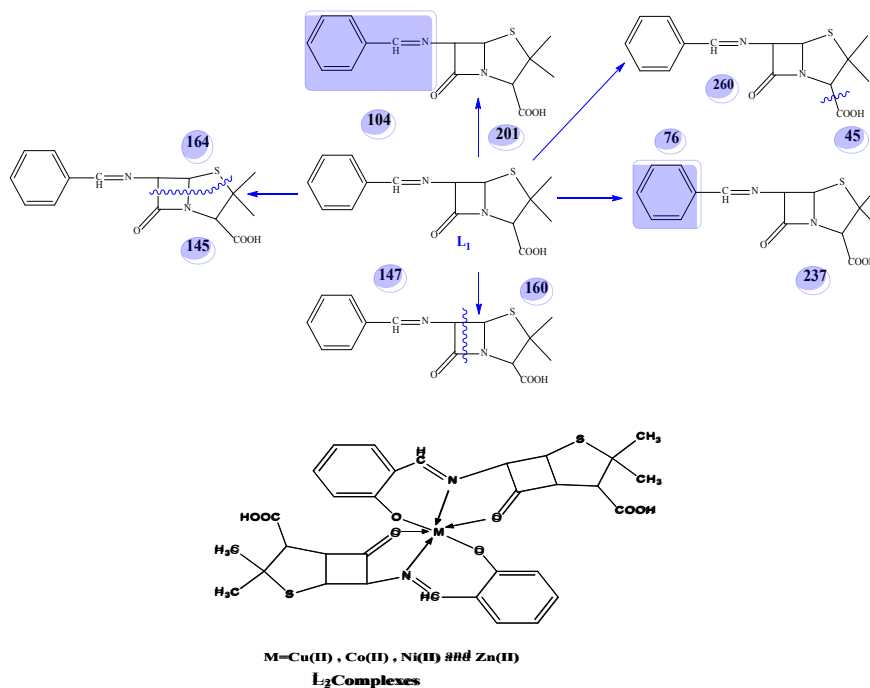
Magnetic Susceptibility properties of all complexes measure and compare them with the literature [21,22], which has been reached to form the vacuum of these complexes was found to be of the shape octahedral as for molar connectivity every complexes recipe is ionic (neutral) and Table 6 illustrates the magnetic susceptibility and molar

Compound Symbol	M. Wt.	MP °C	Color
L	320	136-138	Brown Orange
Cu [L] ₂	703.54	143-145	Light Green
Co [L] ₂	698.69	127-129	Green
Ni [L] ₂	756.93	128-130	Brown
Zn [L] ₂	705.39	160-162	Light Brown

Table 1: Data ligand and complexes show chemical and physical characterizations.

Compound	$\nu(\text{OH})$	$\nu(\text{C-H})_{\text{alph}}$	$\nu(\text{C-H})_{\text{alph}}$	$\nu(\text{C}=\text{O})$ Lactam	(C=N Azomethene)	M-N M-O
L	3259	2974	3064	1720	1629	-
Cu[L] ₂	3190	2976	3064	1726	1635	588 451
Co[L] ₂	3232	2974	3070	1728	1635	580 449
Ni[L] ₂	3236	2976	3064	1726	1631	594 440
Zn[L] ₂	3379	2974	3070	1728	1635	532 45

Table 2: FTIR data of schiff base ligand and metal complexes.



Scheme 1: Fragmentation pattern of ligand.

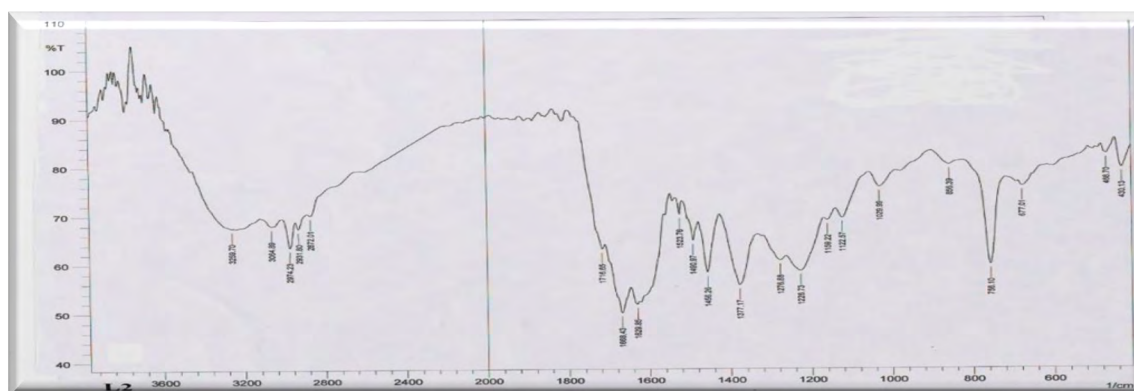


Figure 1: FTIR spectra of ligand L₂.

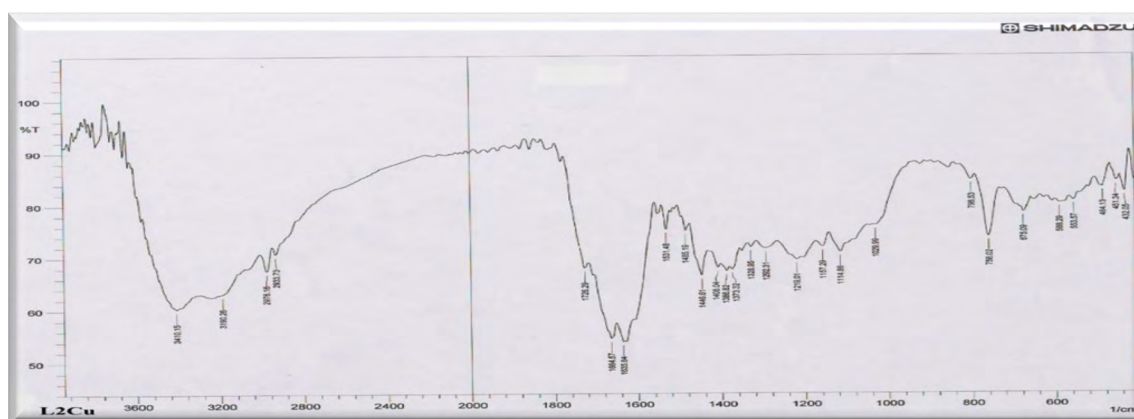


Figure 2: FTIR spectra of copper (ii) complex.

Complexes	Absorption Band λ_{max}	Assignment
L	2,88,270	$n-\pi^*$, $\pi-\pi^*$
Cu[L] ₂	3,78,287	C.T
Co[L] ₂	429	C.T
Ni[L] ₂	409	C.T
Zn[L] ₂	4,66,424	C.T

Table 3: Electronic spectra data of Schiff base ligand and metal complexes.

Compound	¹ H-NMR
L	CH ₃ (1.5 ppm, S, 6H)
	Aromatic (7.2-7.8 ppm, m, 5H)
	Azomethene (8.6 ppm, S, H)
	CHN (4.1 ppm, d, H)
	CH CO (4.3 ppm, d, H)
	DM SO (3.5, 2.5 ppm)

Table 4: Proton nuclear magnetic resonance spectra of the schiff base ligand.

Compound	¹³ C-NMR
L	(aromatic) 124, 130, 124, 130, 116, 168
	(azomethene) 153
	(COOH) 167
	(CH ₃) 25
	(CH) 79
	(β -Lactam) 80
	(CH) 64
	(CHN) 76
	(CHCO) 62

Table 5: Carbon nuclear magnetic resonance spectra of the schiff base ligand.

conductivity results for the complexes and ligand [23-25].

Anti-bacterial activity

The prepared complexes of ligand with Cu, Co, Ni and Zn were studied against biological activity of four different types of bacteria (*Staphylococcus aureus*, *Streptococcus facials*, *Pseudomonas aeruginosa* and *Proteus mirabilis*), at the concentrations of 1×10^{-6} , 1×10^{-5} , 1×10^{-4} , 1×10^{-3} M and compare to the inhibitory effect of the original ligand (L₂) toward same types of bacteria. These bacteria were isolated from the most active plantation of numerous type of ulcer samples (grades III and II burn ulcers, diabetes foot ulcer, in addition to bed ulcers).

The four complexes recorded an elvation in the inhibition ability comparsion to the original ligad which was precarcor of these complexes at different studied concentrations when the ligand and its complexes were applied on the plantation of *Staphylococcus aureus* as shown in Figure 7 and Table 7.

The complex of copper illustrated the highest inhibitory effect among the four complexes, on the other hand the complex of zinc showed approximate inhibitory effectes at the concentration of 1×10^{-6} to 1×10^{-4} M, then the ability of inhibition was increased at the greatest tested concentration (1×10^{-3} M), results of the inhibition ability of the cobalt and nickel complexes were so convergent at the four examined concentrations (Figure 7).

Figure 7 illustrates that the four complexes were failed to record an inhibitory data exceed those which recorded by original ligand even at the greatest tested concentration (1×10^{-3} M) when they applied on the plantation of *Streptococcus facials* (Table 8 shows the details information about the biological activity of *Streptococcus facials* at the original ligand and the four prepared complexes had been added).

The first concentration of the prepared complexes couldn't show significant elvation in the inhibition zone comparison to those at their corresponding ligand, as observed in the Figure 7, then; the ability of inhibition for the complexes towered plantation of *Pseudomonas aeruginosa* was grgually increased simultaneously with the increase of the complexes' concentrations (Table 9 and Figure 7).

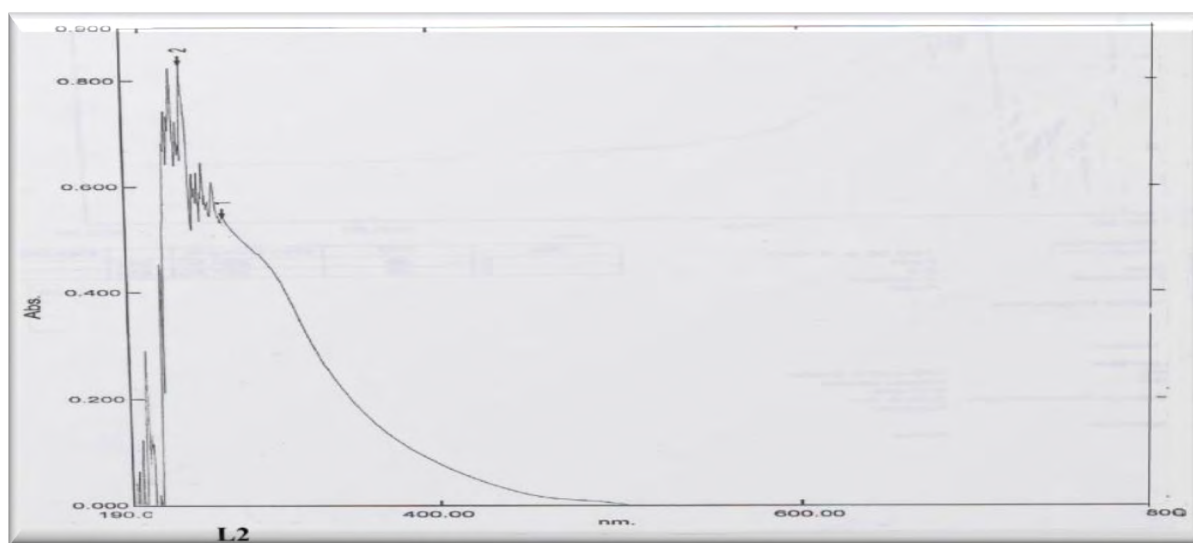


Figure 3: Electronic spectra of schiff base ligand.

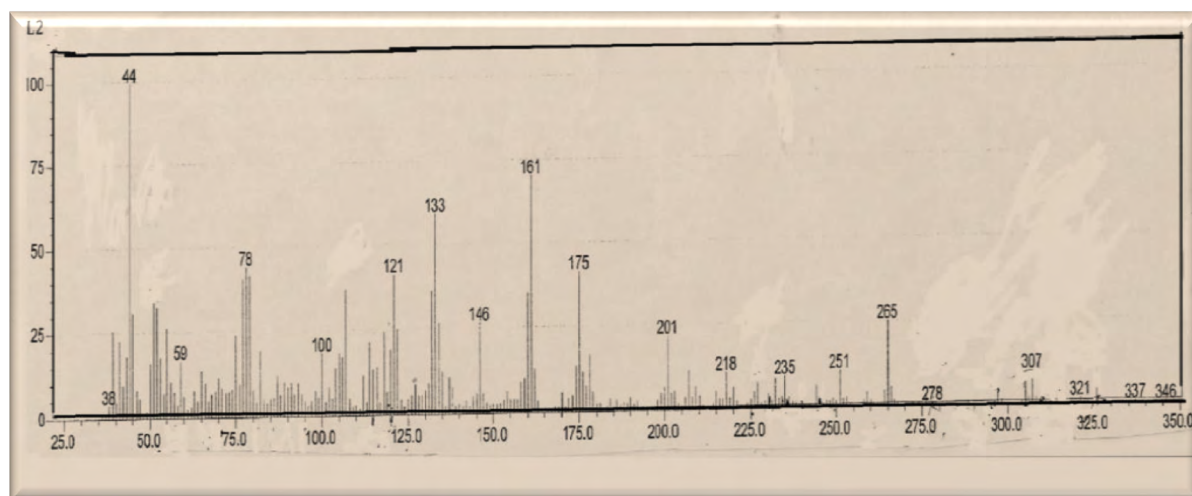


Figure 4: Mass spectrum of ligand.

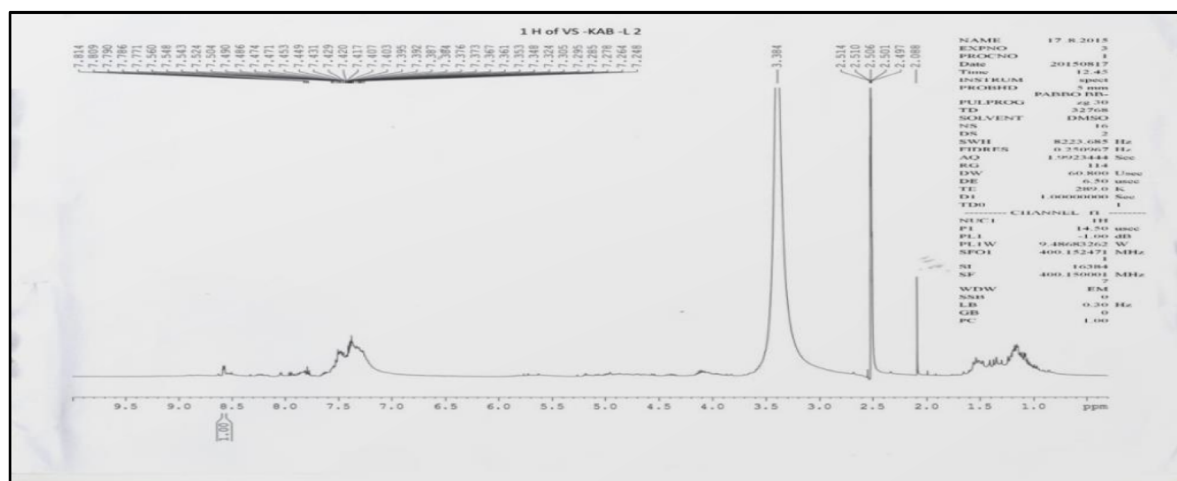


Figure 5: ¹H-NMR spectrum of ligand.

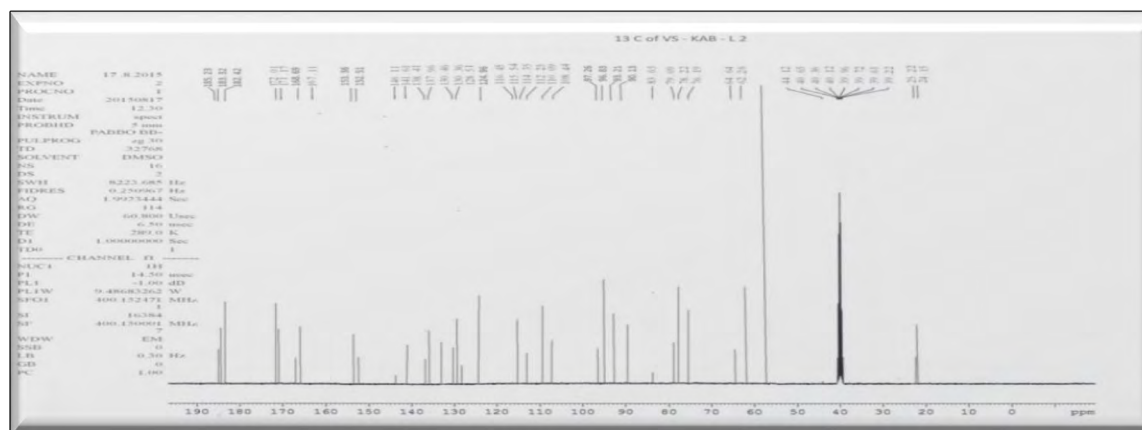


Figure 6: ¹³C-NMR spectrum of ligand.

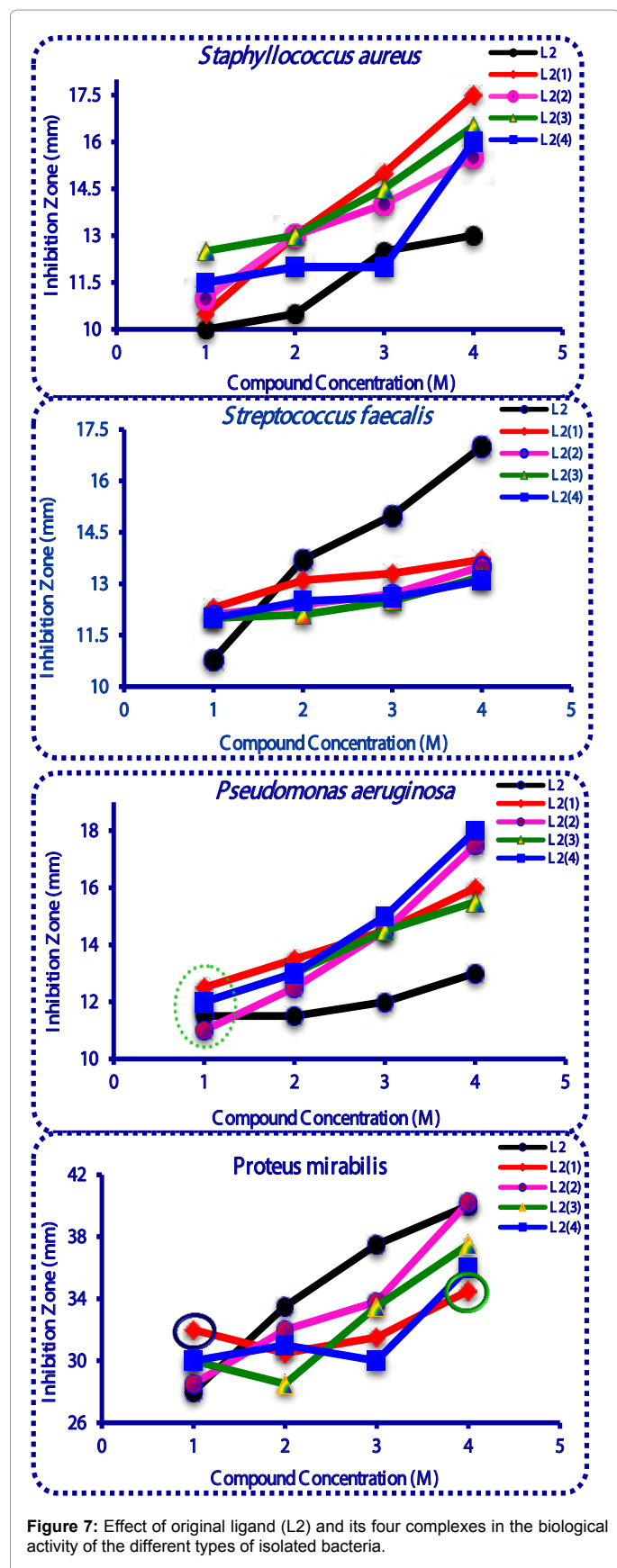


Figure 7: Effect of original ligand (L2) and its four complexes in the biological activity of the different types of isolated bacteria.

Figure 7 exposes the ability of inhibition data for the ligand and its complexes at they applied in the plantation of *Proteus mirabilis*, the complex of cobalt in addition to the original ligand were illustrated the highest inhibitory effect followed by the complexes of nickel then zinc, respectively, while complex of copper demonstrated approximate inhibitory effects at different concentrations, as shown in Table 10 and Figure 7.

Conclusions

The prepared ligand and its complexes illustrated a good inhibitory ability towards the three varieties bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*), while the four complexes failed to be suggested in treatment of ulcers that caused by *Streptococcus facials*. So, the prepared compounds could be good alternatives to the common drugs which are used in treatment of ulcers.

Complexes	Magnetic moment	Molar Conductivity	Geometry
Cu[L] ₂	1.7	5.9	Octahedral
Co[L] ₂	4	8.2	Octahedral
Ni[L] ₂	3.01	8.5	Octahedral
Zn[L] ₂	Diamagnetic	5.8	Octahedral

Table 6: Magnetic moments and molar conductivity data of schiff base ligand and metal complexes.

Concentration (M)	Compound Symbol				
	L	L (1)	L (2)	L (3)	L (4)
1 × 10 ⁻⁶	-	-	-	-	-
1 × 10 ⁻⁵	-	+	+	+	-
1 × 10 ⁻⁴	-	++	+	++	-
1 × 10 ⁻³	+	+++	+++	+++	+++

Table 7: Vital effect of ligand and complexes in vital work of bacterial *Staphylococcus aureus*.

Concentration (M)	Compound Symbol				
	L	L (1)	L (2)	L (3)	L (4)
1 × 10 ⁻⁶	-	-	-	-	-
1 × 10 ⁻⁵	+	-	-	-	-
1 × 10 ⁻⁴	++	+	-	-	-
1 × 10 ⁻³	+++	+	+	+	+

Table 8: Vital effect of the ligand and complexes in the biological activity of *Streptococcus facialis*.

Concentration (M)	Compound Symbol				
	L	L(1)	L(2)	L(3)	L(4)
1 × 10 ⁻⁶	-	-	-	-	-
1 × 10 ⁻⁵	-	+	-	-	-
1 × 10 ⁻⁴	-	++	++	++	++
1 × 10 ⁻³	+	+++	+++	+++	+++

Table 9: Vital effect of the ligand and complexes in the biological activity of *Pseudomonas aeruginosa*.

Concentration (M)	Compound Symbol				
	L	L(1)	L(2)	L(3)	L(4)
1 × 10 ⁻⁶	+	++	+	+	+
1 × 10 ⁻⁵	++	+	++	+	+
1 × 10 ⁻⁴	+++	++	++	++	+
1 × 10 ⁻³	+++	++	+++	+++	+++

Table 10: Vital effect of the ligand and complexes in the biological activity of *Proteus mirabilis*.

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