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Article

Exploring the Antimicrobial Activity of some Oreganometallic complexes Derived from 6- methoxy -2- amino Benzothiazole

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Abstract:

The Oregano Metallic complexes of Cobalt (II) and Nickel (II) were prepared by Complexation reaction with new organic compound Derived from (6- methoxy -2- amino Benzothiazole) type Schiff base **(DTIMP)** . The structures of these organometallic complexes were verified by several spectroscopic techniques ;by using Carbon , Hydrogen and Nitrogen Elemental analysis technique , FT-IR spectroscopy , UV-Vis. spectroscopy. and in order to determine the proportion of elements in their complexes, the technique of (FAAS) spectroscopy was used. a DMSO at a concentration of (1×10^{-3} M) was Used as a solvent to measure the electrolytic property of complexes..

The Comparison of the ligand spectrum **(DTIMP)** with the organometallic complexes in infrared technique showed a difference suitable for explaining the Complexation process ;this was also supported by the interpretations obtained from the UV-vis spectra of the ligand and its organometallic complexes, where the complexes showed high-intensity electronic transitions due to charge transfer of the type (M→L). Also, estimating the percentage of elements, confirming the correctness of their chemical composition, and studying the electrolytic properties, which showed that the complexes are non-electrolytes, supports the previous spectral interpretations. So the behavior of the didentate ligand type N,O donor atoms with mono negative charge .Their proposed geometrical shapes, where the octahedral structure was proposed .

The antibacterial activity of the organometallic complexes under study was studied, and two types of Gram-positive and Gram-negative pathogenic bacteria were selected., ***Staph. aurous*** and ***Escherichia coli*** (G -) respectively, by using the Well diffusion method, with three concentrations of (100, 500, 1000) ppm in DMSO as solvent the sensitivity of these bacteria was studied . As a result of this study, it was shown that the metal complexes had a higher bacterial activity than the ligand, based on the inhibition zone value measured in millimeters.

Keywords: Antimicrobial Activity , Spectral Identification, Magnetic Susceptibility .

Introduction

Oreganometallic chemistry remains a distinctive branch that continues to attract the interest and enthusiasm of scientists and researchers. Its applications have proven effective in therapeutic drug development, particularly in the treatment of serious diseases, in addition to contributing significant advancements to various pharmaceutical preparations.[1] . Schiff base ligands and the metal complexes they generate represent a cornerstone in coordination chemistry, offering broad applications in disciplines ranging from biology and chemistry to materials science.[2,3]. These compounds are characterized by their adaptable coordination behavior and their wide spectrum of structural and functional properties. This introduction focuses on outlining the fundamental principles and defining characteristics of Schiff base ligands along with their corresponding metal complexes. [4].

Schiff base compounds, acknowledged for their broad spectrum of biological activities, have emerged as important candidates in antibacterial research due to their potential pharmacological properties. Several Schiff base derivatives have exhibited noteworthy antibacterial efficacy against a variety of microbial strains. [5-8].

Schiff ligands contain the azomethine group, which plays a crucial role in coordination chemistry due to its excellent binding properties and significance in the formation of metal complexes.[9,10].

In recent years, a substantial number of diverse donor ligands containing the azomethine group have been synthesized, reflecting significant advancements in coordination chemistry research. [11-13].

Certain antibacterial activity azo Schiff base compounds and their complexes were found [14-17]. This class of compounds contains an azo-imine moiety, which is π-acidic and acts as an effective stabilizer for metals in low oxidation states [11,12]. This behavior is attributed to the presence of a π*-molecular orbital centered on the (R-CH=N-R) group. Consequently, numerous derivatives of these compounds have been synthesized, and their potential as polydentate chelating ligands has been thoroughly investigated. [18].

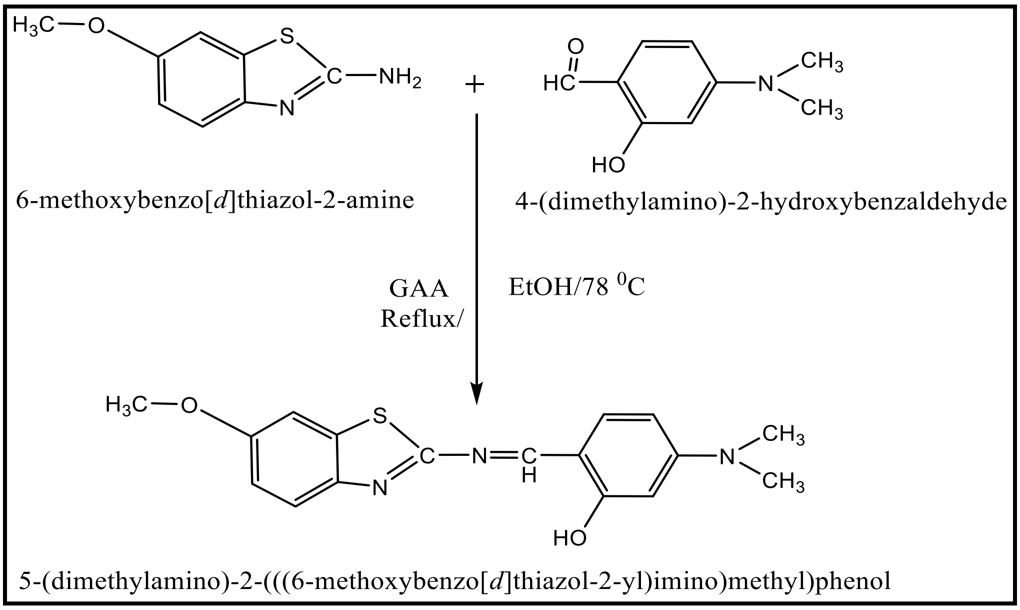
Materials and Methods

All chemicals were procured from Merck, BDH, and Sigma-Aldrich and were used as received without further purification. Melting points of the ligands and their corresponding complexes were measured using a model 9300 apparatus., ^{1}HNMR spectra were recorded as solution in DMSO-d6 as solvent using (varian 500MHZ Spectrophotometer) and Mass Spectra were recorded on Shimadzu Agilent Technologies 5975C. UV-Visible spectra were recorded using a Shimadzu double-beam spectrophotometer, model 1700. Magnetic susceptibility measurements were performed on an MSB-MKI magnetic balance employing the Faraday method, with diamagnetic corrections applied using Pascal's constants. Infrared (IR) spectra were obtained on a Shimadzu FTIR 8400 spectrometer, using KBr pellets in the range of 4000–400 cm^{-1}. Elemental analyses for carbon, hydrogen, and nitrogen (C.H.N) were conducted using a EURO 2012 EA 300 analyzer.

Synthesis of the new Schiff base ligand (DTIMP):

The synthesis of the new Schiff base ligand (DTIMP) was carried out by dissolving 0.002 mol (0.330 g) of 4-dimethylamino-2-hydroxybenzaldehyde in 15 mL of absolute ethanol in a 250 mL round-bottom flask, followed by the addition of 2–3 drops of glacial acetic acid. The mixture was allowed to stand at room temperature for 5 minutes. Subsequently, a solution of 0.002 mol (0.266 g) of 6-methoxy-2-

aminobenzothiazole in 15 mL of absolute ethanol was added, and the resulting solution was refluxed at 78 °C for 15 hours. The progress of the reaction was monitored by thin-layer chromatography (TLC) using a solvent system of 0.5 mL methanol: 4.5 mL benzene. After completion, the product was cooled, dried, collected, and recrystallized from hot absolute ethanol. The physical properties of the ligand are summarized in Table 1, and Scheme 1 illustrates the preparation steps of the new Schiff base ligand. [20].



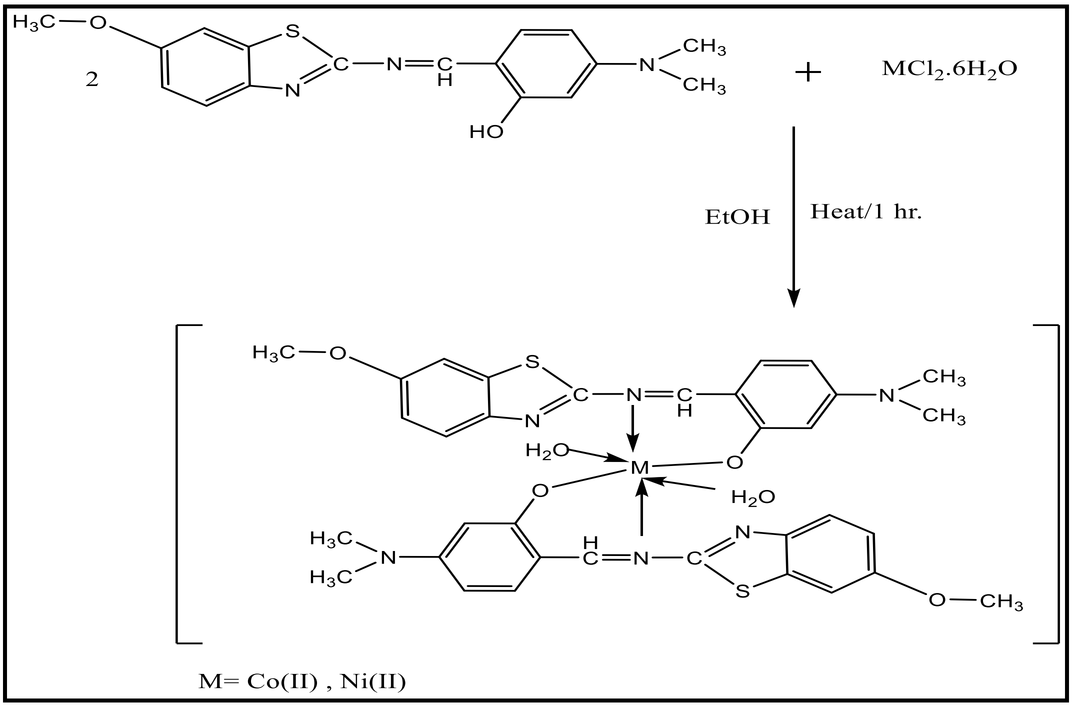
Scheme-1: Synthesis of (DTIMP)

Synthesis of Co(II) and Ni(II) metal complexes:

Cobalt (II) complex was prepared by adding (0.00015mol) of $CoCl_2.6H_2O$ dissolved in (15 mL) of absolute ethanol to the ligand solution prepared by dissolving (0.0003mol, 0.0327g) in (15 mL) of absolute ethanol at a molar ratio of (2:1) (ligand: metal) with stirring and escalation for 1 hour at (78°C). The solid complex was observed to precipitate, then the solution was left to cool and dry, and the precipitate was collected and crystallized using hot ethanol, and its melting point was measured [21].

The nickel(II) complex was synthesized by adding 0.00015 mol of $NiCl_2\cdot 6H_2O$, dissolved in 15 mL of absolute ethanol, to a solution of the ligand (0.0003 mol, 0.0327 g) in 15 mL of absolute ethanol, maintaining a molar ratio of 2:1 (ligand\:metal). The reaction mixture was stirred and refluxed at 78 °C for 1 hour, during which the solid complex precipitated. The solution was then allowed to cool, and the resulting precipitate was collected, dried, and recrystallized from hot ethanol. The melting point of the complex was subsequently determined. [22].

The physical properties of the studied complexes are presented in Table 1, while the scheme illustrates the procedure for synthesizing the organometallic complexes with the ligand (DTIMP)



Scheme- 2 Organometallic complexes preparation

Table (1) The physical properties of the newly synthesized Schiff base ligand and its corresponding metal complexes were characterized.

Compounds	Colors	M.Wt	m.p°C	Yield%	R_f
$C_{17}H_{17}N_3O_2S$	Light Brown	327.40	157-159	79	0.56
$[Co\ (C_{17}H_{16}N_3O_2S\ )_2(H_2O)_2]$	Olive	747.75	160-163	74	0.75
$[Ni\ (C_{17}H_{16}N_3O_2S\ )_2(H_2O)_2]$	Red	747.51	141-143	81	0.60

Antimicrobial Activity Test

1. Preparation of the culture medium:

Mueller-Hinton agar was prepared according to the instructions of the Indian company (Hi media) by dissolving (38) g of the agar in one liter of distilled water in a glass flask and mixing well. Then the mixture was heated on a heater until the agar dissolved. This was followed by placing the agar in the sterilizer (Autoclave) equipped by the Japanese company (Hirayama) at a temperature of (121) C and under a pressure of 15 pounds/inch2 for (15) minutes. Then pour the medium into sterile Petri dishes at a rate of (15-20) ml per dish and leave it until solidification is complete. After that, place the dishes in a (Memmert) incubator equipped by the German company (Memmert) for (24) hours at a temperature of (37) C to ensure that there is no contamination in them [23].

2 -Preparation of solutions:

Solutions of the above-mentioned complexes, copper (II) and silver (I) nanoparticles with the ligand (DTIMP) under study, were prepared at concentrations of (1000, 500, 100) ppm by dissolving the appropriate weight of each compound in (10) ml of DMSO for the complexes, in addition to making a control plate containing 99% ethanol

3 -Source of bacteria: Two isolates of pathogenic bacteria were used, isolated and laboratory diagnosed using biological and microscopic tests obtained from the Amin Laboratory for Advanced Biotechnology and Research / Holy Shrine of Imam Ali. These isolates are considered common pathogens of many diseases in humans, and they show high resistance to many antibiotics. It included Staphylococcus aureus, a representative of Gram Positive Bacteria, and Escherichia coli, a representative of Gram Negative Bacteria, the latter of which is highly resistant to many antibiotics.

4- Method of inoculation and inhibition of bacterial isolates and calculation of inhibition zones: The drilling method was adopted, where sterile Petri dishes containing the solid culture medium Mueller-Hinton agar prepared and inoculated with the test bacteria by pouring a volume of (0.1) ml of the bacterial suspension (broth) onto the solid culture medium in different directions and then moving the dishes to ensure spreading the bacteria whose sensitivity is to be tested evenly in each dish. The dishes were left for (30) minutes at room temperature to ensure absorption of the nutrient broth liquid, and then holes were made using a cork drill with a diameter of 10 mm, at a rate of three holes for each dish, and to avoid overlapping between the inhibition areas, a distance of (2 cm) cm was left between one spot and another. Then, a volume of (10) microliters of metal complex solutions at prepared concentrations of (1000, 500, 100) ppm was placed using a

(Micropipette). The dishes were incubated after the holes dried in the incubator for (20) hours at a temperature of (37 ^{0}C). After that, the inhibition zones of bacterial growth around each hole for the compounds under study were measured using a millimeter ruler, which appears as a halo free of bacterial growth surrounding the spot of the chemical compound. Each experiment was repeated three times and the average measurement for those experiments was taken.[24]

Results and discussion:

The ligand DTIMP and its Co(II) and Ni(II) complexes were readily soluble in THF, DMSO, methanol, and ethanol (Table 2) and exhibited stability in air. Characterization of the ligand and its metal complexes was performed using elemental analysis (Table 2), molar conductivity measurements, magnetic susceptibility, IR, UV-Vis spectroscopy, as well as mass and ^{1}HNMR spectroscopy for the ligand. The analytical results were in good agreement with the experimental data, indicating a metal-to-ligand ratio of 1:2 (M:L) for both Co(II) and Ni(II) complexes, as presented in Table 2. Magnetic susceptibility measurements at room temperature suggested an octahedral geometry for the chelate complexes, while the low molar conductivity values confirmed their non-electrolytic nature [20].

Table (2) presents the elemental analysis of the newly synthesized Schiff base ligand (DTIMP) and its organometallic metal complexes.

Formula	M.Wt	(Found) Calc. %				
		C%	H%	N%	S%	M%
L= $C_{17}H_{17}N_3O_2S$	327.40	62.37 (62.44)	5.23 (5.12)	12.83 (12.90)	9.79 (9.85)	--------
$[Co(L)_2(H_2O)_2]$	711.72	57.38 (57.58)	4.53 (4.71)	11.81 (11.91)	9.01 (9.11)	8.28 (8.37)
$[Ni(L)_2(H_2O)_2]$	711.48	57.40 (57.52)	4.53 (4.71)	11.81 (11.89)	9.01 (9.11)	8.25 (8.21)

Infrared Spectra studies

The IR spectra of the metal complexes were compared with that of the free ligand to identify the changes occurring upon complexation [18,25], with all data summarized in Table 3.

Table (3) FTIR spectra frequencies for the new Schiff base ligand and its Nano metal complexes in cm^{-1}

Compound	OH	H_2O	N-H	$C\text{-}H_{Aro}$	$C\text{-}H_{alph}$	C=N Schiff	C=N Endo	C=C	C-S	M-N	M-O
L= $C_{17}H_{17}N_3O_2S$	3388	-------	3296	3097	2974	1635	1554	1462	1340	-----	------
$[Co(L)_2(H_2O)_2]$	-----	3410	3290	3090	2980	1627	1518	1411	1344	538	491
$[Ni(L)_2(H_2O)_2]$	------	3404	3294	3092	2974	1622	1589	1402	1346	543	450

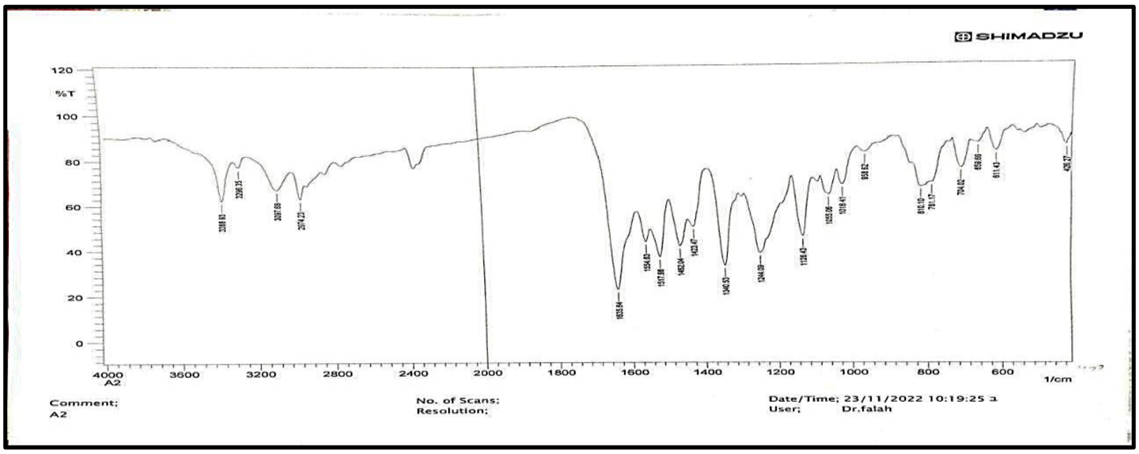


Fig (3): IR-spectra of the ligand

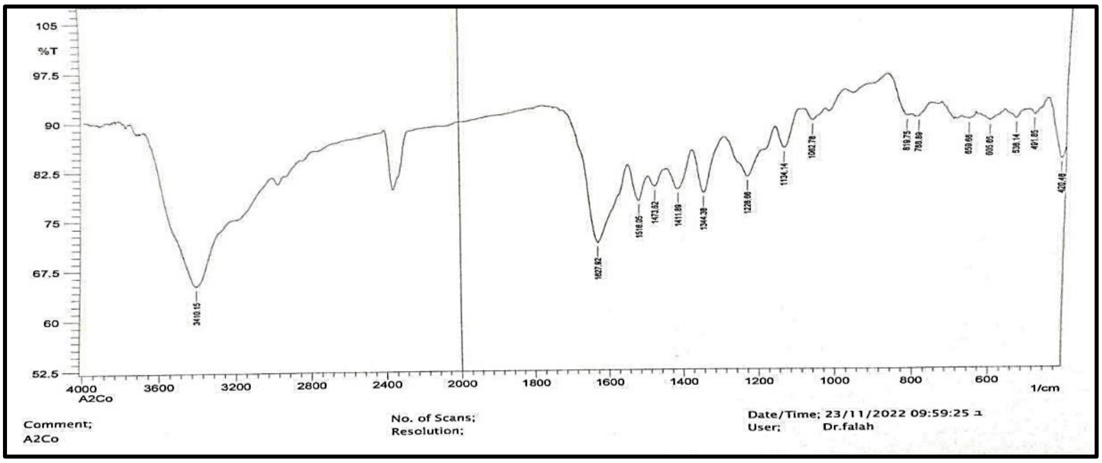


Fig (4): IR-spectra of Co (II) complex

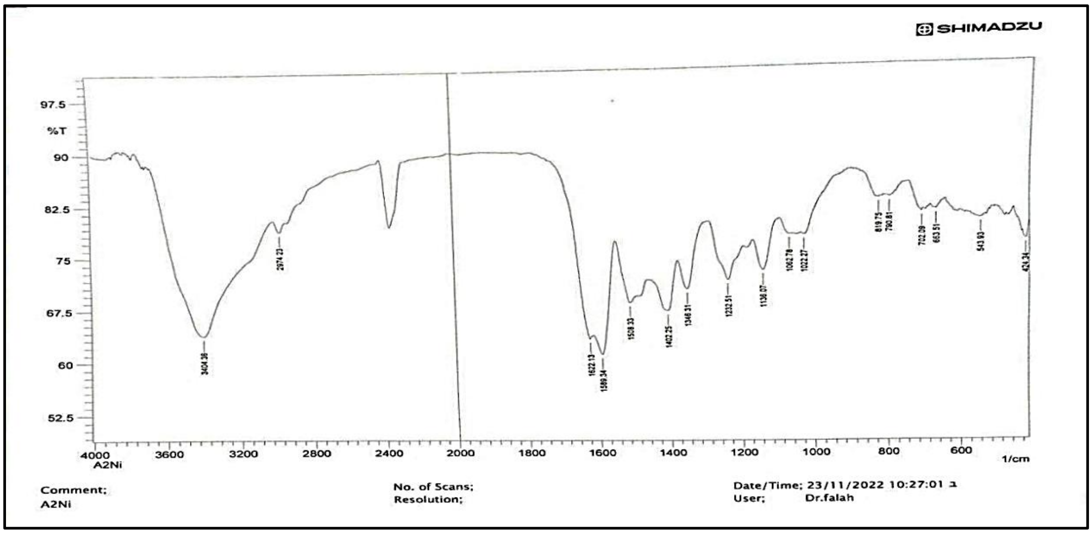


Fig (5): IR-spectra of Ni(II) complex

Magnetic Susceptibility:

The magnetic susceptibility results are summarized in Table 4. The Co(II) complex exhibited an Effective magnetic moment of 3.18 B.M., indicating paramagnetic behavior [25]. The Ni(II) complex showed an Effective magnetic moment of 2.58 B.M., also consistent with paramagnetic characteristics [26]

Molar Conductivity:

Molar conductivity measurements were performed for 1×10^{-3} M solutions of the chelate complexes in DMSO at room temperature, as listed in Table 4. The results indicated non-electrolytic behavior for both Co(II) and Ni(II) complexes, consistent with literature reports on non-ionic metal complexes [26]. To confirm the absence of chloride ions in the coordination sphere, each complex was treated with an alcoholic solution of silver nitrate. No formation of a white precipitate of silver chloride (AgCl) was observed, indicating that chloride ions are not present in the coordination sphere of the prepared complexes [27].

Table (4) Effective magnetic moments and Electrolytic behavior for the Complexes

compounds	μ_{eff} (B.M)	Geometrical	Hybridization	Λ_M(S.cm^2 . mol^{-1})	Solvent	Electrolyte Type
[$Co(L)_2(H_2O)_2$]	3.18	Oh.	Sp^3d^2	18.42	DMSO	Non
[$Ni(L)_2(H_2O)_2$]	2.58	Oh.	Sp^3d^2	16.71	DMSO	Non

Electronic Absorption Spectra:

Electronic absorption spectroscopy is a valuable tool for investigating structural features of compounds. The UV-Vis spectrum of the new ligand (DTIMP) in ethanol exhibited three absorption bands: two at 260 and 344 nm, assigned to π → π* electronic transitions, and one at 440 nm, attributed to n → π* transition, reflecting the presence of double bonds and atoms with lone electron pairs [27].

Comparison of the ligand spectrum with that of the Co(II) complex revealed an absorption band at 455 nm, corresponding to charge transfer type (MLCT), consistent with previous reports [20,27].

The Ni(II) complex revealed an absorption band at 446 nm, corresponding to charge transfer type (MLCT),However, the appearance of new absorption bands not present in the free ligand spectrum indicates successful coordination via charge transfer (C.T) [41].

A red shift in the ligand bands upon Complexation suggests metal to-ligand charge transfer (MLCT), supporting octahedral geometry for the two complexes, as illustrated in Figures 6–8. Table 5 summarizes the electronic transitions of the new azo Schiff base ligand and its metal complexes in cm^{-1}.

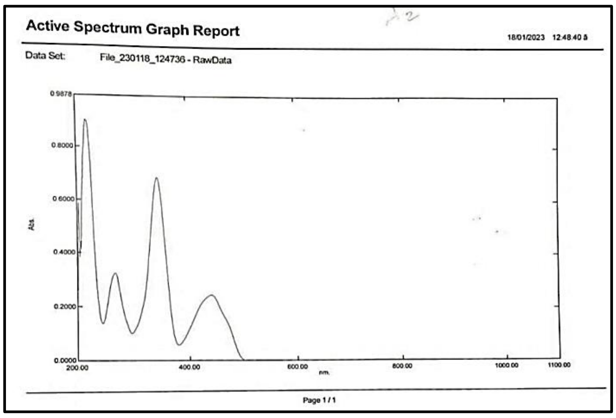

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Fig (6): UV-Vis spectra of new Ligand

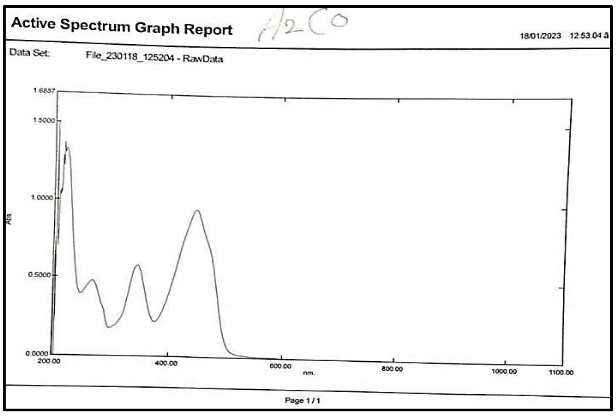
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Fig (7): UV-Vis spectra of Co (II) complex

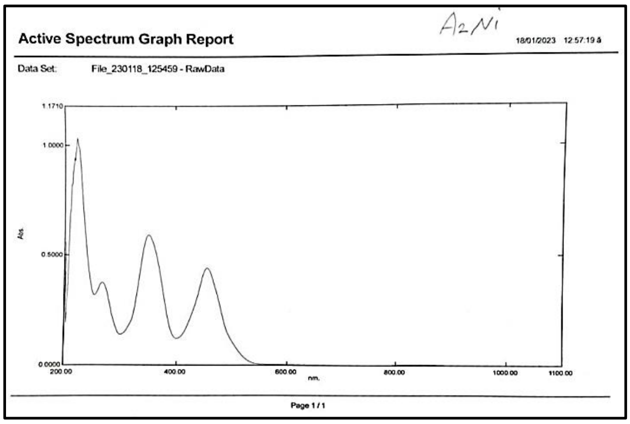
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Fig (8): UV-Vis spectra of Ni(II) complex.

Table (5) Electronic transitions for the new Schiff base ligand and its metal complexes in cm^{-1}

Compounds	Λmax (nm)	Transitions	Geometry	Hybridization
L= $C_{17}H_{17}N_3O_2S$	440 344 266	n →π* π →π* π →π*	----	----
$[Co(L)_2(H_2O)_2]$	455	ML.CT	Octahedral	Sp^3d^2
$[Ni(L)_2(H_2O)_2]$	446	ML.CT	Octahedral	Sp^3

Proposed Structural:

Based on the previously mentioned analytical and spectroscopic measurements of the metal complexes and the diagnosis of the coordination sites available in the organic molecule and how these sites bind to the metal ions, we can conclude that the new azomethine ligand behaves as a mono negatively charged Di chelate ligand with the metal ions selected in this study, as the ligand binds via an oxygen atom of hydroxyl group . And through the nitrogen atom of the azomethine group, since the connection of the two molecules of the di chelate ligand with the cobalt (II) and Ni(II) ions provides six coordination bonds with the presence of two H_2O molecules to form the octahedral shape. Figures (9) shows the proposed geometrical shapes of Co(II) and Ni(II) complexes.

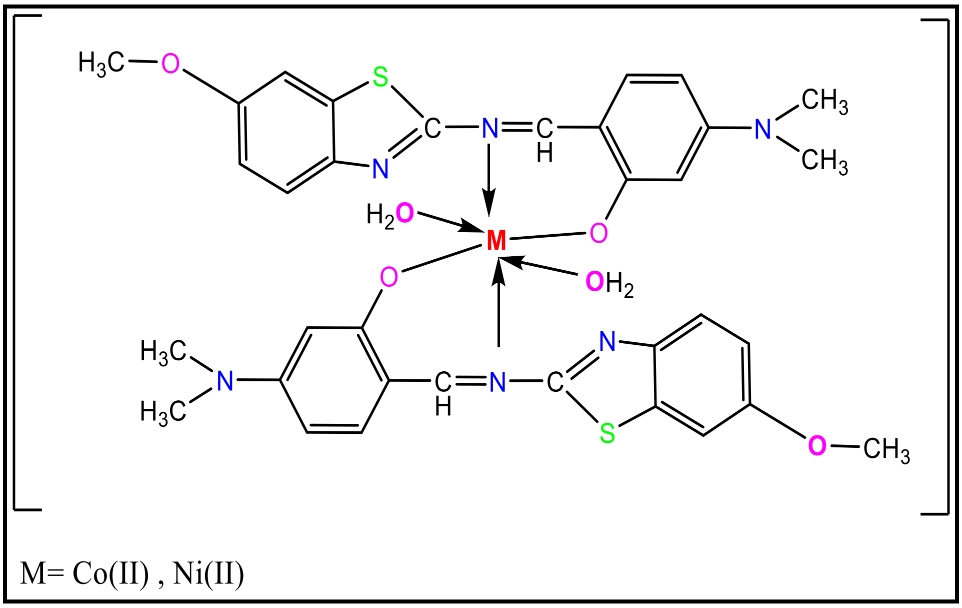

Fig.(9): Proposed Structural of the complexes

Efficacy of the complexes as Antimicrobial against (Gram positive)bacteria and (Gram negative) bacteria

The **Antimicrobial against (Gram positive)bacteria for the complex [Co(L)$_2$(H$_2$O)$_2$] :**showed the greatest inhibitory effect at the highest concentration of (1000 ppm) as its inhibition diameter reached (22 mm), while at a concentration of (500 ppm) a difference in the rate of the complex inhibition diameter was observed as it was (19 mm), while the lowest rate of the inhibition diameter was recorded for the concentrations of (100 ppm) which reached (14 mm).

As for the complex **[Ni(L)$_2$(H$_2$O)$_2$]**, it showed the greatest inhibitory effect at the highest concentration of (1000 ppm) as its inhibition diameter reached (30 mm), while at a concentration of (500 ppm) it recorded a diameter of (25 mm), while the lowest rate of the inhibition diameter was recorded for the concentrations of (100 ppm) which reached (19 mm).

It can be concluded that the metal complexes at a concentration of 1000 ppm exhibit strong antibacterial activity and may potentially affect other bacterial strains not tested in this study. However, their efficacy in vivo may differ from the observed in vitro results, as biological systems involve complex metabolic and physiological processes that can influence the therapeutic outcome [28].

The highest results were from Gram+ positive bacteria , while Gram-negative bacteria that recorded a lower success rate of distillation lines for all nodes and in all tracers[29]. The reason for this is due to the presence of a double generation in each bacterial cell wall. Gram-negative bacteria are characterized by a complex cell envelope composed of two layers: an inner layer of phospholipid proteins and an outer layer of lipopolysaccharides. This structure creates a distinctive cell wall that limits the penetration of antibiotics and other therapeutic agents. In contrast, Gram-positive bacteria possess a thick peptidoglycan layer composed of amino acids and monosaccharides, rendering them generally more susceptible to drugs and antibiotics [29].The activity of the metal complexes can be attributed to their lipophilic nature, which, along with chelation, enhances their ability to penetrate bacterial cell membranes \[31]. The complexes demonstrated antimicrobial activity by inhibiting microbial growth, likely through interactions that block essential active sites \[32,33]. Table 6 presents the inhibition zones of the complexes at concentrations of 1000, 500, and 100 ppm against the tested bacterial strains,and shows in figures (10 -12).

Table (6). Inhibition zones of Co(II) and Ni(II) complexes at concentrations of 1000, 500, and 100 ppm against the tested bacterial strains.

Compound **Bacteria**	**(+) G** ***Staph.aureus***	**(-) G** ***E.coli***
	At 1000 ppm	
$[Co(L)_2(H_2O)_2]$	22	18
$[Ni(L)_2(H_2O)_2]$	30	25
	At 500 ppm	
$[Co(L)_2(H_2O)_2]$	19	15
$[Ni(L)_2(H_2O)_2]$	25	20
	At 100 ppm	
$[Co(L)_2(H_2O)_2]$	14	10
$[Ni(L)_2(H_2O)_2]$	19	15

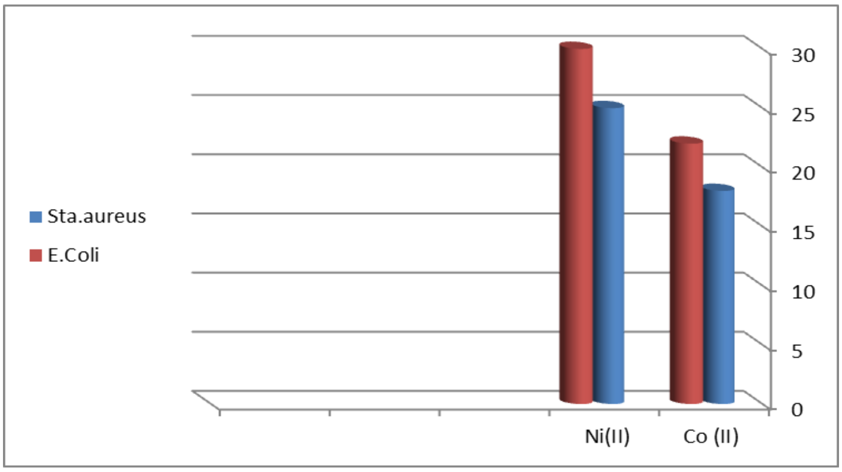

Fig.(10): Inhibition zones of Co(II) and Ni(II) complexes at a concentration of (1000) ppm for the bacteria under study.

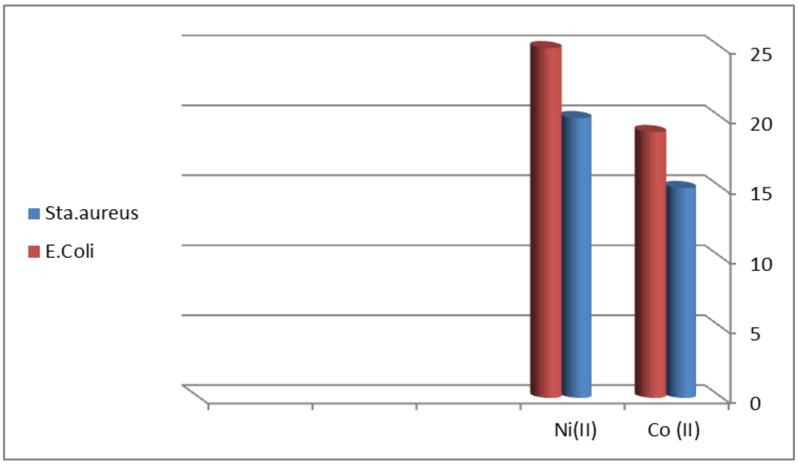

Fig.(11): Inhibition zones of Co(II) and Ni(II) complexes at a concentration of (500) ppm for the bacteria under study.

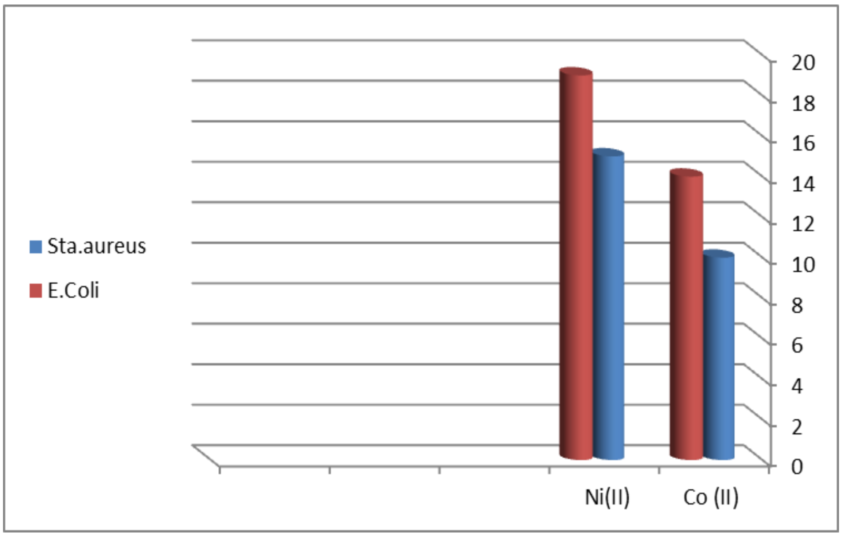

Fig.(12): Inhibition zones of Co(II) and Ni(II) complexes at a concentration of (100) ppm for the bacteria under study.

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