SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL STUDIES OF A NEW TETRADENTATE NITROGEN (N_4) LIGAND AND ITS TRANSITION METAL COMPLEXES

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Abstract: *A new tetradentate nitrogen (N_4) ligand was synthesized from cis-1,2,3,6-tetrahydrophthalic anhydride and thiosemicarbazide, and its complexes with Cr(III), Mn(II), Fe(II), Co(II), Ni(II), and Cu(II) were prepared and characterized. Elemental analyses and molar conductance confirmed the proposed structures, with all complexes being non-electrolytes except $[Cr(L)Cl_2]Cl$. IR spectra indicated coordination through nitrogen atoms, supported by M–N stretching bands. Magnetic and UV–Vis data suggested octahedral geometries around the metal centers. The 1H-NMR spectrum of the ligand confirmed the expected macrocyclic framework. Antibacterial studies against Klebsiella pneumoniae and Staphylococcus aureus revealed moderate activity for the ligand and its complexes, with $[Cu(L)Cl_2]$ showing the highest activity against S. aureus. The results demonstrate that N_4 ligands form stable coordination complexes with potential applications in bioinorganic and medicinal chemistry.*
Keywords: *Tetradentate nitrogen, ligand, transition metal, complexes, thiosemicarbazide, tetrahydrophthalic anhydride*

Introduction

Tetradentate nitrogen (N_4) ligands represent an important class of compounds in coordination chemistry due to their strong chelating ability and their capacity to stabilize transition metal ions in various oxidation states [1]. These ligands impose well-defined geometries around the metal center and enhance both thermodynamic and kinetic stability of the resulting complexes. Furthermore, their incorporation into extended molecular frameworks provides structural rigidity and tunable electronic properties, making them versatile platforms in modern inorganic and materials chemistry [2].

In homogeneous catalysis, N_4 tetradentate ligands play a crucial role in stabilizing high-valent metal intermediates and promoting selective oxidation reactions such as olefin epoxidation and hydroxylation. For example, Mn(II) and Co(II) complexes bearing pyridine-based N_4 ligands have demonstrated high catalytic efficiency, where both steric and electronic factors significantly influence reactivity and selectivity [3].

Beyond catalysis, these ligands have attracted considerable attention in bioinorganic and medicinal chemistry. N_4 systems can act as structural and functional models for non-heme metalloenzymes, mimicking their coordination environment and electronic behavior. In addition, their strong metal-binding affinity has enabled applications in radiopharmaceuticals and diagnostic imaging. Several N_4 Schiff base complexes have also exhibited promising antimicrobial and anticancer activities, highlighting their biological relevance [4].

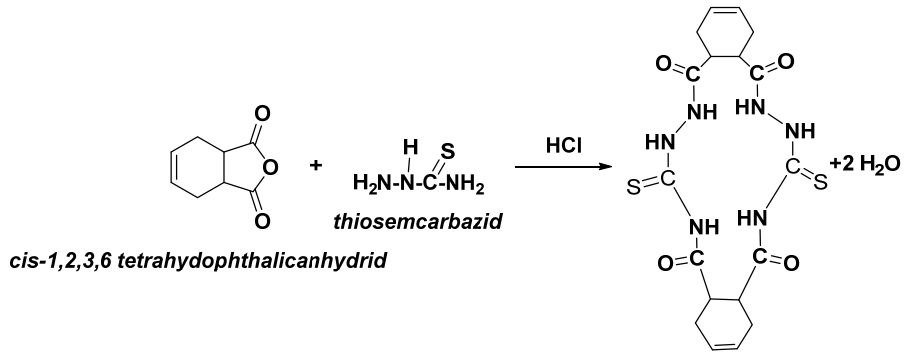
Recently, N_4-based metal sites have been widely explored in sustainable energy applications. In particular, atomically dispersed Fe–N_4 and Ni–N_4 centers embedded in carbon materials have emerged as highly efficient catalysts for oxygen reduction and CO_2 electroreduction reactions. Their performance is attributed to the precise control of the coordination environment and the modulation of electronic properties provided by the tetradentate ligand framework [5-7].

Despite these advances, the design of new N_4 ligands with tailored structural features remains an important challenge, especially for improving biological activity and structure–activity relationships. In this context, the present work focuses on the synthesis of a new tetradentate nitrogen ligand derived from cis-1,2,3,6-tetrahydrophthalic anhydride and thiosemicarbazide.

The novelty of this study lies in the development of a ligand with a distinct donor environment and structural configuration, which is expected to influence its coordination behavior and biological properties. Its complexes with Cr(III), Mn(II), Fe(II), Co(II), Ni(II), and Cu(II) ions were synthesized and systematically characterized. Furthermore, their antibacterial activities were evaluated in order to establish correlations between structure and biological performance.

Experimental part

Preparation of ligand (L). In a 50 mL conical flask equipped with a magnetic stirrer, cis-1,2,3,6-tetrahydrophthalic anhydride (0.01 mol, 1.50 g) was dissolved in absolute ethanol (20 mL). A solution of thiosemicarbazide (0.01 mol, 0.911 g) in absolute ethanol (10 mL) was then added, followed by the addition of three drops of concentrated hydrochloric acid (Scheme). The reaction mixture was refluxed under continuous stirring for 7 h. Upon completion of the reaction, an off-white solid precipitate formed and was collected by filtration, washed several times with small portions of cold ethanol followed by diethyl ether, and dried under vacuum. The crude product was purified by recrystallization from hot ethanol.



Scheme. Preparation of ligand

Preparation of the metal complexes. A hot ethanolic solution of the ligand L (0.001 mol, 0.45 g) in ethanol (20 mL) was added dropwise, with continuous stirring, to a hot ethanolic solution (20 mL) containing the corresponding metal salt (0.001 mol): $CrCl_3 \cdot 6H_2O$ (0.266 g), $MnCl_2 \cdot 4H_2O$ (0.197 g), $FeCl_2 \cdot 4H_2O$ (0.198 g), $CoCl_2 \cdot 6H_2O$ (0.240 g), $NiCl_2 \cdot 6H_2O$ (0.237 g), or $CuCl_2 \cdot 2H_2O$ (0.170 g). The resulting mixture was refluxed for 5 h under continuous stirring. Upon cooling to room temperature, solid metal complexes of various colors precipitated. The products were isolated by filtration, washed thoroughly with cold ethanol, and dried under vacuum.

Result and discussion

The tetradentate nitrogen ligand (L) was successfully synthesized via the condensation reaction between cis-1,2,3,6-tetrahydrophthalic anhydride and thiosemicarbazide in ethanolic medium under acidic conditions. The reaction proceeded smoothly, yielding an off-white solid in good yield, indicating the efficiency of the synthetic route and the stability of the resulting ligand framework. The ligand structure is consistent with the formation of a macrocyclic-like system containing multiple nitrogen donor sites capable of coordinating transition metal ions.

Elemental Analysis and Molar Conductance. The analytical and physicochemical data of the ligand and its metal complexes are summarized in Table 1. The obtained elemental analysis values (C, H, N) are in good agreement with the calculated values, confirming the proposed stoichiometry of the ligand and its corresponding complexes.

Molar conductance measurements in DMSO revealed that most complexes exhibit low conductivity values, indicating their non-electrolytic nature [8]. An exception was observed for the Cr(III) complex, which showed a higher conductance value consistent with a 1:2 electrolyte behavior.

This suggests the presence of an outer-sphere chloride ion in this complex, while the remaining complexes are likely neutral species. These findings support the proposed coordination mode and formulation of the complexes.

Table 1. Molar conductance and element analysis data of the complexes

No	Compound	color	M.P., ^{o}C	Yield, %	Molar conductance (cm^2 Mol^{-1} Ω^{-1})	Element analysis calculated (found)		
						C	H	N
L	$C_{18}O_4S_2N_6H_{22}$	Off white	120-122	80	------	48 (47.7)	4.92 (4.58)	18.69 (17.49)
1	$[Cr(L)Cl_2]Cl$	Dark green	106-109	78	33.8	35.51 (34.98)	3.62 (3.47)	13.81 (12.99)
2	$[Mn(L)Cl_2]$	Pale yellow	68-70	82	16.2	37.51 (35.98)	3.84 3.25)	14.59 (14.01)
3	$[Fe(L)Cl_2]$	Dark red	158-160	85	13.6	37.45 (36.09)	3.84 (3.11)	14.56 (14.29)
4	$[Co(L)Cl_2]$	Green	160-163	91	8.9	37.25 (35.99)	3.79 (3.09)	14.48 (13.85)
5	$[Ni(L)Cl_2]$	Light green	130-133	89	18.3	37.27 (37.00)	3.82 (3.21)	14.49 (14.08)
6	$[Cu(L)Cl_2]$	Green	172-175	79	12.3	36.96 (36.10)	3.79 (3.55)	14.37 (13.76)

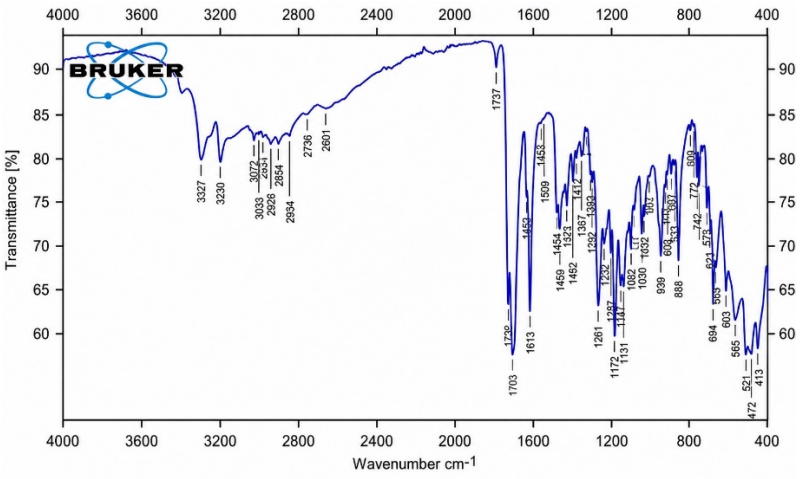
IR spectra. The IR spectrum of the free ligand (Table 2, Fig. 1) provides important insights into its coordination behavior. The absence of bands corresponding to free primary amine groups confirms the completion of the condensation reaction. Characteristic bands associated with amide (C=O), thioamide, and N–H groups are clearly observed [9, 10].

Upon complexation, significant changes in the IR spectra were detected. The amide II and amide III bands exhibited noticeable shifts in frequency, indicating the involvement of nitrogen atoms in coordination with the metal ions. Additionally, the N–H stretching vibration, originally observed at 3297 cm^{-1} in the free ligand, shifted to lower frequencies (3143–3266 cm^{-1}) in the complexes. This redshift reflects a decrease in bond strength due to coordination of the nitrogen lone pair to the metal center [11].

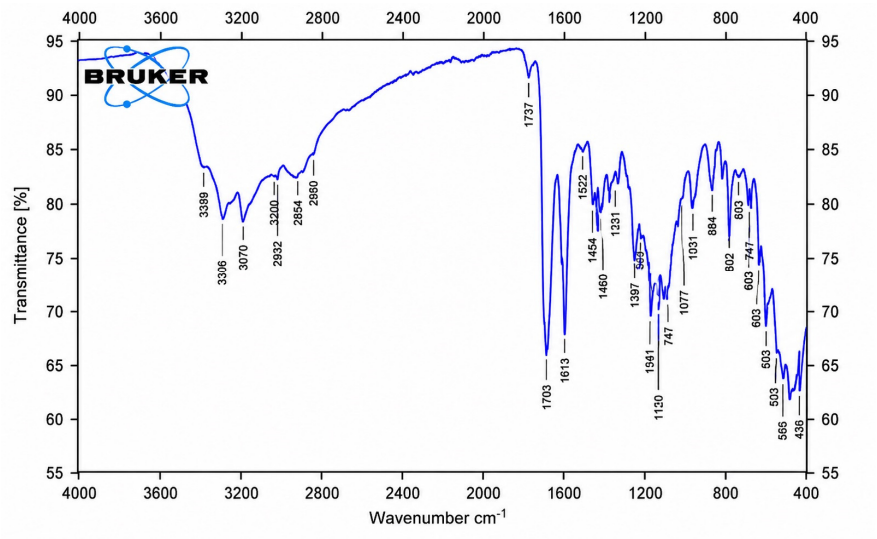
Furthermore, new bands appearing in the low-frequency region (426–487 cm^{-1}) are attributed to M–N stretching vibrations, providing strong evidence for metal–nitrogen bond formation. Overall, the IR spectral data confirm that the ligand coordinates through nitrogen donor atoms, forming stable chelate complexes [12].

Table 2. Selected IR bands and their assignment in cm^{-1}

No.	Compound	υ(C=O) amide I	υ(C-N) + (N-H) amide II	υ(N-H) amide III	Thioamide I and II		υ(N-H)	υ(M-N)
	L	1613	1539	1323	755	1392	3297	------
1	$[Cr(L)Cl_2]Cl$	1615	1578	1347	753	1380	3156	487
2	$[Mn(L)Cl_2]$	1617	1554	1368	754	1389	3145	439
3	$[Fe(L)Cl_2]$	1612	1597	1370	751	1390	3264	440
4	$[Co(L)Cl_2]$	1621	1566	1345	754	1398	3151	431
5	$[Ni(L)Cl_2]$	1613	1518	1347	755	1391	3266	435
6	$[Cu(L)Cl_2]$	1615	1557	1375	755	1375	3143	426



(a)



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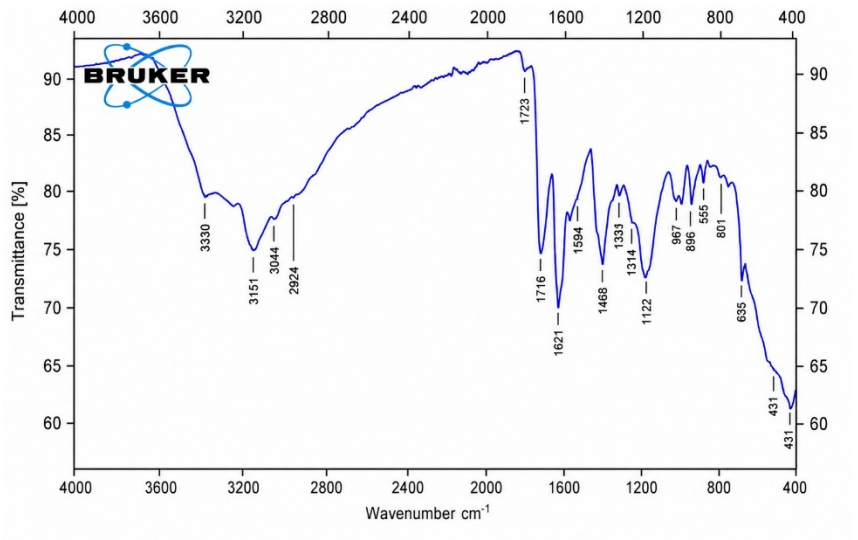


(c)

Fig. 1. IR-Spectra of (a) (L), (b) [Ni(L)Cl_2], and (c) [Co(L)Cl_2]

The magnetic susceptibility and UV-visible spectroscopy. The electronic spectral data combined with magnetic susceptibility measurements provide valuable information about the geometry of the complexes.

The effective magnetic moment (μ_{eff}) of Cr(III) complex (1) was 3.99 B.M. for d^3 electronic configuration [13], and the electronic spectra at room temperature showed bands at 17123, 22783, and 38759 cm^{-1} which were attributed to the $^4A_2g \rightarrow {}^4T_2g$ υ_1, $^4A_2g \rightarrow {}^4T_1g$ (F) υ_2 and $^4A_2g \rightarrow {}^4T_1g$ (P) υ_3, respectively. The position of electronic spectra bands and the value of μ_{eff} indicate that the complex has octahedral geometry [14, 15].

Mn(II) complex (2) has a magnetic moment value of 5.85 B.M., and the electronic spectra exhibit bands of 37885, 31566, and 36527 cm^{-1} due to charge transfer. These values were well in accordance with the complex having an octahedral structure with five unpaired electrons [3, 4].

The electronic spectra of iron (II) complex (3) showed a band at 11164 cm^{-1}, which is attributed to the $^5T_2g \rightarrow {}^5Eg$ transition and could be assigned to a distorted octahedral structure and μ_{eff}= 5.09 B.M. confirms the proposed configuration [16].

The Co(II) complex (4) exhibits a magnetic moment value lying in 4.49 B.M., corresponding to three unpaired electrons, a higher value due to orbital contribution. The electronic spectra of this complex showed an absorption in the regions (10238), (15933), (18751), and 32428 cm^{-1} these bands may be assigned to the transitions $^4T_1g(F) \rightarrow {}^4T_2g(F)$ υ_1, $^4T_1g \rightarrow {}^4A_2g$ υ_2, and $^4T_1g(F) \rightarrow {}^4T_1g(P)$ υ_3, respectively. The last band may be due to charge transfer. These bands indicated distorted octahedral geometry around the Co(II) complex [2].

The magnetic moment value of the Ni(II) complex (5) showed 3.02 BM. This value is in turn with a high-spin configuration and octahedral environment around the Ni(II) ion in the complex [17]. The electronic spectrum of the complex show three bands 10198, 15386, 25784 cm^{-1}, corresponding to $3A_2g(F) \rightarrow 3T_2g(F)$ υ_1, $3A_2g(F) \rightarrow 3T_2g(F)$ υ_2, and $3A_2g(F) \rightarrow 3T_2g(F)$ υ_2, respectively.

The μ_{eff} value of Cu(II) complex is 2.1 B.M., which corresponds to one unpaired electron, and displays one band in the electronic spectra at 10883 cm^{-1}, which is assigned to the $2E_2g \rightarrow 2T_2g$ transition in the distorted octahedral structure around the Cu(II) ion [18].

Fig. 2 represents suggested structures of synthesized complexes.

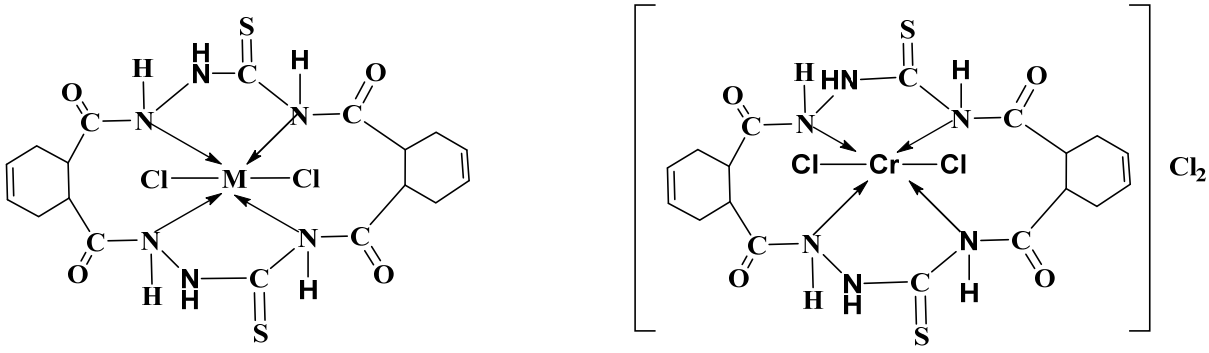


Fig. 2. Suggested structures of complexes

^{1}H-NMR Spectra. The ^{1}H-NMR spectrum of the synthesized ligand (400 MHz, DMSO-d_6) shows signals consistent with the proposed macrocyclic structure (Fig. 3). A distinct signal was observed at ~5.84-5.91 ppm, attributed to alkene (-CH=CH-) protons within the non-aromatic ring [19], a range consistent with standard values for isolated double bond protons. Signals were also observed in the ~ 2.85-2.95 ppm range, attributed to aliphatic (CH_2) group protons attached to electron-withdrawing atoms or groups, resulting in a shift towards the lower field [20]. Additionally, several signals appeared in the 8.53-8.62 ppm range, attributed to NH protons attached to amide (-CONH-) groups where the electron-withdrawing effect of the carbonyl group removes electron shielding and increases the chemical shift value [21]. Signals were also recorded at ~10.27-10.35 ppm

and ~10.90-10.97 ppm, which are attributed to NH protons bound to thioamide groups (-CSNH-). This large shift is attributed to the combined effect of the electronegativity of the sulfur atom and the possibility of internal or intermolecular hydrogen bonds. Notably, there are no signals in the 6.5-8.0 ppm range, confirming the absence of an aromatic system in the compound. Therefore, the signal distribution and their chemical position show good agreement with the proposed structure and support its validity in terms of the proton electronic environment [21, 22].

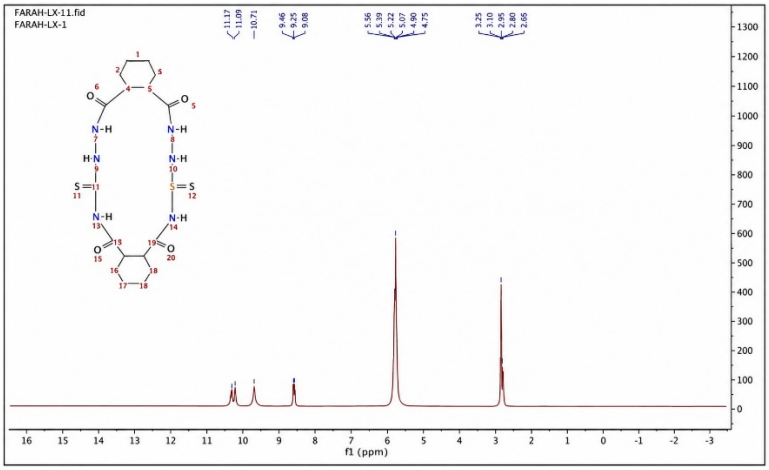


Fig. 3. ^{1}H-NMR spectra for the ligand

Microbiological investigations (Table 3). Mueller-Hinton agar medium was prepared according to the manufacturer's instructions. Specifically, 38 g of agar was dissolved in 1 L of boiling distilled water, and the pH was adjusted to 7.2, the medium was sterilized by autoclaving at 121°C and 150 psi for 15 min. After cooling to approximately 50° C, the sterile medium was poured into Petri dishes to a uniform thickness of 2 mm and allowed to solidify. The plates were subsequently incubated at 37° C for 24h to confirm sterility before being used in antibacterial assays.

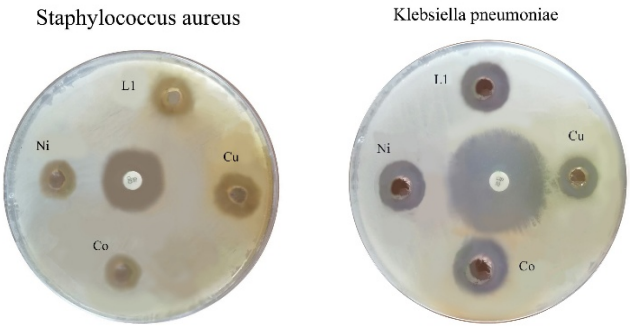
The antimicrobial activity of ligand (L_1) and its Co(II), Ni(II), and Cu(II) complexes was evaluated by the disk diffusion method on *Klebsiella pneumoniae* (Gram-negative) and Staphylococcus aureus (Gram-positive). The free ligand exhibited moderate inhibition zones (16 mm and 14 mm, respectively), confirming its intrinsic antibacterial potential. The positive control produced significantly larger zones (30 mm and 20 mm), validating the assay conditions [23].

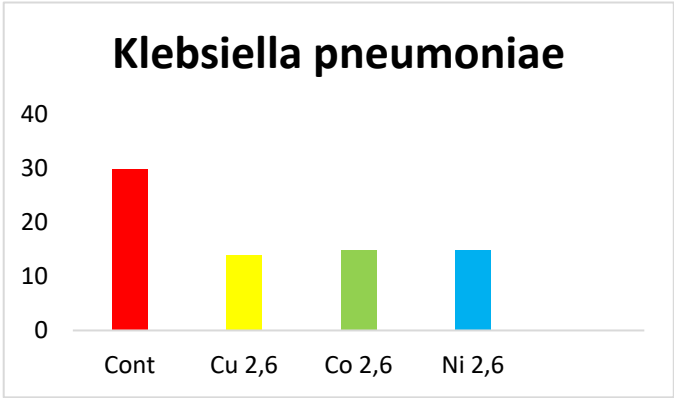
Complexation produced variable effects depending on the bacterial strain. [Co(L)Cl_2] slightly enhanced activity against *Klebsiella* (18 mm) but reduced it against *Staphylococcus* (13 mm). [Ni(L)Cl_2] showed no significant change, while [Cu(L)Cl_2] improved activity against *Staphylococcus* (16 mm) but decreased it against *Klebsiella* (14 mm) [24].

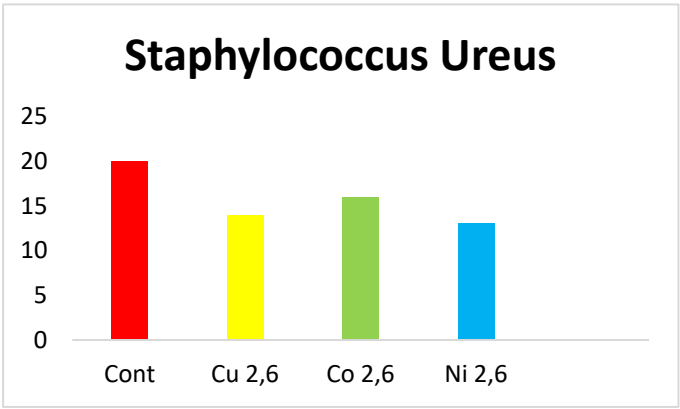
These trends indicate that metal coordination can modulate lipophilicity, stability and membrane permeability, leading to species-dependent activity. The higher effect of Cu(II) against Gram-positive *Staphylococcus* reflects easier penetration compared to Gram-negative *Klebsiella*. Overall, the ligand and complexes exhibit measurable but lower potency than standard antibiotics [25, 26].

Table 3. The effectiveness area of bacterial inhibition in mm for the prepared complexes

Complexes	**Symbol**	***Klebsiella Pneumoniae***	***Staphylococcus Ureus***
L	L1	16	14
Control	Cont.	30	20
[Co(L)Cl_2]	Co	18	16
[Ni(L)Cl_2]	Ni	19	14
[Cu(L)Cl_2]	Cu	18	16







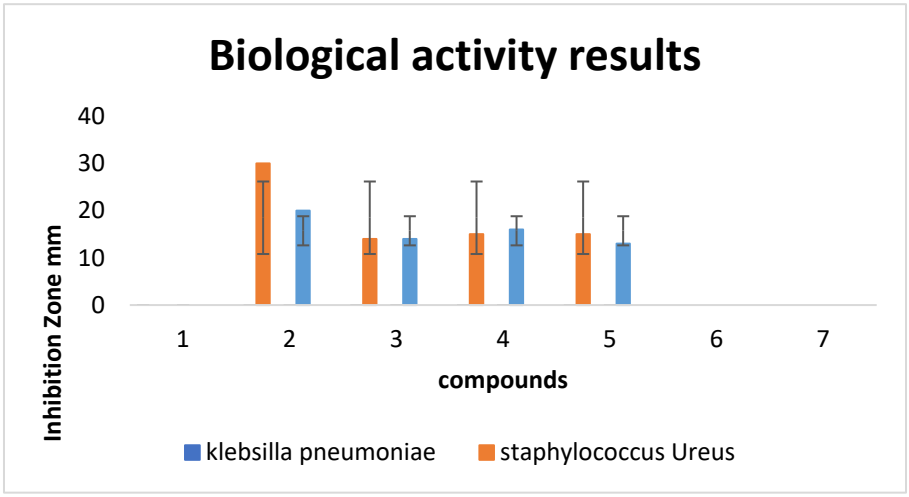


Fig. 4. Antimicrobial action of component agents against *Staph. Aureus* and *Klebsiella pneumonia*

The results of the microbial activity shown in Figure 4 demonstrated a significant superiority of the prepared metal complexes in inhibiting the growth of the tested bacteria compared to the free

ligand (L), which is directly consistent with the recorded minimum inhibitory concentration (MIC). This increase in activity can be explained based on the theory of chelation, where the compatibility of Overton′s concept and Overton′s theory of the metal ion with the ligand leads to a decrease in polarity and an increase in the lipophilicity of the complex, which facilitates its penetration of the lipid cell membrane of microbes and its involvement in cellular bioprocesses. The error bars included in the graph also reflect the statistical accuracy and reliability of the results resulting from the replication of measurements, which confirms the efficacy of these complexes as promising inhibitory agents at low concentrations.

Conclusion

The present study successfully demonstrated the synthesis of a novel tetradentate nitrogen (N_4) ligand and its coordination with various transition metal ions. Spectroscopic and magnetic analyses confirmed coordination through nitrogen donor atoms and suggested distorted octahedral geometries for all complexes. The physicochemical data supported the proposed structures and stability of the complexes. Biological investigations revealed moderate antibacterial activity, with enhanced performance observed upon metal coordination, particularly in the Cu(II) complex. These findings emphasize the importance of ligand design in tuning both structural and biological properties, suggesting potential applications in coordination chemistry and medicinal research.

Conflicts of Interest. The authors declare no conflict of interest.

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