Egypt. J. Chem. **Vol. 64**, No. 10 pp. 5703 -5712 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Preparation ,Characterization and Study of Complexes Containing Beta-lactam Group with Some Transitional Elements and their Biological Activity

Ibtisam M Ali[a], Mohammed Hamid Said[b], Wafaa AlWazni[c]

[a]*Department of Chemistry, College of Science, Kerbala University, Kerbala - 56001. Iraq*

[b]*Department of Chemistry, College of Science for women, University of Babylon, Babylon - 51002. Iraq*

[c] *The Department of Biology, College of Science, Kerbala University, Kerbala - 56001, Iraq*

Abstract

The reaction of 6-amino pencillanic acid with Schiff base "(Z)-3-((3-hydroxybenzylidene) amino) phenol)" has formed a novel Azo–azomethine ligand (HL) "(7-((Z)-(2-hydroxy-4-((Z)-((3-hydroxyphenyl) imino) methyl) phenyl) diazenyl)-3, 3-dimethyl-6-oxo-2-thiabicyclo [3.2.0] heptane-4-carboxylic acid". The ligand (HL) is characterized by ^{1}H-NMR, ^{13} C-NMR, Mass Spectrometry, CHNs, Uv-Visible, and FT-IR. This new Ligand (HL) is used to prepare numerous complexes using transition metal ions Fe (II) Cu (II), and Ni (II). These complexes' properties are studied using different techniques such as Mass Spectroscopy, Atomic Absorption, FT-IR, UV-Visible, Magnetic Moment, and Molars Conductivity. All these techniques indicate 1:2 (M: L) stoichiometry's synthesized complexes. Azo-azomethine ligand (HL) acts as a tridentate ligand to form the octahedral shape complexes with sp^3d^2, d^2sp^3 hybridization. These new ligands and complexes are studied for their biological activity. The results show that the minimum inhibition concentration (MIC) of the prepared compounds has inhibition activity against *Staphylococcus aureus* bacteria and obviously affects the ability of pathogenic bacteria (*S. aureus*) to attain virulence factors such as Biofilm and hemolysin toxin

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

1. Introduction

Due to their distinctive properties and significance in chemical and biological applications, the transition metallic complexes are extensively studied by several chemical researchers [1]. Specifically, the azo-azomethine compound coordinated with metallic elements has several advantages such as biological and analytical efficacy. These compounds are characterized by high ability, effectiveness, and having antimicrobial [2], analytical [3], and biochemical reagents [4]. The bioactivity of the azo-azomethine metals complexes is indicated by recent studies as an antioxidant and other pharmacological properties [5]. The distinguishing property of the biological activity related to the azo-azomethine ligand is the possession of donor atoms such as O, N, and S, which provide different models for bonding with metallic ions [6].

These are significant compounds as they are featured with numerous ways of coordination done by various atoms in the Azo-azomethine ligand with metal ions. They also can act as multi-dentate because they have more than one donor site of electrons [7]. The 6-amino pencillanic acid is regarded as one of the most significant antibiotics, as the biological effectiveness of this compound is due to the availability of a beta-lactam ring in its compound [8]. Importantly, this beta-lactam ring is biologically effective against bacteria that continue to develop mechanisms that decrease these antibiotics' effectiveness [9].

Also, it is still very essential to conduct pieces of research and papers in the area of developing the composition of antibiotics [10]. Numerous recent researches are deeply concerned with developing these antibiotics by adding active groups to their composition [11] or by linking them with metal ions to produce complexes featured with biological activity [12]. However, the current work deals with developing the" 6-amino-penicillin acid" compound by synthesizing a new organic compound and then bonding with some metal ions to form new

*Corresponding author e-mail: ibtisam.m@uokerbala.edu.iq

Receive Date: 18 April 2021, Revise Date: 19 May 2021, Accept Date: 23 May 2021

DOI: 10.21608/EJCHEM.2021.72890.3614

effectively biological complexes, study them chemically [13], and analyze their biological activity [14]. Besides, this study shows a newly formed organic compound, i.e. (ligand HL) that exhibits a three-dentate behavior. When consistent with metals with a molar ratio of 1:2 (M: L), the biological study also shows the high efficiecy of this ligand (HL) and its complexes withCu (II) Ni (II) Fe(II) ions against a type of bacteria is *S. aureus*.

2. Experimental

2.1. Materials and Reagents

"3-amino phenol", "m-Hydroxybenzyldehyde" (BDH), acetic acid (CDH), and (6-amino pencillanic acid (Sigma) were used without purification. Sulpheric acid (CDH), Sodium Hydroxide (BDH), Sodium Nitrite, distilled water; Nickel Chloride (BDH) and Absolut Ethanol were purchased from (CDH) as required.

2.2. Instrumentation

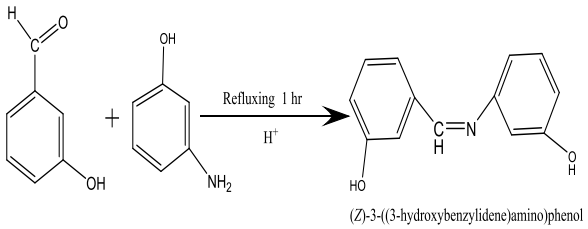
Melting points were identified at Stuart melting point SMP10. An elemental study of Elemental Research (EA) of a Perkin-Elmer CHNS 6000 was performed. Then, FTIR spectra were analyzed with Shimadzu FTIR 8400 in a spectrum of 4000-400 cm-1 using KBr disks. Also, 3000 Nano optimums were used to achieve UV &Visible spectrum utilized at room temperature. A Varian- 400 MHz was for measured ^{1}HNMR and ^{13}CNMR spectra. At room temperature at DMSO d^{6} Metal, the content of the complexes measured using flame atomic absorption techniques by Agilent 30A at the Department of Chemistry at the Mustansiriyah University.

2.3.1. Synthesis of the Schiff bases

Schiff base compound was prepared by dissolving (2 g, 0.0176 mol) 3-amino phenol in 10 mL of absolute ethanol then mixing it with (2.15g 0.0176 mol) of" m-hydroxybenzyldehyde" to prepare (Schiff base). The reaction was refluxed for 1 hour being stirred and the proper pH was adjusted to ≈ 4 by adding some drops of glacial acetic acid, noting that this process is followed up by a pH paper test). As stated by [15], the color of the reaction mixture was changed and precipitates were formed after cooling and evaporating the solvent. Schiff base compound was recrystallized using ethanol solvents to give the titled compounds as shown in scheme (1).

2.3.2. Synthesis of the ligand (HL)

At first, a solution of (2 g, 9.2 $x10^{-3}$mol) "6-amino pencillanic acid" was prepared by dissolving it in (10% H_2so_4in a baker 100 mL), and a "sodium nitrite" solution was prepared and concentrated 10% by dissolving (1 g, in 100 mL distilled water). Then, the two solutions were mixed being stirred, and maintained at the temperature (0-5Ċ). Next, the mixture was lifted for 15 minutes to complete the diazonium process. A solution was prepared by dissolving (2.2g - $9.2x10^{-3}$mol) of "(Z)-3-((3-hydroxybenzylidene) amino). Phenol" (Schiff base compound) prepared in the (2.3.1) section was dissolved in (10% NaOH). This solution was dissolved in the diazonium salt solution with stirring. The precipitate was filtered and washed several times with distilled water and dried at room temperature as shown in scheme (2). [16]

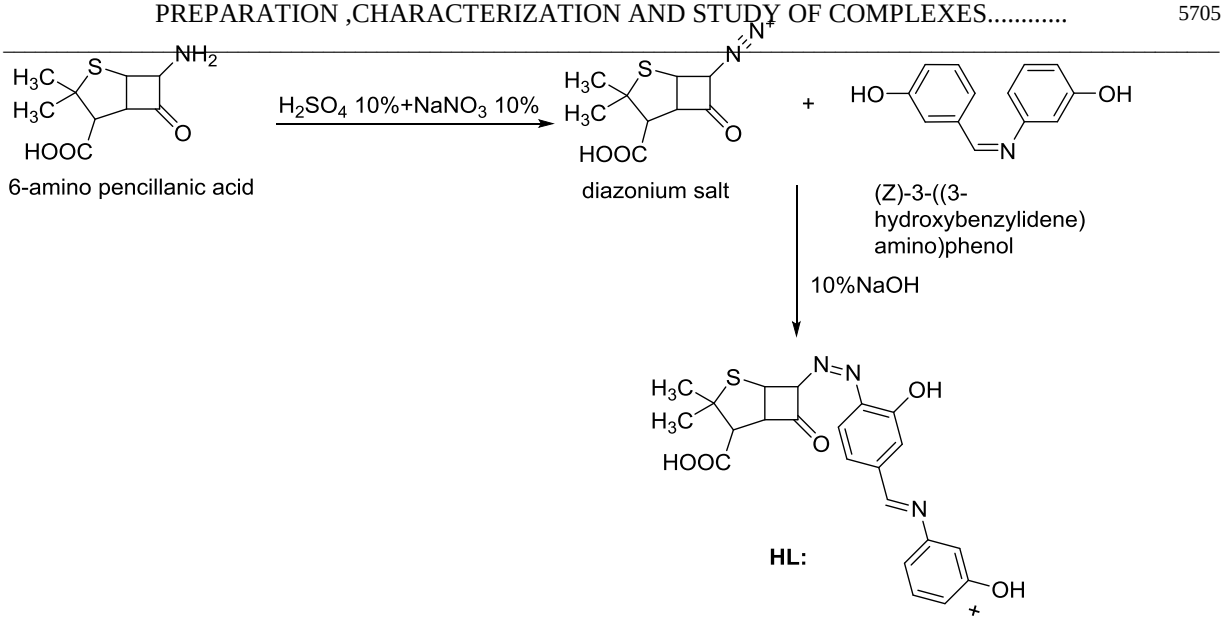


(Z)-3-((3-hydroxybenzylidene)amino)phenol

Scheme (1) Schematic diagram of synthesis of the Schiffbase(Z)-3-((hydroxybenzylidene)amino)phenol

2.3.3. Complex preparation of HL

Complexes were also prepared by dissolving (2.5 g, $5.8x10^{-3}$ mol) of (HL) in 10 mL of absolute ethanol and then was mixed with in the (0.5 g, $2.93x10^{-3}$ mol) desired metal ion ($CuCl_2.2H_2O$), (0.68g, $2.93x10^{-3}$ mol) $NiCl_2.6\ H_2O$), and (0.58 g, $0.93x10^{-3}$mol) ($FeCl_2.4H_2O$). The mixture was refluxed for 1 hour and the color was also changed. The resulting precipitates were formed after evaporating the solvent and recrystallizing it with two solvents, i.e. acetone and absolute ethanol [17].



Scheme (2) Schematic diagram of synthetic route to get the target ligand.

2.3.4. Biological activity

A- Bacterial isolates

Staphylococcus auruas bacteria were obtained from the microbiology lab at the Department of Biology at the College of Science at Kerbala University. The bacterial strains were biochemically diagnosed according to methods mentioned by Collee[18]and API staph system applied according to manufacturer's instructions. The isolated bacteria used in this study were selected depending on their ability to produce deep violet colors on the bottom and wall of the tubes used to determine the biofilm-forming as mentioned in Mather [19]. Then, the ability of these bacterial strains to produce hemolysin on blood agar plates was determined as described by Collee [20].

B-Antibacterial activity of Ligand (HL) and its complexes on the isolation bacteria

The selected bacterial strain of the bacterial suspension density was adjusted using 0.5 McFarland as a standard and was cultured on muller-Hinton agar, and agar well diffusion method. The antibacterial activity of Ligand (HL) and its complexes were determined according to Egharevba [21]. The holes with 8 mm diameter were done by corky bore and were carefully filled with 100 µl from each concentration of the ligand (0.005, 0.01, 0.02, 0.04, 0.06, and 0.08g/mL) by using micropipettes. Distilled water was added to one hole to be as control. Then, the Petri dishes were incubated at 37 Ċ for 24 hours. After the incubation period, each plate was observed and the inhibition zone was measured by the ruler; this was repeated three times for each concentration. The minimum inhibitory concentration (MIC) of Ligand (HL) and its complexes were determined according to [22] represented by the last concentration with little or no visible growth.

C-Effect of Ligand (HL) and there complexes on the bacterial virulence factors

MIC of Ligand (HL) and its complexes were added to the bacterial suspension, where the deamination biofilm activity and hemolysin production of bacteria cell was repeated after treated with these compounds.

3.Results AND Discussion:

3.1 A characteristic of the ligand and the metal complexes Physical properties:

The physical properties of ligand (L) and $[Cu(L)_2]$ $[Ni(L)_2]$, $[Fe(L)_2]$ complexes given in Table (1). The (L) and complexes were colored in Brown color and were non-hygroscopic. The ligand melted at a temperature104 Ċ and the $[Ni(L)_2]$ complex have 365 Ċ while $[Cu(L)_2]$, $[Fe(L)_2]$ are decomposed. Those have a good yield between 56% and 64%.

The solubility of the ligand and complexes were measured and the results are listed in Table (1) the molar conductivity values of the ligand and their complexes in the range of 1.5 to 25 (mol-1 ohm-1 cm^2) were evaluated in DMSO, as these values show that they are nonelectrolyte [23, 24]

Table (1) The physical properties and Solubility:.

Comp	M.wt g/mol	m.p Ċ	color	Yield%	Solubility					
					D.water	Methanol	Ethanol	CHCl3	DMF	DMSO
(L)	439	104	Brown	64%	_	+++	+++	+++	+++	+++
$[Cu(L)_2]$	941	decomposition	Brown-orang	56%	_	_	_	_	+++	+++
$[Ni(L)_2]$	936	365	Brown-Yellow	64%	_	_	_	_	+	+
$[Fe(L)_2]$	933.8	decomposition	Brown-orang	61%	_	_	_	_	+++	+++

(-) non soluble (+) The partial soluble (+++) High solubility

3.2 Metal and Elemental Micro analysis (C.H.N.S)

Micro elemental analysis (C.H.N.S.) for ligand and the atomic absorption of the synthesized complexes have played a key role in predicting the final structure and formula of the compound. With a metal analysis, the result shows that the elemental analysis of (L) ligand is (59.4, 4.2, 9.6 7.15%) for the (C%, H%, N%, S%) respectively and the atomic absorption of complexes $[Cu(L)_2]$ $[Ni(L)_2]$ and $[Fe(L)_2]$ are (5.8, 4.98, 5.1%).

3.3 Electronic spectra:

The UV- visible spectrum was determinedat in DMSO at wave lengths ranged (250-700nm) range and the results are explaind in Table (2) and Figure (1). The electronic spectra of the prepared ligand and $[Cu(L)_2]$, $[Ni(L)_2]$ and $[Fe(L)_2]$ complexes showed two peaks at 270 nm and 380 nm assigned to n→π*and π→ π*transitions that belong to Azo,azomethine groups of ligand[25].

The FTIR of synthesized ligand (L) showed absorption bands belonging to the Hydroxyl group at 3348 cm^{-1}, carbonyl of lactam group ν (C=O) at 1643 cm^{-1}, and azo group ν(N=N) at 1525 cm^{-1}. The hydroxyl,carbonyl, and azo groups were shifted to lower wavenumber, along with the occurrence of a change in shape and the location of absorption. This shows the participation in the coordination process in all complexes $[Cu(L)_2]$, $[Ni(L)_2]$ and$[Fe(L)_2]$ and this Absorptionmaxima were shifted to the higher values at the sites (295,365, 365)

nm in the complexes $[Cu(L)_2]$ $[Ni(L)_2]$ and $[Fe(L)_2]$ respectively , along with the occurrence of a change in the shape of peak upon coordination process. The electronic transient of $[Cu(L)_2]$ complex has wide bands at 475nm. It is compatible with the transient $^2Eg \rightarrow {}^2T_2g$ of many octahedral coppers (II) complexes, where a blue shift is shown due to coordination of Octahedral and the band at 293 and its return to the charge transition (C.T). In the Electronic spectra of hexagonal nickel (II) complex, two bands (536nm, 288nm) appeared which corresponds to ^3A_2g (F) _ ^3T_1g (p) and charge transition (CT). The UV-visible spectrum of the ferrous (II) complex derived from the (L) showed two absorption peaks at the site (410 nm) and (291nm) due to the CT electron transition and ($^5T_2g \rightarrow {}^5Eg$) [26, 27].

3.4 FTIR Spectra:

is in agreement with the mentioned in the literature [28,29]. However, the band at1606 cm^{-1} of the azomethine group (C=N) did not participate in the coordination process because it was not situated in a favorable position to form a 4 or 5-member ring to support the coordinate process. Important characteristic peaks in the FT-IR spectra were also explained in Table(3)andFigure(2).

Table (2) The electronic spectral data of the ligands and the complexes (nm)

No	Compounds	absorption nm	band assignment	Geometry
1	L	272 ,380	π-π*, n-π*	_
2	$[Cu(L)_2]$	293 ,475	CT , $^2Eg \rightarrow {}^2T2g$	octahedral
3	$[Ni(L)_2]$	288 ,536	CT , $^3A2g_{(F)} \rightarrow {}^3T1g_{(p)}$	octahedral
4	$[Fe(L)_2]$	291 ,410	CT , $^5A2g_{(F)} \rightarrow {}^5T1g_{(P)}$	octahedral

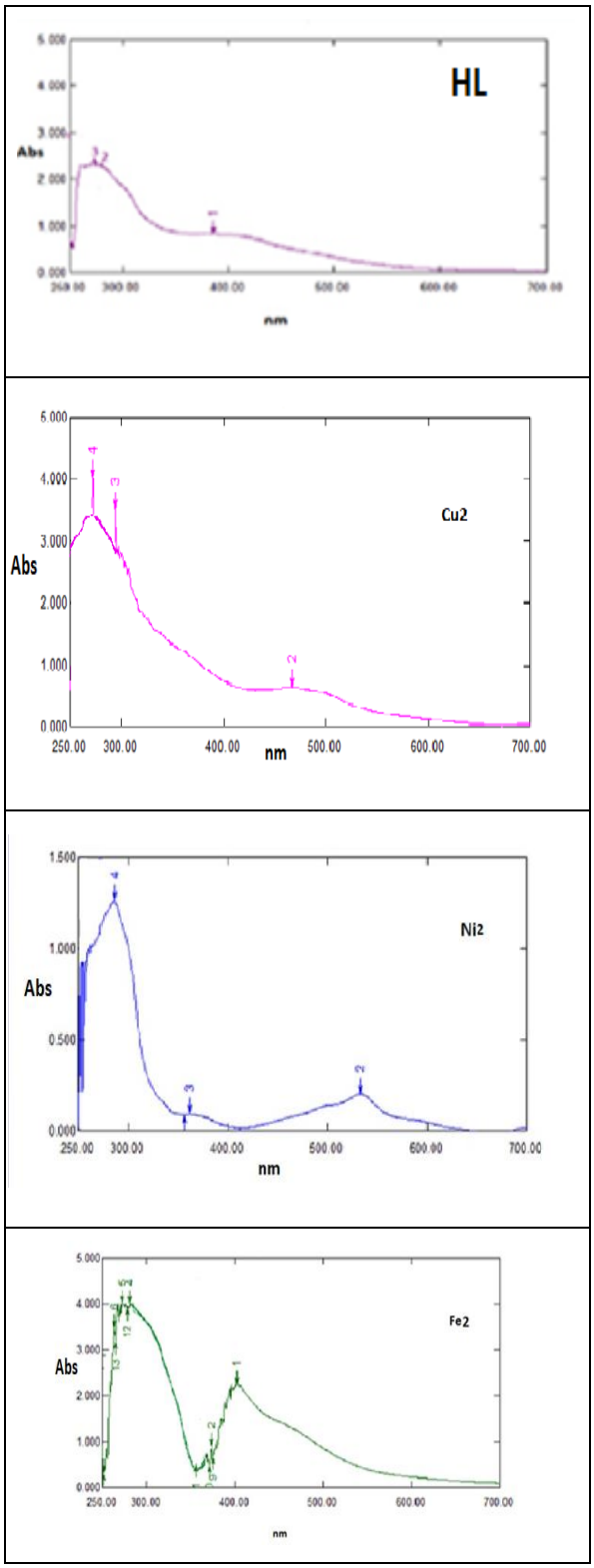


Fig (1) .The Uv-Visible spectrum of ligand (L) and [$Cu(L)_2$], [$Ni(L)_2$], [$Fe(L)_2$]

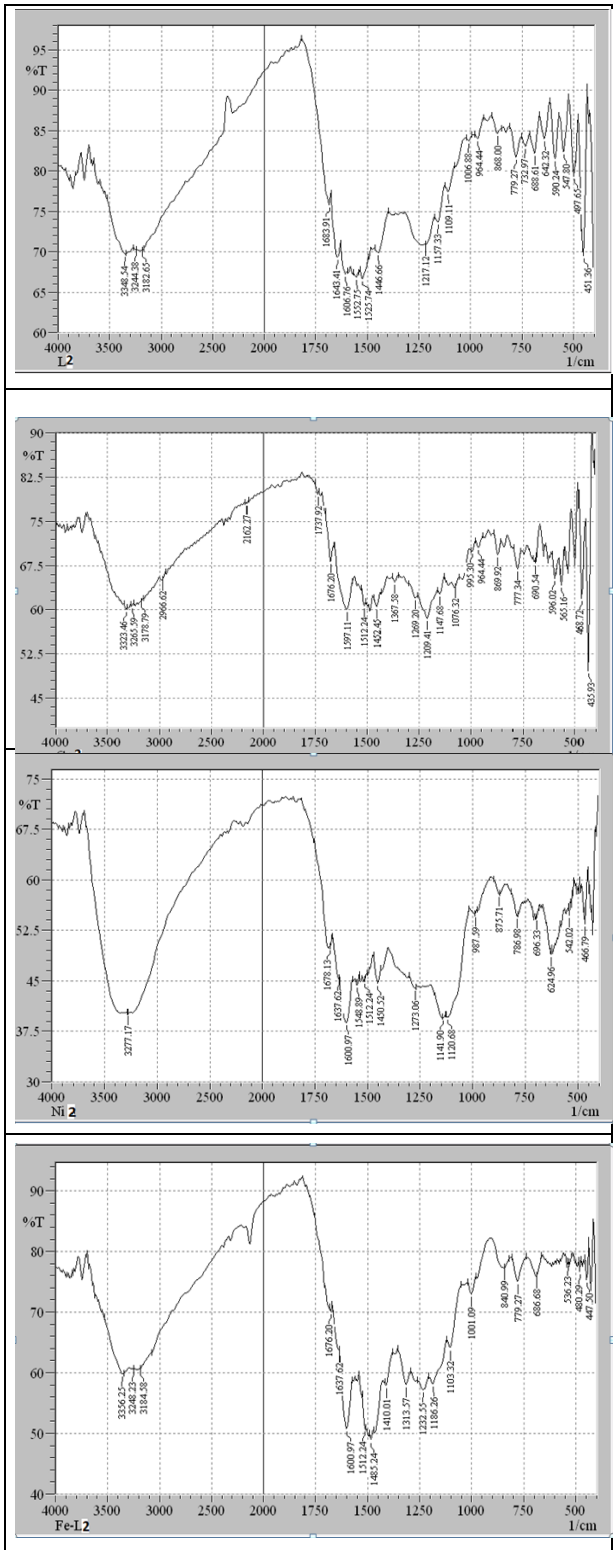


Fig (2). The FTIR spectrum of(L)ligand and complexes.

Table (3) some selected FTIR spectral data of the Schiff base and the complexes (cm^{-1})

Compounds	ν(O-H)	N(COOH)	ν (C=O) Lactam	ν (C=N)	ν(N=N)	ν(M-O)	ν(M-O)	ν(M-N)
L	3348	1683	1643	1606	1525	_	_	_
$[Cu(L)_2]$	3323	1676	1640	1597	1512	468	565	596
$[Ni(L)_2]$	3277	1678	1637	1600	1512	466	542	642
$[Fe(L)_2]$	3356	1676	1637	1600	1512	447	480	536

3.5 Mass spectroscopy

The mass spectrum of the ligand (L) showed a mother M^+ ion peak m/e at 437.8 which is equivalent to its molecular weight. The fragmentation peaks at m/e 345, 239, and 313m/e are ascribed to the cleavage[$C_{15}H_{14}N_4O_5S$],[$C_{14}H_9N_4O_5$] ,[$C_{12}H_9N_4O_2S$] ,respectively. It showed the correctness of the molecular structures of the ligan.

The Scheme (3)shows the Ligand has taken multiple important fractionation paths, namely: azomethine carbonyl azo group combined with the formation of positive molecular ions and other positively charged fractions[30,31].

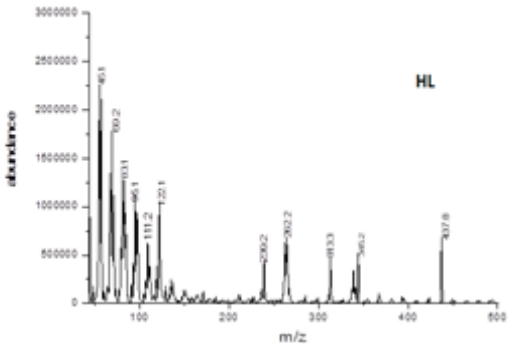
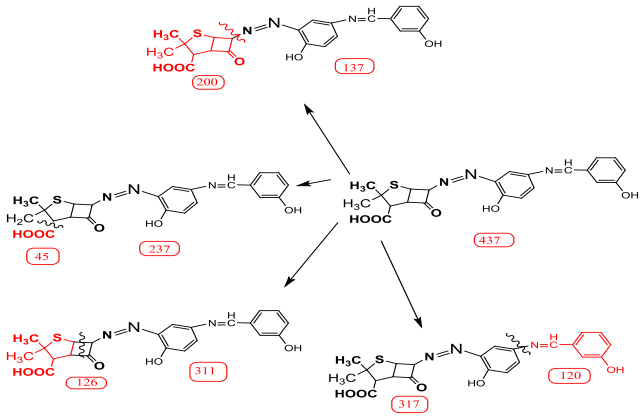


Fig (3) . The prepared ligand mass spectrum.



Scheme (3) the fragmention pattern of ligand (L)

3.6 Magnetic susceptibility:

Magnetic Susceptibility properties of the complexes [Cu(L)2], [Ni(L)2] and[Fe(L)2] measured are (1.8, 3.2 and 5.4) MB respectively compared them with the literature [32,33] which showed that forming these complexes was found to be the octahedral shape. The results of analyses and measurement of organic compounds used as a ligand can coordinate with metal ions through the oxygen atoms of the hydroxyl, carbonyl groups, and nitrogen atom of the azo group, where the ligand (L) acts as a tridentate ligand to form the octahedral shape complexes with sp3d2, d2sp3Hybridization,as show in figure(4).

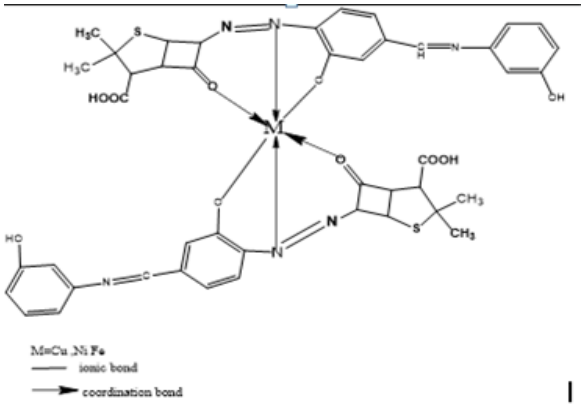


Fig (4). The suggested prepared complexes of (L):

3.7 Biological activity of (L) and complexes:

A.The MIc of (L),[Cu(L)2],[Ni(L)2] and[Fe(L)2]

The MIC of (L),[Cu(L)2],[Ni(L)2],[Fe(L)2] was achieved against S. aureus bacteria compared with distilled water as a control group. The minimum inhibition concentration (MIC) for (L), [Cu(L)2],[Ni(L)2], and [Fe(L)2] were (0.01, 0.005,0.02, 0.02, respectively. This component provided clear activity against pathogenic bacteria as listed in Table (5) modes and bonding with metal ions.

B. Antibacterial activity of (L) and complexe

The MIC of (L),[$Cu(L)_2$] ,[$Ni(L)_2$],[$Fe(L)_2$] was achieved against *S. aureus* bacteria in the compulsion to distilled water as control as shown in Figure (5). The synthesized ligand and their complexes of penicillin derivatives showed a significant inhibition zone estimated(10mm, 19mm, 10 mm) for each of the (L),[Cu ($L)_2$] and [$Fe(L)_2$], respectively.

However,[$Ni(L)_2$] showed narrow inhibition zone area compared with the other compounds. The results indicate that the complexes showed more activity and the ligands did not have any activity against the same microorganisms under experimental conditions. Through biochemical and morphologic modifications, the bacteria achieve resistance to antibiotics. This suggests that the Chelation facilitates the ability of a complex to significantly cross a cell membrane Chelation and decreases the polarity of the metal ion as a result of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring [34]. Such Chelation improves the lipophilic character of the central metal atom, which subsequently favors its permeation through the cell membrane's lipid layer. The variation in the effectiveness of various compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in the ribosome of microbial cells [35,36].

Having studied the ability of *S. aureus* bacteria to provide biofilm and hemolysin, the current results obviously explained that these bacteria strains were active producers for these virulence factors after treating them with these bacterial suspensions with MIC of (L),[$Cu(L)_2$],[$Ni(L)_2$] and ,[$Fe(L)_2$].

The results also explained that *S. aurous* bacteria lost their capacity to provide these virulence factors (biofilm & hemolysin enzyme) after their treatment with the MIC bacteria as shown in Figure (5) and Table (6) where the ligand (L) and its complexes reduced the biofilm & hemolysin enzyme. This proposes that Chelation can make the complex's capacity decrease virulence factor formation due to reduction in the polarity of the metal ion by partial positive charge with donor groups [37,38].

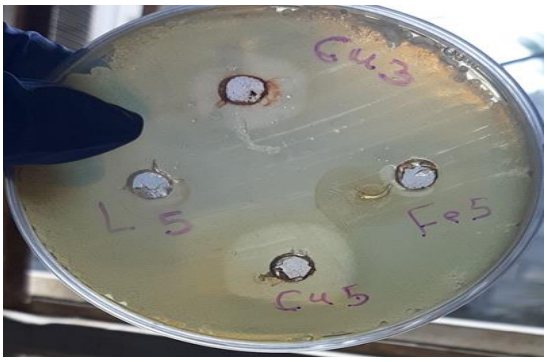


Fig (5). The inhibition zone for ligand (L) and its complexes against *S. aureus* bacteria.

Table (5) The minimum inhibitory concentration (MIC) g/mL of (L) and complexes

Name of pathogen	Compounds	Minimum 0.005	Inhibition 0.01	MIC 0.02	0.04	0.06	0.08
S. aureus.	(HL)	-	+	-	-	-	-
	[$Cu(L)_2$]	+	-	-	-	-	-
	[$Ni(L)_2$]	-	-	+	-	-	-
	[$Fe(L)_2$]	-	-	+	-	-	-

\+ positive for ligand and complexes, - negative for ligand and complexes

Table (6) The hemolysin & biofilm of ligand (L) and its complexes.

Pathogen virulence Factor	*Staphylococcus aurous*	Minimum 0.005	Inhipitory 0.01	Concentration 0.02	MIC 0.04	0.06	0.08
Himolysin	(L)	–	–	–	–	–	–
	$[Cu(L)_2]$	+	–	–	–	–	–
	$[Ni(L)_2]$	–	–	+	–	–	–
	$[Fe(L)_2]$	–	–	+	–	+	–
Biofilm	(L)	–	+	–	–	–	–
	$[Cu(L)_2]$	+	–	–	–	–	–
	$[Ni(L)_2]$	–	–	+	–	–	–
	$[Fe(L)_2]$	–	–	+	–	–	-

Fig (7). the effect before and after treated with ligand (L) and its complexes.

.

Conclusion

the results of analyzes and measurement of organic compounds that uses as ligand can be co-ordinated with metal ions Through the oxygen atoms of the hydroxyl ,carbonyl groups and nitrogen atom of the azo group, where the ligand (L) acts as a tridentate ligand to form the octahedral shape complexes with sp^3d^2, d^2sp^3Hybridization were The prepared ligand (L)and there complexes, $[Cu(L)_2]$,$[Ni(L)_2]$,$[Fe(L)_2]$In a nutshell, the newly prepared ligand (L) and their complexes of $[Cu(L)_2]$,$[Ni(L)_2]$,$[Fe(L)_2]$ demonstrated a good inhibitory ability towards the (*Staphylococcus aureus*) bacteria. Also, the presence of the metal ions in the complexes reducej the biofilm & hemolysin enzyme. Thus, the prepared compounds could be good replacements for the public drugs used in the treatment of ulcers and other related diseases.

References

1. Davies, J. A. (1996). Synthetic coordination chemistry: principles and practice. World Scientific.
2. Sahan, F., Kose, M., Hepokur, C., Karakas, D., & Kurtoglu, M. (2019). New azo- azomethine-based transition metal complexes: Synthesis, spectroscopy, solid- state structure, density functional theory calculations and anticancer studies. Applied Organometallic Chemistry, 33(7), e4954.
3. Berhanu, A. L., Mohiuddin, I., Malik, A. K., Aulakh, J. S., Kumar, V., & Kim, K. H. (2019). A review of the applications of Schiff bases as optical chemical sensors. TrAC Trends in Analytical Chemistry, 116, 74-91.

4.Warra, A. A. (2011). Transition metal complexes and their application in drugs and cosmetics-a

Review. Journal of Chemical and Pharmaceutical Research, 3(4), 951-958.

5. Sarigul, M., Sari, A., Kose, M., McKee, V., Elmastas, M., Demirtas, I., &Kurtoglu, M. (2016). New bio-active azo-azomethine based Cu (II) complexes. InorganicaChimicaActa, 444, 166-175.
6. Abdi, K., Nowroozi, A., Mansouri-Torshizi, H., & Malekmohammadi, R. (2020). A Zn (II) complex of tetraaza macrocyclic Schiff base ligand. Synthesis, characterization, its experimental and therotical interaction studies with CT-DNA and BSA. Nashrieh Shimi va Mohandesi Shimi Iran.
7. Liu, X., Manzur, C., Novoa, N., Celedón, S., Carrillo, D., &Hamon, J. R. (2018). Multidentate unsymmetrically-substituted Schiff bases and their metal complexes: Synthesis, functional materials properties, and applications to catalysis. Coordination Chemistry Reviews, 357, 144-172.
8. KONG, K. F., Schneper, L., &Mathee, K. (2010). Beta- lactam antibiotics: from antibiosis to resistance and bacteriology. Apmis, 118(1), 1-36.
9. Paul, M., Lador, A., Grozinsky- Glasberg, S., &Leibovici, L. (2014). Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. Cochrane Database of Systematic Reviews, (1).
10. Sundareshan, V., &Khardori, N. (2020). Diagnostic microbiology from the beginning to the future: regional antibiograms as public health tools to slow down antibiotic resistance. The Indian Journal of Pediatrics, 87(1), 48-50.
11. Lund, F., &Tybring, L. (1972). 6β-amidinopenicillanic acids—a new group of antibiotics. Nature New Biology, 236(66), 135-137.
12. Shehata, M. R., Shoukry, M. M., &Barakat, M. H. (2004). Coordination properties of 6-aminopenicillanic acid: binary and ternary complexes involving biorelevant ligands. Journal of Coordination Chemistry, 57(16), 1369-1386.
13. Crea, F., Cucinotta, D., De Stefano, C., Milea, D., Sammartano, S., &Vianelli, G. (2012). Modeling solubility, acid–base properties and activity coefficients of amoxicillin, ampicillin and (+) 6-aminopenicillanic acid, in NaCl (aq) at different ionic strengths and temperatures. European journal of pharmaceutical sciences, 47(4), 661-677.
14. Alabdali, A. J., Sabah, Y. H., &Mohsien, R. A. (2016). Synthesis, Characterization and Biological Evaluation of Penicillin Derivatives Complexes with Some Transition Metal Ions. Int. J. Curr. Microbiol. App. Sci, 5(12), 321-332.
15. Al Zoubi, W., Al- Hamdani, A. A. S., Ahmed, S. D., &Ko, Y. G. (2018). A new azo- Schiff base: Synthesis, characterization, biological activity and theoretical studies of its complexes. Applied Organometallic Chemistry, 32(1), e3895.
16. Azam, M., Al-Resayes, S. I., Trzesowska-Kruszynska, A., Kruszynski, R., Shakeel, F., Soliman, S. M., ... & Wabaidur, S. M. (2020). Zn (II) complex derived from bidentate Schiff base ligand: Synthesis, characterization, DFT studies and evaluation of anti-inflammatory activity. Journal of Molecular Structure, 1201, 127177.
17. Adnan, S. (2020). Synthesis, Spectral Characterization and Anticancer Studies of Novel Azo Schiff Base And its Complexes with Ag (I), Au (lll) And Pt (lV) ions. Egyptian Journal of Chemistry, 63(12), 4749-4756.

DOI:https://dx.doi.org/10.21608/ejchem.2020.23312.2438

18. Karam, M. R. A., Habibi, M., &Bouzari, S. (2018). Relationships between virulence factors and antimicrobial resistance among Escherichia coli isolated from urinary tract infections and commensal isolates in Tehran, Iran. Osong public health and research perspectives, 9(5), 217.
19. Adwan, G., &Mhanna, M. (2009). Synergistic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens. Asian Pacific Journal of Tropical Medicine, 2(3), 46-51.
20. Ahmad, I., Mehmood, Z., & Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. Journal of ethnopharmacology, 62(2), 183-193.
21. Aiyegoro, O., Adewusi, A., Oyedemi, S., Akinpelu, D., & Okoh, A. (2011). Interactions of antibiotics and methanolic crude extracts of Afzelia Africana (Smith.) against drug resistance bacterial isolates. International journal of molecular sciences, 12(7), 4477-4487.
22. Khdur, R. A., & Zimam, E. H. (2018). Synthesis and characterization of some new β-lactam derivatives from azo sulphadiazine and its biological evaluation as anticancer. Oriental Journal of Chemistry, 34(1), 371.

23. Adnan, S., & Neamah, R. (2020). Study the Biological Activity for Shiff Base and B–Lactam Compounds that Synthesis and Identification from Pyrimidine Derivatives. International Journal of Pharmaceutical and Clinical RESEARCH, 11(01), 80-87.
24. Mumtaz, A., Mahmud, T., Elsegood, M. R., & Weaver, G. W. (2016). Synthesis and characterization of new Schiff base transition metal complexes derived from drug together with biological potential study. J Nucl Med Radiat Ther, 7(310), 2.
25. Awad, A. A., Hasson, M. M., & faron Alfarhani, B. (2019, September). Synthesis and characterization of a new Schiff base ligand type N2O2 and their cobalt (II), nickel (II), copper (II), and zinc (II) complexes. In Journal of Physics: Conference Series (Vol. 1294, No. 5, p. 052040). IOP Publishing..
26. Salehi, M., Faghani, F., Kubicki, M., & Bayat, M. (2018). New complexes of Ni (II) and Cu (II) with tridentate ONO Schiff base ligand: synthesis, crystal structures, electrochemical and theoretical investigation. Journal of the Iranian Chemical Society, 15(10), 2229-2240.
27. Gönül, İ. (2019). Synthesis and structural characterization of ONO type tridentate ligands and their Co (II) and Ni (II) complexes: Investigation of electrical conductivity and antioxidant properties. Inorganica Chimica Acta, 495, 119027.
28. Yamashita, H., Koikawa, M., & Tokii, T. (2000). Synthesis, structure, and magnetic properties of tetranuclear copper (II) complexes of tridentate dianionic ligands with an ONO donor set. Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 342(1), 63-68.
30. Ismail, A. H., & Al-Bairmani, H. K. (2020). Nano-synthesis, spectroscopic characterisation and antibacterial activity of some metal complexes derived from Theophylline. Egyptian Journal of Chemistry, 63(12), 4951-4962. DOI:https://dx.doi.org/10.21608/ejchem.2020.32582.2690
31. Saleh, M., Ayoub, A. I., & Hammady, A. O. (2020). Synthesis biological studies of some new heterocyclic compound derived from 2-chloro-3-formyl quinoline and 4-(benzyl sulfonyl) acetophenone. Egyptian Journal of Chemistry, 63(12), 4-6. DOI:https://dx.doi.org/10.21608/ejchem.2020.26354.2535
32. Ispir, E. (2009). The synthesis, characterization, electrochemical character, catalytic and antimicrobial activity of novel, azo-containing Schiff bases and their metal complexes. Dyes and Pigments, 82(1), 13-19.
33. Lotfi, A. (2020). Synthesis and characterization of novel mixed complexes derived from 2-aminomethylbenzimidazole and 2-(1H-Benzimidazol-2-yl) aniline and theoretical prediction of toxicity. Egyptian Journal of Chemistry, 63(12), 4757-4767. DOI:https://dx.doi.org/10.21608/ejchem.2020.24418.2453
34. Ispir, E. (2009). The synthesis, characterization, electrochemical character, catalytic and antimicrobial activity of novel, azo-containing Schiff bases and their metal complexes. Dyes and Pigments, 82(1), 13-19.
35. Dahi, M. A., & Jarad, A. J. (2020, November). Synthesis, characterization and biological evaluation of thiazolyl azo ligand complexes with some metal ions. In Journal of Physics: Conference Series (Vol. 1664, No. 1, p. 012090). IOP Publishing.
36. Mulye, S. S., Maurya, A. S., Kamble, S. A., Deshmukh, P. V., Yadav, L. S., Mishra, R. K., & Jain, A. (2020). Medicinal and Phytochemical Analysis of Alcoholic Whole Fruit Extracts of Actinidia Deliciosa. Journal of Scientific Research, 64(1).
37. Bose, S., Khodke, M., Basak, S., & Mallick, S. K. (2009). Detection of biofilm producing staphylococci: need of the hour. Journal of clinical and diagnostic research, 3(6), 1915-1920.
38. Halim, R. M. A., Kassem, N. N., & Mahmoud, B. S. (2018). Detection of biofilm producing staphylococci among different clinical isolates and its relation to methicillin susceptibility. Open access Macedonian journal of medical sciences, 6(8), 1335.