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Synthesis, Spectroscopic Investigation, and Antibacterial Studies of Ternary Salen Copper(II) Complexes

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Abstract: Three mononuclear ternary copper(II) complexes of the type, $[CuL^1L^2]$, where H_2L^1 is the Salen-type ligand N-(salicylidene)-(*O*-hydroxyacetophenone)ethylenediamine and L^2 represents 2,2'-bipyridine, 1,10-phenanthroline, or pyrrolidine dithiocarbamate, were successfully synthesized and thoroughly characterized. The complexes were prepared via a one-pot reaction involving copper(II) chloride dihydrate, the co-ligand, and the Schiff base. Comprehensive characterization was performed using FT-IR, UV-visible, and mass spectroscopies, alongside conductometric and magnetic susceptibility measurements, and elemental analysis. Analytical and spectral data confirmed that the Salen ligand coordinates as a tetradentate ONNO chelating ligand, forming an octahedral geometry around the copper(II) center. Furthermore, the *in vitro* antibacterial efficacy of these ternary copper(II) complexes was evaluated against pathogenic Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The free ligand exhibited limited activity, while the complexes generally showed enhanced antibacterial properties. Notably, complex 3 displayed moderate activity against *S. aureus* (10–15 mm inhibition zones), and all three complexes demonstrated moderate activity against *E. coli* (12–22 mm inhibition zones). These findings highlight the potential of these copper(II) chelates as effective antimicrobial agents.

Keywords: Ternary complexes, Salen Schiff base, Spectroscopic characterization, Antibacterial activity, Copper(II).

Introduction:

Schiff bases constitute a vital category of chelating organic ligands with a prominent role in coordination chemistry. Their significance stems largely from their straightforward synthesis and their robust capacity to form stable complexes with a wide array of transition metal ions [1-4]. These properties have made them widely applicable in several fields, including analytical chemistry [5], where Schiff bases act as complexing agents used in the removal of heavy metals. They can also function as electrochemical catalysts [6], in the homogeneous or heterogeneous catalysis of alkane oxidation, and in the preparation of epoxides from alkenes [7]. Schiff bases are good biosensors [8], and play an important function as corrosion inhibitors [9]. Many Schiff bases have also shown high biological activity, and it has been observed that their effectiveness increases when they form complexes with various metal ions, resulting in compounds with very high biological activity [10, 11]. They have been used as anticancer agents [12], antifungal agents [13], antibacterial agents [14], and antioxidants [15]. Schiff base ligands and their corresponding metal coordination complexes are also of great importance due to their ability to bind with oxygen in any oxidation-reduction system, thereby oxidizing DNA [16]. Salen ligand (a Schiff base resulting from the condensation reaction between one molecule of ethylenediamine and two molecules of salicylaldehyde) has become the common name for most tetradentate imine Schiff bases, whether symmetrical or asymmetrical, derived from diamines or dialdehydes/diketones [17].

Since metal Salen complexes are easily prepared through direct reactions and have structures and chemical properties similar to those of porphyrins, the chemistry of Salen-type complexes has long attracted the attention of chemists. Salen type ligands are classified as tetradentate Schiff bases with donor atoms N_2O_2, and they coordinate with a large number of metal ions, forming complexes that have a square planar, square pyramidal, or octahedral shape [18]. Within complexes characterized by square planar and square pyramidal geometries, the four donor atoms typically maintain a near-planar arrangement around the central metal ion. Furthermore, these systems may exhibit a configuration where the metal center is displaced from the plane defined by the N_2O_2 donor set, occupying an axial or apical position relative to the coordinating atoms. [19]. Based on X-ray analysis, it was found that Salen complexes with cobalt(II) [20], copper(II) [21], and platinum(II) [22] are square planar. However, several examples of M(Salen) complexes were found to be distorted toward a tetrahedral shape

[23]. While it was found that Salen complexes with metal ions in the oxidation state (+3), such as Al, Sc, Cr, Fe, and Co [24], form square pyramidal structures, Salen binds in a planar manner through the four donor atoms N_2O_2, with the fifth site occupied by another monodentate ligand. For example, in the Salen complex with Al(III), Salen binds via N and O atoms, with the Al(III) canter situated above the N_2O_2 basal plane, occupying the fifth site with an ethyl ligand [25]. In addition to the above, a large number of metal ions form octahedral complexes with Salen ligands in the presence of co-ligands, resulting in three possible configurations that the Salen ligand exhibits conformational flexibility as a function of the oxidation number of the central metal ion and the type of substituent groups in the Salen-type ligand. These configurations are *trans*, *cis-α*, and *cis-β* (Figure 1) [26].

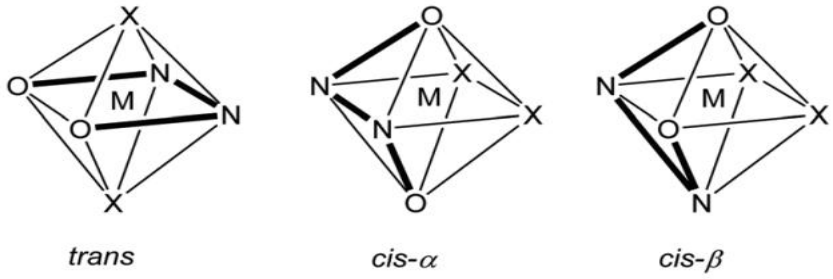


Fig.1 *Trans*, *cis* and *cis-β* conformation of Salen

Instrumentation

IR spectra acquired using a Perkin Elmer FT-IR Spectrometer-USA, 4000–650 cm^{-1}). The Molar conductivity of the complexes was measured in DMSO (10^{-4} M) solution at room temperature using Jenway 4510 conductivity meter. The electronic spectra of ligand and complexes were measured by a UV –Visible Spectrophotometer (Agilent Technologies Cary60) with a 1.0 cm cell model in DMSO (10^{-5} M). The melting point were recorded using Stuart Melting point SM10. Perkin Elmer type 2400 equipment was used to do elemental analysis. Magnetic susceptibilities at room temperature were measured using a Sherwood Scientific magnetic balance, Cambridge science, England, Model no.MKI, Serial no. MSBI/230/95/680. The FAB mass spectra were recorded using a Micromass Autospec spectrometer.

Synthesis of Ligand

The ligand N-(saliclidene)-(*O*-hydroxyacetophenone) ethylenediamine was synthesized according to literature procedures with some modifications [27]. (20 mmole, 2.73 g) of 2-hydroxyacetophenone was dissolved in 10 mL of ethanol, and a solution of salicylaldehyde (20 mmole, 2.44 g) dissolved in 10 mL of ethanol was gradually added to it. The mixture was placed in an ice bath with continuous stirring for 30 minutes. A solution of ethylenediamine (20 mmole, 1.2 g) dissolved in 20 mL of ethanol was added dropwise, and the yellow product was collected after filtration, washing, and drying in the oven at 60 °C.

Synthesis of complexes

The complexes [CuL(bipy)] (1), [CuL(phen)] (2) and [Cu(HL)(PDTC)] (3) were synthesized by the following procedure: (1mmole, 0.1435 g) of copper(II) chloride dihydrate was dissolved in 5mL of methanol, and a methanol solution of the coligand (2,2-bibyridine, 1,10-phenanthroline or ammonium pyrrolidine dithiocarbamate) (1mmole) was gradually added with continuous stirring for 30 minutes. A solution of the Schiff base (H_2L) (1mmole, 0.282g) dissolved in 5mL methanol was added, the resulting mixture was refluxed for two hours. The resulting powder was filtered, washed with water and methanol, and stored in a desiccator.

Biological Activity

The biological activity of the prepared compounds was tested against two pathogenic bacteria, Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, using the agar well diffusion method. All the isolates used are pathogenic antibiotic-resistant isolates. The bacterial suspensions were prepared, and their turbidity adjusted to match a McFarland (0.5) standard. Using cotton swabs, the bacterial samples were introduced onto Muller Hinton plates, and then the plates were allowed to dry slightly. Wells with a diameter of 6 mm were made in the inoculated agar plates using a cork borer. Solutions with concentrations of 25, 50, 100, and 300 mg/l of each compound were prepared separately using DMSO as a solvent. The agar wells were injected with 70 µL of the solutions under study. After that, the plates were incubated for twenty-four hours at 37°C. The effectiveness of the compound was determined by measuring the diameter of the inhibition zone around the wells compared to the DMSO solvent used as a negative control. The effectiveness of each compound with each type of bacteria was tested in duplicates, and

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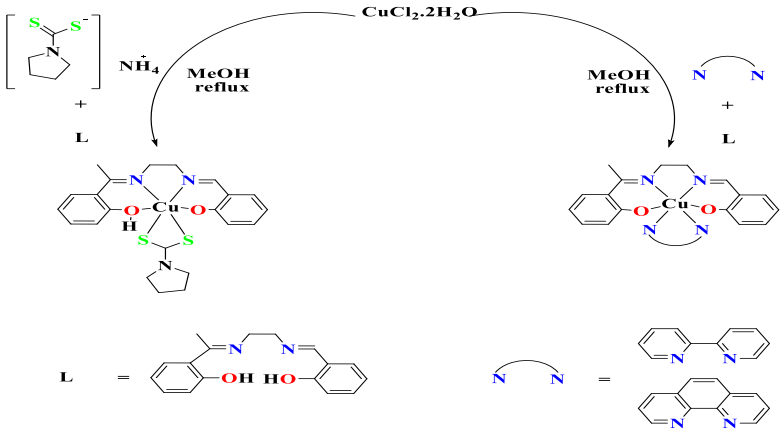
the average diameter of the inhibition zone was adopted [28].

Result and Discussion

In this work, copper(II) mixed ligand complexes of Schiff base ligand with NN and SS donor ligands are synthesized (Scheme 1) and spectrally characterized. Table 1 highlights the physical properties and analytical data of the prepared complexes. The resulting complexes showed good solubility in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), but were insoluble in other common organic solvents. They also showed remarkable stability in their solid state at ambient temperature. The low molar conductance values of the synthesized complexes (10 – 30 Ω^{-1} cm^{-2} mol^{-1}) support the conclusion that these complexes are non-electrolytes [29]. The effective magnetic susceptibility data of the complexes agreed well with those of octahedral Cu(II) complexes [30]. The CHN elemental analysis results of the ligand and its complexes, (Table 1), which showed good agreement with the proper theoretical values, provided additional support for the proposed chemical formula.

Table 1: Analytical data and physical characteristics of ternary copper(II) complexes

Compound	Color	Yield %	Λ_m	μ_{eff}	Anal.Calc. (Found)%		
					N	H	C
H_2L	Yellow	65	-	-	9.92 (9.93)	6.43 (6.41)	72.32 (72.30)
[CuL(bipy)](1)	Green	52	10	1.80	11.20 (11.19)	4.84 (4.83)	64.85 (64.82)
[CuL(phen)] (2)	Green	47	14	1.85	10.69 (10.67)	4.62 (4.60)	66.46 (66.44)
[Cu(HL)(PDTC)] (3)	Brown	70	30	1.93	8.56 (8.55)	5.13 (5.11)	53.80 (53.79)



Scheme 1 Synthesis of ternary Salen copper(II) complexes

Spectroscopic Properties

The characteristic FT-IR spectroscopic data for the free H_2L ligand and its corresponding mixed-ligand copper(II) complexes (1–3) are summarized in Table 2. To elucidate the coordination mode of the ligand to the copper(II) center, the vibrational spectra of the free ligand were compared with those of the synthesized complexes. Consistent with previously reported findings [31], the IR spectrum of the H_2L ligand exhibited a prominent band at 1606 cm^{-1} assigned to the azomethine ν(CH=N) stretching vibration, alongside a phenolic ν(C−O) stretching band at 1281 cm^{-1}. Upon complexation, the azomethine stretching frequency shifted from 1606 cm^{-1} to higher wavenumbers—specifically 1625, 1620, and 1618 cm^{-1} for complexes 1, 2, and 3, respectively—

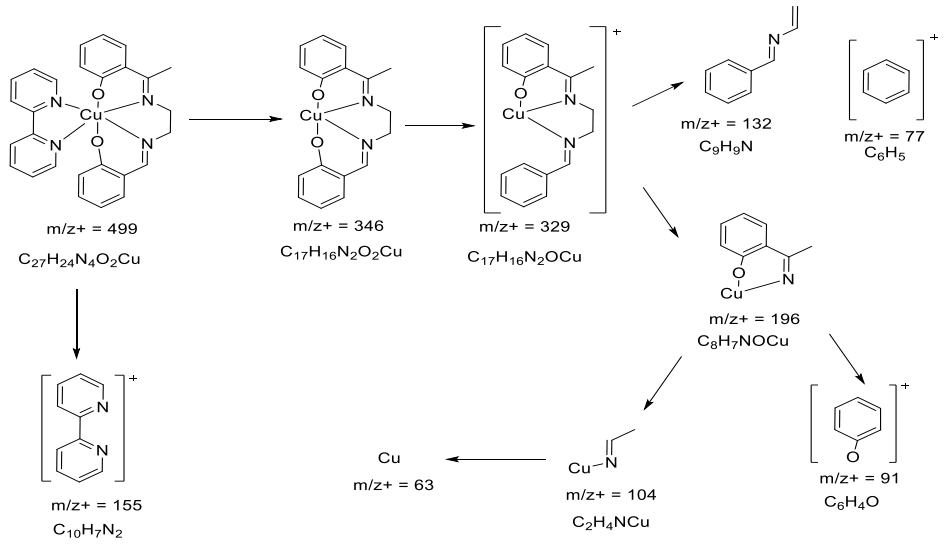
indicating the involvement of the azomethine nitrogen in metal coordination.

Table 2 Characteristic IR absorptions of Salen Schiff base and ternary copper(II) complexes

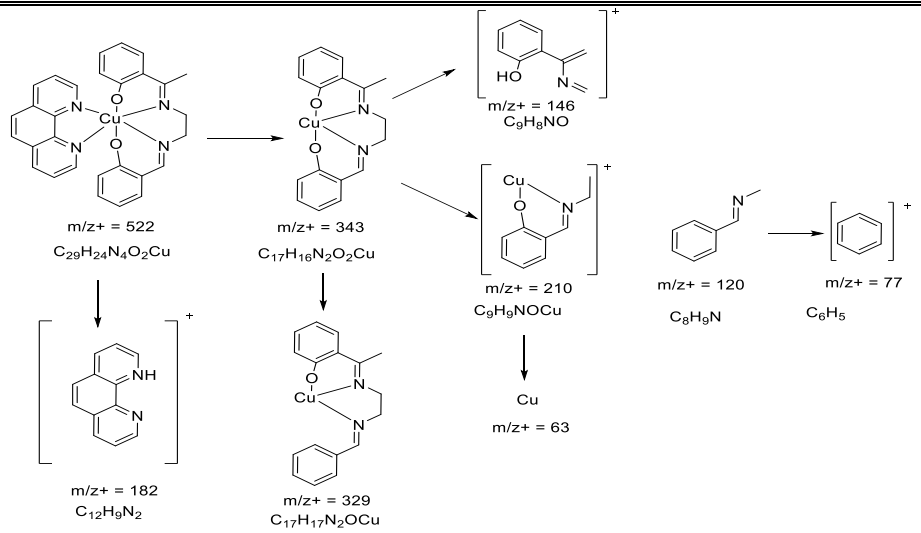
Compound	C=N	C=C	C-O	Bands for co ligand
H_2L	1606	1575	1281	-
1	1625	1597	1190	1529 1447 1326
2	1620	1588	1141	1573 1445 1326
3	1618	1597	1125	1467 905

This shift indicates the coordination of the nitrogen of the azomethine group with the copper(II) ion in the prepared complexes [32]. Additionally, infrared spectra of the prepared complexes showed that the absorption band corresponding to the stretching vibration of the v(C-O) bond shifted to a lower frequency (1190, 1141, and 1125 cm^{-1} in complexes 1, 2, and 3, respectively), suggesting that the phenolic oxygen atom participated in coordination with the Cu ion. The development of additional bands at about 1529 cm^{-1} and 1447 cm^{-1}, which correspond to the stretching vibrations of the C=C and C=N groups in the ligands 2,2-bipyridine and 1,10-phenanthroline, was another characteristic of the copper mixed ligand complexes 1 and 2 [33,34]. Moreover, the complex [Cu(HL)(PDTC)] (3) exhibited two bands at 1467 cm^{-1} and 905 cm^{-1}. These bands correspond to the stretching vibrations of the v (N–CSS) and v (–CSS) groups in the ligand pyrrolidinedithiocarbamate [35].

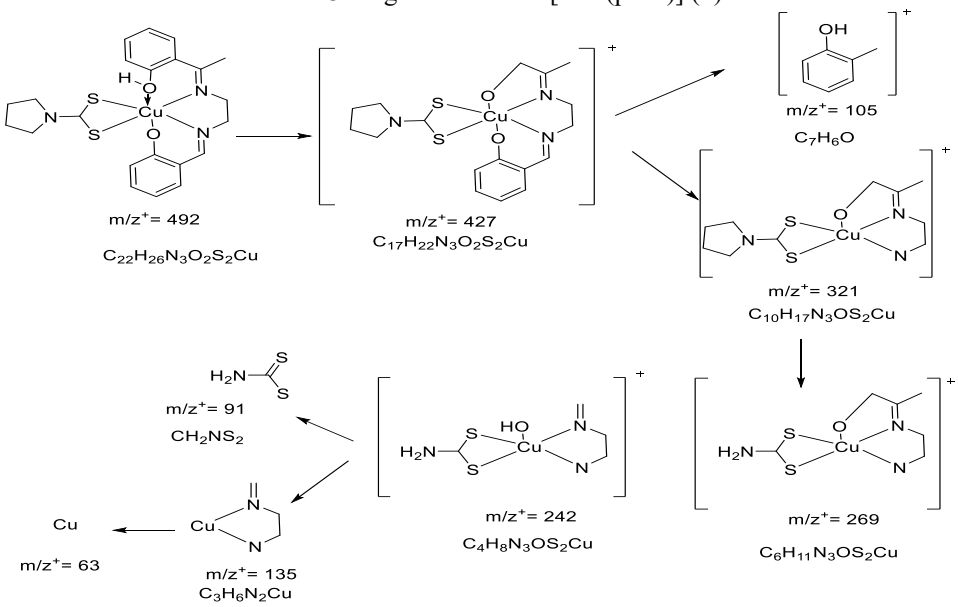
The mass spectrum and fragmentation for complex 1 (Fig. 2) showed a peak at $m/z^+ = 499$ consistent to its molecular weight, and also showed several fragmentation peaks at (m/z) at 346, 329, 196, 155, 132, 104, 91, 77, and 63 (Scheme 2). The m/z value of 499 and its initial fraction m/z 155 confirmed the presence of the coligand, 2,2-bipyridine molecules with the [CuL(bipy)] metal complex as $C_{27}H_{24}N_4O_2Cu$. The calculated mass of complex 2 ($C_{29}H_{24}N_4O_2Cu$) is 523, consistent with its molecular ion peak at m/z 522 (scheme 3). Apart from this parent peak, subsequent fractions were found with various m/z values at the masses of 343, 329, 210, 182, 146, 120, 77, and 63. Thus, MS spectral findings (Fig. 3) lead to the firm synthesis of the ternary complex [CuL(phen) with the proposed structure, which is shown in Scheme 1). A molecular ion peak was visible in compound 3's mass spectra (Fig. 3) at m/z 492, along with further fragments at m/z 427, 321, 269, 242, 179, 135, 105, 91, and 63, suggesting the proper fragmentations (Scheme 4) display the expected peaks corresponding to the molecular ions. The structure of ternary copper(II) complexes was in good accordance with the reasonable empirical formula deduced from the elemental analysis results, according to the mass spectral investigations mentioned above.



Scheme 2 Fragmentations of [CuL(bipy)] (1)



Scheme 3 Fragmentations of [CuL(phen)] (2)



Scheme 4 Fragmentations of [Cu(HL)(PDTC)] (3)

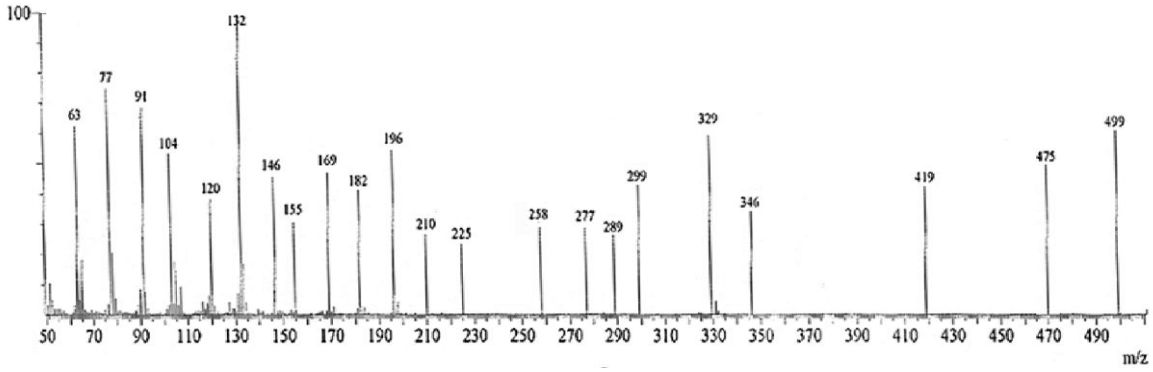


Fig. 2 Mass spectra of complex 1

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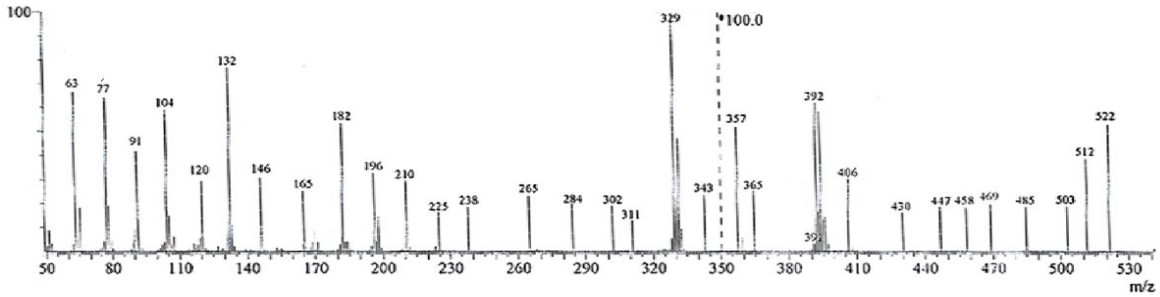


Fig. 3 Mass spectra of complex 2

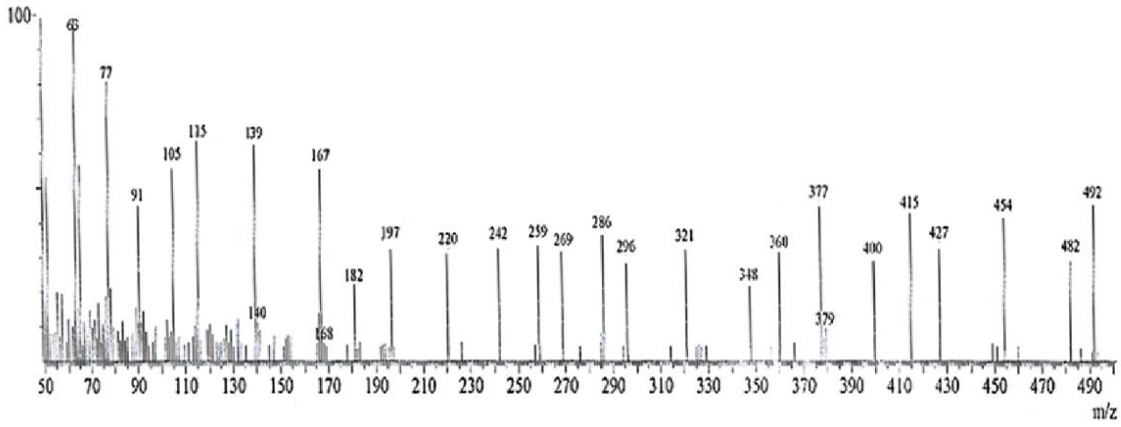


Fig. 4 Mass spectra of complex 3

In the region of 200-800 nm at ambient temperature, the UV-visible spectra of the free ligand H_2L and its ternary copper(II) complexes was examined in DMSO solvent at a concentration of 1 $\times 10^{-5}$ M (Fig. 5). The UV-visible spectrum of the ligand showed an absorption peak at 258 nm corresponding to the $\pi \rightarrow \pi^*$ transition in the benzenoid ring and the azomethine group. Furthermore, the peak observed at 313 nm is ascribed to $n \rightarrow \pi*$ transitions originating from the lone pairs of the azomethine nitrogen and phenolic oxygen atoms [36]. These bands have shifted toward longer wavelengths in the electronic spectra of the copper(II) complexes due to the binding of the nitrogen and oxygen atoms in the ligand to the copper ions. In addition to these ligand-specific bands, the additional absorption features observed in the electronic spectra of the complexes (Table 3) are ascribed to (MLCT) transitions, involving the migration of electron density from the metal orbitals to the vacant $\pi*$ orbitals of the co-ligand. [37].

Table 3 UV-Vis absorption maxima and electronic transitions for the Salen ligand and ternary Cu(II) complexes.

Compound	λ (nm)	Transition
H_2L^1	258	$\pi \rightarrow \pi^*$
	313	$n \rightarrow \pi^*$
1	270	$\pi \rightarrow \pi^*$
	362	$n \rightarrow \pi^*$
2	270	$\pi \rightarrow \pi^*$
	362	$n \rightarrow \pi^*$
3	272	$\pi \rightarrow \pi^*$
	365	$n \rightarrow \pi^*$
	438	$M \rightarrow L$

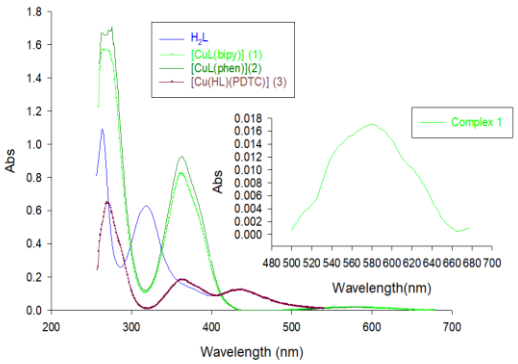


Fig. 5 Electronic spectra of H_2L and its ternary copper(II) complexes

In vitro antibacterial screening

Escherichia coli (Gram negative) and *Staphylococcus aureus* (Gram positive) were the two types of bacteria used to assess the biological activity of produced compounds. Solutions with concentrations of 25, 50, 100, and 300 mg/ml of the prepared compounds in DMSO solvent were prepared. The standard drug was amoxicillin and DMSO solvent was also used as a negative control to study its effect on bacterial growth under the same conditions to avoid solvent interactions. The effect of the ligands and the prepared complexes on two types of bacteria was studied using the well diffusion method in bacterial plates, and Table 4 and Figure 6 show the study results. Screening results indicated that the Schiff base ligand lacked activity against *S. aureus* but possessed slight inhibitory potential toward *E. coli*. The ternary copper(II) complexes showed variable antibacterial activity against the microbes, and increased with increasing the concentration. In contrast to complexes **1** and **2**, which remained inactive against *S. aureus*, complex **3** exhibited moderate antibacterial activity, with inhibition zones ranging from 10 to 15 mm. However, all the three complexes showed moderate activity against *E.Coli* in the similar concentrations with inhibitory zones range of 12–22 mm. According to the data in the table, the copper complexes more active than the free ligand. It has been stated in reports that the improved antibacterial activity of the metal complexes is thought to be caused by a decrease in polarizability [38]. Moreover, [CuL(bipy)] (1) and [CuL(phen)] (2) showed efficacy against *E.coli* (Gram-negative bacteria) but did not show any effect on *S.aureus* (Gram-positive bacteria).

The reasons for the absence of inhibition zones for these complexes against *S. aureus* could be bacterial selectivity, as the cell wall of Gram-positive bacteria contains a thick layer of peptidoglycan, which may prevent these complexes from penetrating and reaching the target sites within the cell. In contrast, the cell wall of the Gram-negative bacterium *E.coli* contains a thin layer of peptidoglycan. Previous reports have indicated that copper(II) complexes derived from Schiff bases often exhibit selective antibacterial activity, demonstrating superior efficacy against Gram-negative bacteria relative to Gram-positive strains. This differential susceptibility is primarily ascribed to fundamental disparities in cell wall architecture and membrane permeability between these two bacterial classes [39, 40].

Table 4 Inhibition zone diameters (mm) of the ternary copper(II) complexes against selected bacterial strains.

Compounds	Bacterial Zone of Inhibition (mm)							
	Concentration in mg/ml							
	Gram Positive				Gram Negative			
	Staphylococcus aureus				*Escherichia coli*			
	25	50	100	300	25	50	100	300
DMSO (control)	-	-	-	-	-	-	-	-
H_2L	-	-	-	-	5	8	8	10
1	-	-	-	-	13	12	13	15
2	-	-	-	-	13	13	14	14
3	10	12	14	15	15	15	16	22
Amoxicillin	15	22	30	35	18	20	27	40

Conclusion

Three new ternary copper(II) Salen-type complexes were developed and structurally elucidated. Utilizing a streamlined one-pot procedure, these compounds were synthesized from copper(II) chloride dihydrate and the respective co-ligands, bypassing the need for intermediate purification. Characterization studies established that the Salen ligand functions as a tetradentate ONNO-donor, facilitating the formation of an octahedral coordination environment around the copper(II) center. Furthermore, the *in vitro* antibacterial screening demonstrated that complexation with copper(II) markedly improves the antimicrobial potency of the system, with the ternary complexes outperforming the free ligand.

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تحضير ودراسة طيفية وتقييم النشاط المضاد للبكتيريا لمُعقدات النحاس الثلاثية المحتوية على ليكاند سالين

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الملخص: تم تحضير معقدات ثلاثية للنحاس(II) أحادية النواة من النوع $[CuL^1L^2]$، حيث H_2L^1 هو ليكاند من نوع N-(سالسيليدين)-*O*-هيدروكسي أسيتوفينون)إيثيلين داي أمين ، L^2 يمثل 2,2'-ثنائي بيريدين، 1,10-فينانثرولين، أو بيروليدين ثنائي ثيوكربامات. تم تحضير المعقدات من خلال تفاعل وعاء واحد يشمل كلوريد النحاس(II) ثنائي الماء، الليكاند المساعد، وقاعدة شيف. تم إجراء تشخيص المعقدات المحضرة باستخدام طيف الأشعة تحت الحمراء FT-IR، الأشعة فوق البنفسجية-المرئية، ومطيافية الكتلة، إلى جانب قياسات التوصيلية وقياسات القابلية المغناطيسية، والتحليل العنصري. أكدت البيانات التحليلية والطيفية أن ليكاند السالين يتناسق كليكاند رباعي الأسنان ONNO، مكونًا هندسة ثمانية السطوح حول مركز النحاس(II). علاوة على ذلك، تم تقييم الفعالية المضادة للبكتيريا في المختبر لهذه المعقدات الثلاثية من النحاس(II) ضد البكتيريا الموجبة الجرام (*Staphylococcus aureus*) والسالبة الجرام (*Escherichia coli*). أظهر الليكاند الحر نشاطًا محدودًا، بينما أظهرت المعقدات خصائص مضادة للبكتيريا جيدة. من الجدير بالذكر أن المركب 3 أظهر نشاطًا معتدلًا ضد *S. aureus* (مناطق تثبيط تتراوح بين 10-15 مم)، وأظهرت المعقدات الثلاثة المحضرة نشاطًا معتدلًا ضد *E. coli* (مناطق تثبيط تتراوح بين 12-22 مم). تُبرز هذه النتائج إمكانيات هذه المعقدات النحاسية (II) كعوامل مضادة للميكروبات فعالة.

الكلمات المفتاحية: المعقدات الثلاثية، قاعدة شيف سالين، التشخيص الطيفي، النشاط المضاد للبكتيريا، النحاس(II).