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Symmetrical N_2O_2 Tetradentate Schiff Base Complexes: Antibacterial Properties

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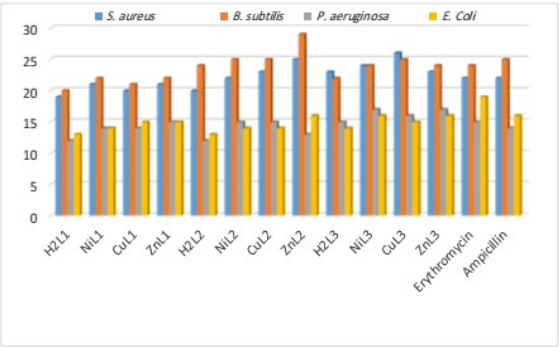
Abstract: Three Schiff bases, **(H_2L^n)**, were successfully synthesized by condensing different 3,5-dihalosalicylaldehydes (chloro, bromo, and iodo as haloatoms) with 2,2-dimethyl-1,3-propanediamine. Furthermore, their transition metal complexes, **(ML^n)**, were prepared by refluxing the Schiff bases with corresponding metal(II) acetate in methanol. To thoroughly analyze the synthesized compounds, various spectroscopic techniques, such as: FT-IR and ^{1}H NMR, along with analytical methods like CHN analysis were employed. To assess their antibacterial activity in vitro, the compounds were tested against two categories of bacteria: *Staphylococcus aureus* and *Bacillus cereus* representing Gram-positive bacteria, and *Escherichia coli* and *Pseudomonas aeruginosa* representing Gram-negative bacteria. Ampicillin and Erythromycin were used as reference antibacterial drugs.



Keywords: Tetradentate Schiff base, Metal complexes, Spectral characterization, Antibacterial activity

1. INTRODUCTION

When an amine reacts with an aldehyde or ketone through a condensation reaction, a chemical compound called Schiff base is formed. This process generates a carbon-nitrogen double bond (C=N).[1] This double bond characterizes the Schiff base functional group. The late 19th century saw the emergence of a class of compounds first described by the German chemist Hugo Schiff, from whom they derived their name in coordination chemistry. Schiff bases play a vital role as essential chelating ligands. These compounds can form stable complexes with transition metals such as copper, zinc, nickel, and cobalt by binding through their imine nitrogen. As a consequence, various metal complexes containing Schiff bases that exhibit different coordination numbers and geometrical arrangements can be prepared.[2,3]

The diverse chemical and structural properties of Schiff base complexes enable them to be utilized across a broad spectrum of fields. Some of the key applications of Schiff base complexes include materials science,[4] photophysical applications,[5,6] dye sensitizers,[7] nanotechnology,[8] catalyst,[9-11] and so on.

Schiff base complexes display a diverse array of biological effects, encompassing antimicrobial,[12-14] antiviral,[15] antitumor,[16] antimalarial,[17] and antioxidant activities.[18] These chemical entities have been subject to scrutiny regarding their potential utility as therapeutic agents in diverse medical contexts.[19,20] The existence of a Schiff base ligand within the complex can alter its biological activity through its impact on the interaction with target biomolecules or enzymes.

Schiff bases often display chelating properties, engaging in the formation of a stable ring structure with the metal ion. This chelation behavior can enhance the complex's stability and solubility, thus influencing its biological activity.[21] By engaging in chelation, the metal ion's reactivity can be regulated, which, in turn, impacts its interaction with other biomolecules. The adaptability of Schiff base complexes in coordination chemistry and their customizable properties render them highly valuable in numerous scientific and technological domains. Researchers are actively conducting extensive investigations to unveil their potential applications across various realms of medicine and biotechnology.

2. EXPERIMENTAL

Materials and methods

In the current work, laboratory-grade chemicals of high purity are used, which are procured from Alfa Aesar. The ^{1}H NMR spectral data was collected through a BRUKER AVANCE 400 MHz spectrometer. The chemical shift (δ) values were recorded in ppm with reference to tetramethylsilane (TMS). Additionally, a Heraeus CHN-O-FLASH EA 1112 instrument was used to analyze the chemical composition of the synthesized compounds. To assess the vibrational properties of different functional groups in the molecules, the IRPrestige-21 spectrophotometer was used by adopting the KBr pellet method.

Synthesis

Synthesis of symmetrical tetradentate schiff base ligands (H_2L^n). First, 2,2-dimethyl-1,3-propanediamine (1.0 mmol), was dissolved in 20 mL ethanol. Next, this solution was added to a separate solution of 3,5-dihalosalicylaldehyde (2.0 mmol) dissolved in 20 mL ethanol. The resulting mixture was then refluxed for 3 hours. To confirm the sample's purity, thin-layer chromatography (TLC) was used. When the mixture was cooled, it produced a solid yellow product **(H_2L^n)**. The product was filtered, washed with ethanol and ether, and then placed in a dehydrated environment within a desiccator to dry.

H_2L^1: 6,6'-((1*E*,1'*E*)-((2,2-dimethylpropane-1,3-diyl)bis(azanylylidene))bis(methanylylidene)) bis(2,4-dichlorophenol). Yield: 93 %, 0.417 g. Anal. Calc. for $C_{19}H_{18}Cl_4N_2O_2$: C, 50.92; H, 4.05; N, 6.25, Found: C, 51.07; H, 4.09; N, 6.11 %. Selected IR data (KBr, υ/cm^{-1}); 1635 (C=N); 1558, 1467 (C=C); 1213 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 1.11 [3 H, s, (-CH_3)], 3.56 [2 H, s, (-CH_2-N)], 7.19 [2 H_b, d, ^4J = 2.4 Hz], 7.44 [2 H_a, d, ^4J = 2.4 Hz], 8.29 [2 H, s, (-CH=N)], 14.44 [2H, s, br. (-OH)].

H_2L^2: 6,6'-((1*E*,1'*E*)-((2,2-dimethylpropane-1,3-diyl)bis(azanylylidene))bis(methanylylidene)) bis(2,4-dibromophenol). Yield: 83 %, 0.520 g. Anal. Calc. for $C_{19}H_{18}Br_4N_2O_2$: C, 36.46; H, 2.90; N, 4.48, F.und: C, 36.67; H, 2.95; N, 4.34 %. Selected IR data (KBr, υ/cm^{-1}); 1631 (C=N); 1555, 1456 (C=C); 1213 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 1.10 [3 H, s, (-CH_3)], 3.56 [2 H, s, (-CH_2-N)], 7.36 [2 H_b, d, ^4J = 2.4 Hz], 7.73 [2 H_a, d, ^4J = 2.4 Hz], 8.25 [2 H, s, (-CH=N)], 14.56 [2 H, s, br. (-OH)].

H_2L^3: 6,6'-((1*E*,1'*E*)-((2,2-dimethylpropane-1,3-diyl)bis(azanylylidene))bis(methanylylidene)) bis(2,4-diiodophenol). Yield 78 %, 0.635 g. Anal. Calc. for $C_{19}H_{18}I_4N_2O_2$: C, 28.04; H, 2.23; N, 3.44, Found: C, 28.27; H, 2.26; N, 3.28 %. Selected IR data (KBr, υ/cm^{-1}); 1628 (C=N); 1581, 1437 (C=C); 1195 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 1.10 [3 H, s, (-CH_3)], 3.54 [2 H, s, (-CH_2-N)], 7.55 [2 H_b, d, ^4J = 2.4 Hz], 8.08 [2 H_a, d, ^4J = 2.4 Hz], 8.16 [2 H, s, (-CH=N)], 14.69 [2 H, s, br. (-OH)].

Synthesis of ML^n complexes (M = Ni, Cu, Zn & n = 1, 2, 3). First, $M(OAc)_2.nH_2O$ (M = Ni, Cu, Zn) (1.0 mmol), was dissolved in 10 mL methanol. Next, this solution was added to a separate solution of Schiff base ligands (1.0 mmol) in 10 mL methanol. The resulting mixture was then refluxed for 2 hours. Afterwards, the complexes were precipitated as the reaction mixture was allowed to cool down to ambient temperature. These precipitates were subsequently filtered using suction and then washed thrice with methanol for purification. Finally, the purified complexes were dried under vacuum conditions.

NiL^1: Yield 74 %, 0.374 g. Anal. Calc. for $C_{19}H_{16}Cl_4N_2NiO_2$: C, 45.20; H, 3.19; N, 5.55, Found: C, 45.37; H, 3.22; N, 5.41 %. Selected IR data (KBr, υ/cm^{-1}); 1626 (C=N); 1522, 1452 (C=C); 1321 (C-O); 509 (Ni-O); 442 (Ni-N). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.91 [3 H, s, (-CH_3)], 3.45 [2 H, s, (-CH_2-N)], 7.38 [2 H_b, br.], 7.50 [2 H_a, d, br.], 8.49 [2 H, s, (-CH=N)].

CuL^1: Yield 81 %, 0.413 g. Anal. Calc. for $C_{19}H_{16}Cl_4CuN_2O_2$: C, 44.77; H, 3.16; N, 5.50, Found: C, 44.91; H, 3.20; N, 5.34 %. Selected IR data (KBr, υ/cm^{-1}); 1628 (C=N); 1520, 1446 (C=C); 1317 (C-O); 557 (Cu-O); 436 (Cu-N).

ZnL^1: Yield 63 %, 0.322 g. Anal. Calc. for $C_{19}H_{16}Cl_4N_2O_2Zn$: C, 44.61; H, 3.15; N, 5.48, Found: C, 44.86; H, 3.18; N, 5.33 %. Selected IR data (KBr, υ/cm^{-1}); 1630 (C=N); 1521, 1444 (C=C); 1313 (C-O); 559 (Zn-O); 463 (Zn-N). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.84 [3 H, s, (-CH_3)], 3.46 [2 H, s, (-CH_2-N)], 7.16 [2 H_b, d, ^4J = 2.8 Hz], 7.33 [2 H_a, d, ^4J = 2.8 Hz], 8.16 [2 H, s, (-CH=N)].

NiL^2: Yield 68 %, 0.464 g. Anal. Calc. for $C_{19}H_{16}Br_4N_2NiO_2$: C, 33.43; H, 2.36; N, 4.10, Found: C, 33.57; H, 2.38; N, 3.91 %. Selected IR data (KBr, υ/cm^{-1}); 1622 (C=N); 1510, 1442 (C=C); 1317 (C-O); 503 (Ni-O); 445 (Ni-N). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.91 [3 H, s, (-CH_3)], 3.45 [2 H, s, (-CH_2-N)], 7.54 [2 H_b, br.], 7.65 [2 H_a, d, br.], 8.29 [2 H, s, (-CH=N)].

CuL^2: Yield 73 %, 0.502 g. Anal. Calc. for $C_{19}H_{16}Br_4CuN_2O_2$: C, 33.19; H, 2.35; N, 4.07, Found: C, 33.31; H, 2.39; N, 3.89 %. Selected IR data (KBr, υ/cm^{-1}); 1628 (C=N); 1508, 1440 (C=C); 1311 (C-O); 553 (Cu-O); 484 (Cu-N).

ZnL^2: Yield 59 %, 0.407 g. Anal. Calc. for $C_{19}H_{16}Br_4N_2O_2Zn$: C, 33.11; H, 2.34; N, 4.06, Found: C, 33.36; H, 2.38; N, 3.93 %. Selected IR data (KBr, υ/cm^{-1}); 1629 (C=N); 1508, 1440 (C=C); 1309 (C-O); 549 (Zn-O); 447 (Zn-N). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.83 [3 H, s, (-CH_3)], 3.48 [2 H, s, (-CH_2-N)], 7.30 [2 H_b, d, ^4J = 2.4 Hz], 7.55 [2 H_a, d, ^4J = 2.4 Hz], 8.13 [2 H, s, (-CH=N)].

NiL^3: Yield 66 %, 0.575 g. Anal. Calc. for $C_{19}H_{16}I_4N_2NiO_2$: C, 26.21; H, 1.85; N, 3.22, Found: C, 26.47; H, 1.88; N, 3.11 %. Selected IR data (KBr, υ/cm^{-1}); 1626 (C=N); 1522, 1452 (C=C); 1321 (C-O); 507 (Ni-O); 445 (Ni-N). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.91 [3 H, s, (-CH_3)], 3.45 [2 H, s, (-CH_2-N)], 7.64 [2 H_b, br.], 7.95 [2 H_a, d, br.], 8.38 [2 H, s, (-CH=N)].

CuL^3: Yield 72 %, 0.630 g. Anal. Calc. for $C_{19}H_{16}CuI_4N_2O_2$: C, 26.07; H, 1.84; N, 3.20, Found: C, 26.28; H, 1.88; N, 3.02 %. Selected IR data (KBr, υ/cm^{-1}); 1624 (C=N); 1520, 1448 (C=C); 1311 (C-O); 551 (Cu-O); 476 (Cu-N).

ZnL^3: Yield 57 %, 0.501 g. Anal. Calc. for $C_{19}H_{16}I_4N_2O_2Zn$: C, 26.01; H, 1.84; N, 3.19, Found: C, 26.25; H, 1.89; N, 3.03 %. FT-IR (KBr, cm^{-1}); 1629 (C=N); 1508, 1440 (C=C); 1309 (C-O); 505 (Zn-O); 457 (Zn-N). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 0.81 [3 H, s, (-CH_3)], 3.49 [2 H, s, (-CH_2-N)], 7.41 [2 H_b, d, ^4J = 2.0 Hz], 7.80 [2 H_a, d, ^4J = 2.0 Hz], 8.03 [2 H, s, (-CH=N)].

Antibacterial activity studies

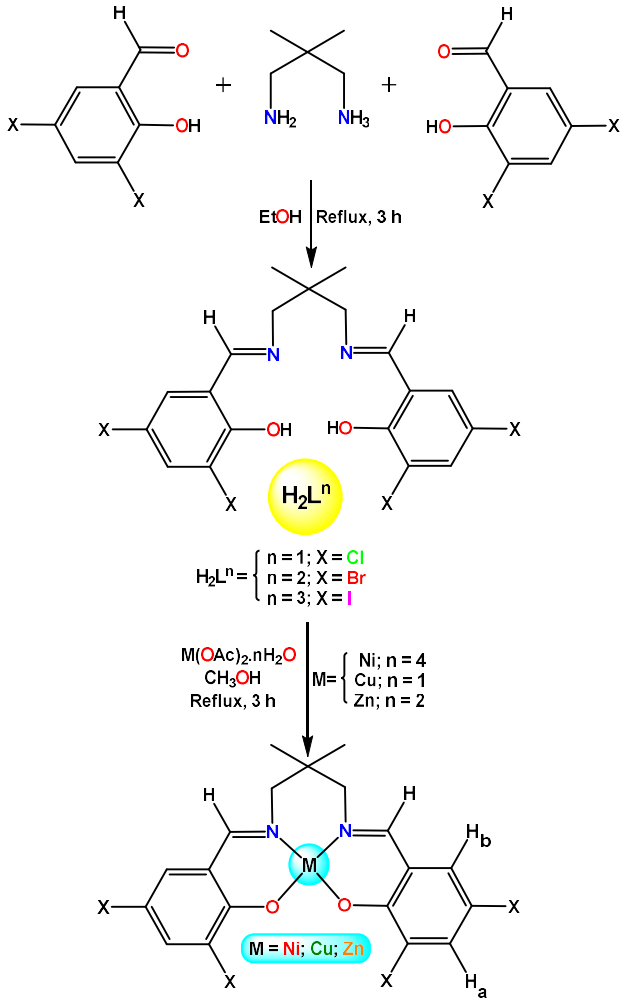
The screening program aims to evaluate the antimicrobial activity of the compounds under investigation. To achieve this, a panel of microorganisms, including Gram-positive bacteria, such as *Staphylococcus aureus* (PTCC1431) and *Bacillus subtilis* (PTCC1015), as well as Gram-negative bacteria like *Pseudomonas aeruginosa* (PTCC1074) and *Escherichia Coli* (PTCC1394), was utilized to test the synthesized Schiff base metal complexes, **(ML^n)**, and the free ligands, **(H_2L^n)**. The disk diffusion method was chosen for its convenience, effectiveness, and cost-efficiency compared to other procedures.[22,23] Dimethyl sulfoxide (DMSO) was selected as the solvent due to its high solubility for various organic and organometallic compounds. Additionally, DMSO served as the negative control in all experiments. The laboratory samples were prepared by dissolving the compounds in DMSO at a concentration of 1 mg/mL, which was subsequently diluted to a concentration of 50 µg/mL. Agar was used as a nutrient medium for bacterial culture preparation. Erythromycin and Ampicillin were used as positive control standard drugs. To ensure sterility, sterile paper discs with a diameter of 7 mm were autoclaved. These discs were aseptically soaked with either the synthesized compounds or DMSO. The discs were then carefully placed in separate Petri dishes containing nutrient agar media that had been inoculated

with the four aforementioned bacterial strains. After incubating for 24 hours at 37 °C, the diameters of the inhibition zones formed by the samples were measured in millimeters.

3. RESULTS AND DISCUSSION

Synthesis

The symmetric tetradentate N_2O_2 Schiff base ligands were synthesized by treating 2,2-dimethyl-1,3-propanediamine with 3,5-dihalosalicylaldehydes in ethanol. The metal complexes were then synthesized by reacting metal acetate with **H_2L^n** in refluxing methanol, as illustrated in Scheme 1.



Scheme 1. Synthesis of Schiff base ligands, **H2Ln**, and their corresponding metal complexes**, MLn**

FT-IR spectra

In the experimental investigation, unique infrared (IR) bands for ligands **(H_2L^n)** and complexes were identified. The coordination sites involved in chelation for the ligands were effectively identified using a comparison study of the FT-IR spectra.

The stretching vibrational band associated with the C=N bond in the Schiff base ligands **(H_2L^n)** experienced a significant change. This band was detected at 1628-1635 cm^{-1} as reported in the literature.[24] However, after complexation, it is shifted to a lower wavenumber value, suggesting that the Schiff base ligands were coordinated with the metal ion through the azomethine nitrogen. As a result of this coordination, the double-bond character was reduced, which agrees with previous findings for similar compounds.[25]

An intriguing observation was made regarding the phenolic ν(C–O) band in the free ligand's spectrum, which exhibited a frequency range of 1323-1293 cm^{-1}. However, in the spectra of the complexes, this band appeared at higher frequencies, indicating that the phenolic oxygen atom acted as an additional coordination site for the Schiff base. Moreover, we observed evidence of oxygen atoms participating in coordination, as seen from the upward shift in the absorption frequency of the phenolic ν(C–O) band in the spectrum of free ligand. As a result of complexation, the C–O stretching vibrational bands in the free ligand shifted towards higher frequency regions, conclusively demonstrating the active involvement of oxygen atoms in the coordination process.[26] It was also observed that interesting bands at lower frequencies are due to interactions between iminic nitrogen and phenolic oxygen, known as M-N and M-O interactions. These findings further support the coordination of the synthesized compounds and improve our comprehension of their coordination behavior.[27]

^{1}H NMR spectral data

In the experimental phase involving the Schiff base ligands and Ni(II) and Zn(II) complexes, ^{1}H NMR spectroscopy was employed for characterization. The experiments were carried out using deuterated chloroform ($CDCl_3$) and deuterated dimethyl sulfoxide (DMSO-d_6) as the solvents. Detailed information regarding the experimental setup can be found in the relevant section. A thorough examination of the chemical entities confirmed that the observed signals matched the expected protons within their respective regions, which provided strong evidence for the proposed compositions of the Schiff base ligands and their complexes. This alignment was further supported by data from infrared (IR) and elemental analyses.

The analysis of integration was successful in accurately identifying the compounds, and the proposed structures of the compounds were matched well with the total number of protons. On complexation, certain resonance signals underwent significant shifts when comparing the chemical shifts of the Schiff base ligands to their corresponding complexes. A noticeable peak at δ = 14.44-14.69 ppm in the ligands' spectra indicated the presence of phenolic protons of ligands. However, this peak disappeared after

complexation, suggesting that the hydroxyl group was deprotonated and oxygen was directly coordinated with the metal atoms.

The spectra of the Ni(II) and Zn(II) complexes were particularly fascinating. The proton resonance of the HC=N group showed upfield and downfield shifts for the nickel and zinc complexes, respectively, indicating chelation through the azomethine nitrogen. Interestingly, the ligands exhibited highly similar spectra, and the expected regions between δ = 7.19 and 8.08 ppm confirmed the presence of all aromatic protons. Upon complexation, the shifts in the positions of signals (δ = 7.16-7.95 ppm) for aromatic protons were observed, while the chemical shift values for aliphatic protons remained relatively unchanged.

Antibacterial activity

Table 1 provides a summary of the results obtained from both qualitative and quantitative assays assessing the antibacterial activity of the test compounds. It is important to mention that, at the tested concentration, DMSO did not show significant antibacterial activity. Under investigation, the biological activity of the compounds remained unaffected by the solvent used.[28,29] It is essential to emphasize the significance of investigating various compounds and identifying active components. Precisely identifying a lead molecule and its drug-like characteristics during the early stages of drug design can offer significant benefits in subsequent drug development efforts.

The study involved testing different types of bacteria, such as Gram-negative *E. coli* and *P. aeruginosa*, and Gram-positive *B. subtilis* and *S. aureus*, to assess how effective Schiff base ligands and their complexes are in combating them. The researchers evaluated the antimicrobial properties of these compounds, and the results are listed in Table 1. Additionally, Figure 1 provides a visual representation of the findings to aid comprehension. The data indicated that Schiff base ligands and their complexes exhibited varying levels of inhibitory effects on the growth of the tested microorganisms. Notably, *S. aureus* was found to be the most susceptible among the tested bacteria. Moreover, all complexes demonstrated higher antibacterial activity values compared to their respective free Schiff base ligands under the same experimental conditions. Notably, the zinc complex demonstrated the highest antimicrobial activity, followed by the copper complex.

Based on previous investigations, it is widely acknowledged that liposolubility plays a crucial role in governing antimicrobial activity.[30,31] The lipid membrane encompassing the cell functions as a selective barrier, permitting only lipid-soluble substances to permeate through. Notably, the free Schiff base ligands exhibit lower antibacterial activity than their corresponding metal complexes.[32-34] One plausible explanation for the reduced activity of the free ligands might be attributed to their lower lipophilicity, leading to decreased diffusion through the lipid membrane, thereby limiting their ability to obstruct or inhibit microorganism growth.[29] The improved antimicrobial properties of Schiff base complexes compared to their corresponding Schiff base ligands can be attributed to various factors, including solubility, conductivity, and metal-ligand bond length.[35] These differences in geometry and steric hindrance enable the complexes to better penetrate and inhibit microorganism cells.

Table 1. Antibacterial activity of synthesized compounds against different strains

Compound	Gram-positive strains		Gram-negative strains	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
H_2L^1	+++	+++	+	+
NiL^1	+++	+++	+	+
CuL^1	+++	+++	+	++
ZnL^1	+++	+++	++	++
H_2L^2	+++	+++	+	+
NiL^2	+++	++++	++	+
CuL^2	+++	++++	++	+
ZnL^2	++++	++++	+	++
H_2L^3	+++	+++	++	+
NiL^3	+++	+++	++	++
CuL^3	++++	++++	++	++
ZnL^3	+++	+++	++	++
Erythromycin	+++	+++	++	++
Ampicillin	+++	++++	+	++

Inhibition values = 10-14 = +
Inhibition values = 15-19 = ++
Inhibition values = 20-24 = +++
Inhibition values = 25-30 = ++++

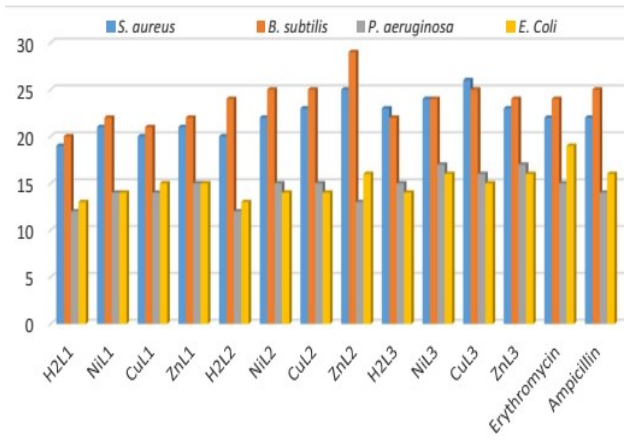


Figure 1. A histogram show the antibacterial evaluation of the investigated Schiff base ligands and their complexes.

It was aimed to enhance the ability of the antimicrobial properties of Schiff base complexes through an exploration of overtone and chelation theory domains.

The intriguing concept of chelation involves reducing the metal ion's polarity by bringing together ligand-associated orbitals and partially engaging donor groups with the metal ion's positive charge. This fascinating phenomenon facilitates more efficient delocalization of p-electrons across the entire chelating ring. As a result, these complexes can better penetrate lipid membranes and hinder metal binding sites in microorganism enzymes, leading to increased efficiency. These findings align with our previous research, which established a connection between a compound's molecular structure and its antibacterial activity, contingent on the specific bacterial strain under examination.[33,36]

Gram-positive bacteria showed heightened sensitivity due to their thicker cell membrane, which differs from the cell membrane of Gram-negative strains. The study's results revealed higher activity in Gram-positive bacteria, which supports previous findings.[37]

4. CONCLUSIONS

In this papar, the synthesis of a series of tetradentate Schiff base ligands with N_2O_2 donor atoms, along with their mononuclear metal complexes are concluded. Once the synthesis was triumphantly accomplished, in a comprehensive characterization process, a diverse range of physicochemical methods are employed. Intriguingly, the metal complexes exhibited a slight but noteworthy enhancement in activity compared to the free Schiff base ligands. The ligands and their metal complexes exhibited higher toxicity levels in gram-positive strains compared to gram-negative strains.

CONFLICTS OF INTEREST

The author affirms that there are no known financial interests or personal relationships that could pose any conflicts and potentially influence the reported findings.

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