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Synthesis and Comparative Antibacterial Studies on Ni (II) and Cu (II) Complexes from some Amino Acid Derivatives

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Abstract

Schiff base ligands Ethane-1,2-diylbis-2-aminoacetimidic acid (L_1) and Ethane-1,2-diylbis-2-aminopropanimidic acid (L_2), along with their corresponding metal (II) complexes of Ni and Cu, were synthesized and characterized through Fourier Transform Infrared (FT-IR) and UV-Visible spectral analysis, melting point/decomposition temperature measurements, solubility assessments, molar conductivity evaluations, and magnetic susceptibility test. The sharp melting points of the ligands and decomposition temperatures of the complexes indicate their purity. The compounds were predominantly insoluble in non-polar organic solvents but soluble in polar solvents, presuming their polar characteristics. The conductivity measurements demonstrate the electrolytic properties of the compounds, correlating with their solubility in polar solvents. Magnetic susceptibility results indicate that the complexes are paramagnetic, exhibiting tetrahedral geometries in all the Ni (II) complexes and distorted octahedral structures in Cu (II) complexes. The antibacterial activities of the compounds were evaluated against gram-positive *Staphylococcus aureus* and *Streptococcus pneumoniae*, as well as gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* isolates, the results generally revealed higher activity in L_1 complexes compared with L_2 complexes.

Keywords: Synthesis, amino acids, Schiff base, antibacterial.

INTRODUCTION

Metals and their complexes are gaining significance in both clinical and commercial settings. Inorganic metal complexes have been employed for the treatment of numerous diseases and conditions for many years. The metal ions in these complexes contribute to activities that improve drug delivery systems, since most organic medications need to interact with metals to demonstrate their effectiveness [1].

Amino acids are organic compounds that serve as the building blocks of proteins. They consist of an amino group (-NH_2), a carboxyl group (-COOH), and a unique side chain R (which might be hydrogen or an organic group) connected to the same carbon atom, also known as the α-carbon atom. It is possible to represent amino acids using the general formula R-CH (NH_2) COOH. The R controls the characteristics of each unique amino acid [2]. Amino acids help the body exchange nutrients between tissues, regulate the body's water balance, and give it energy. Because they can form stable complexes with transition metal ions due to the presence of donor atoms like oxygen and nitrogen, they are therefore perfect chelates or ligands from both chemical and nutritional perspective [3].

Transition metal complexes (also called coordination complexes) are molecules that have groups arranged around a core metal ion. They resemble "lego-molecules" in certain respects since they are easily assembled from smaller parts and can occasionally be changed into new molecules by replacing old ones with new ones. The speed at which these compounds can be assembled and disassembled is among the factors that contribute to their effectiveness in biological and industrial catalysis. The anti-microbial effects of transition metal complexes containing amino acids have been extensively researched. They have shown encouraging results in tests against a variety of bacteria. They fight infection and cancer as well as possess optimal rates of biotransformation and elimination, chemical stability, and selective toxicity. Several active transport systems in the intestinal lumen facilitate the efficient absorption of amino acids. Amino acids exhibit strong biological activity and form stable complexes with ease with most transition metal ions [4].

Schiff bases have been used as chelating ligands in the synthesis of numerous transition metal complexes. They are diverse in structure and can be easily synthesized, typically through the reaction of primary amines with an aldehyde or ketone. Schiff bases are widely employed in a variety of disciplines, including organic, inorganic, and analytical chemistry. Interestingly, a variety of biological and pharmacological functions, including those that are antibacterial, antifungal, analgesic, antiviral, antipyretic, antimalarial, and antiproliferative, highlight the necessity of Schiff base synthesis [5]. It appears that the imine or azomethine group (>C=N–) is essential to their biological actions. This paper reported the synthesis, characterization and antibacterial evaluation of some transition metal complexes derived from glycine and alanine with ethylenediamine.

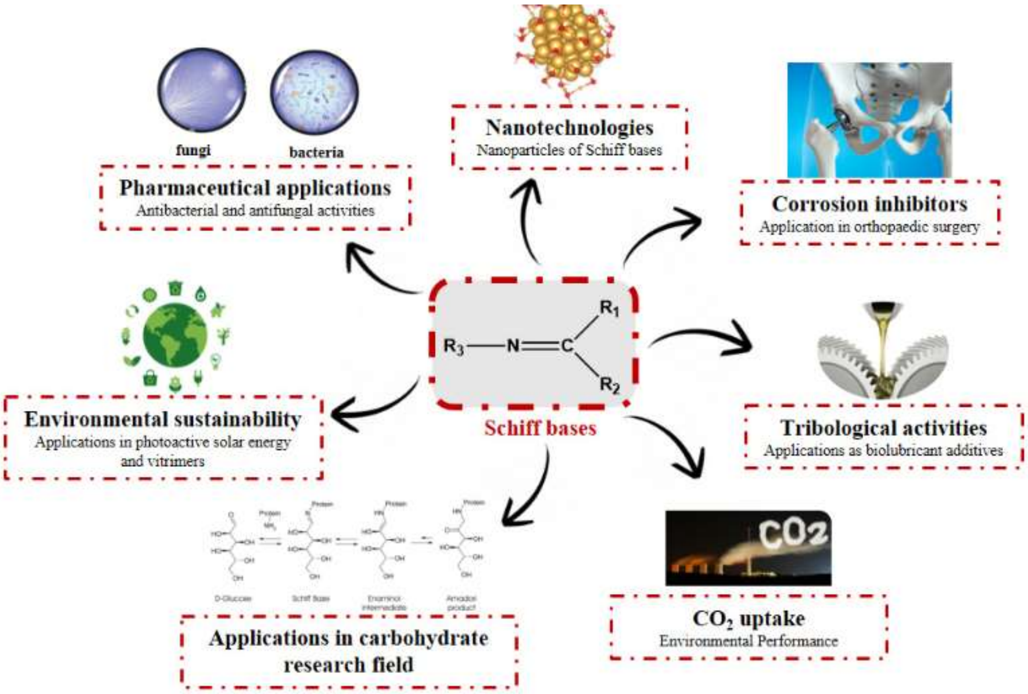

Figure 1: Main properties and uses of Schiff bases [5].

MATERIALS AND METHODS

All the reagents and solvents used were obtained from BDH Chemical Ltd and directly used without further purification. The infrared spectra of the Schiff base ligands and their corresponding metal (II) complexes were obtained using FT-IR spectrophotometer, (Model Cary 630, Agilent Technology) in the range of 4000 – 400 cm^{-1} and 4000 – 350 cm^{-1}, respectively. The UV-Visible absorption spectra were analyzed using UV-Visible spectrophotometer, (Model, T60). While melting point/decomposition temperatures were determined using Barnstead Electrothermal melting point device, the molar conductivities were measured in deionized water (10^{-3} M) using Jenway conductivity meter (model 4010). Similarly, the magnetic susceptibility of the complexes was measured using Sherwood Scientific magnetic susceptibility balance, Mark 1, and the effective magnetic moments were calculated using $\mu_{eff} = 2.828\ (Xm)^{1/2}$ B.M. The bacterial isolates were obtained and identified at the Department of Microbiology, Faculty of Natural and Applied Sciences, Umaru Musa Yaradua University, Katsina.

Synthesis of the Schiff base Ligands

Ethylenediamine (0.05 mol) was dissolved in ethanol (50 mL) and stirred for 10 minutes. The solution was added into a hot ethanolic solution of glycine (0.09 mol), and the stirring continued for 10 minutes. The mixture was then refluxed at 60 °C for 3 hours. The precipitate formed was filtered, washed with ethanol-water mixture at 70:30 ratio, and dried in desiccator over

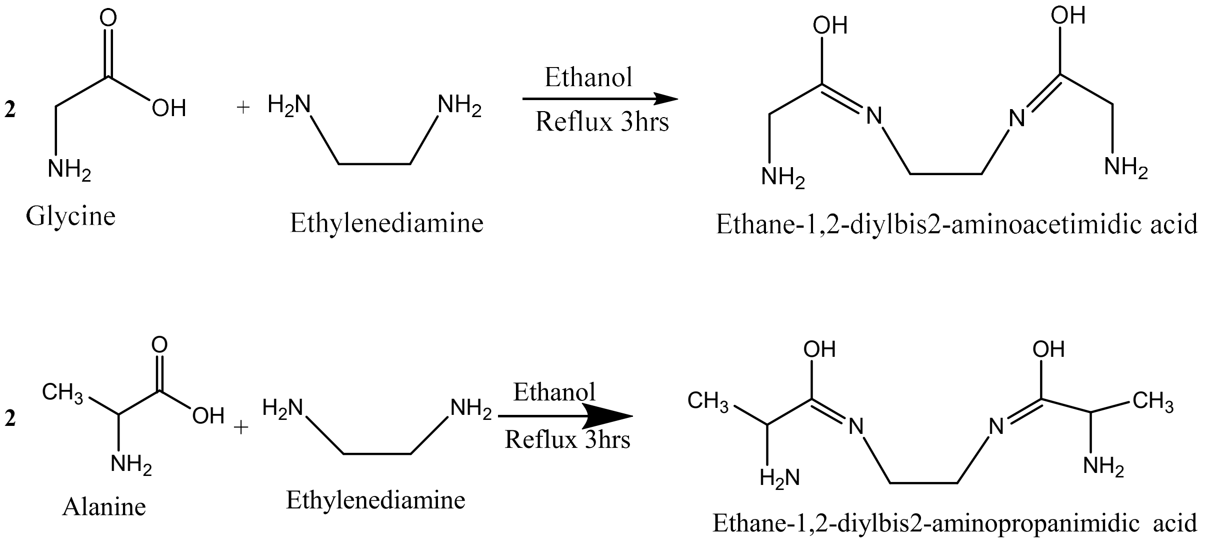

Figure 2: Proposed Structures of the Schiff base Ligands (L_1 and L_2)

phosphorous pentoxide. This procedure was used as a prototype for the preparation of the other Schiff base derived from alanine [6] and [7].

Preparation of the Metal (II) Complexes

The equimolar quantities of the metal salts ($NiCl_2$ and $CuCl_2$), (0.001 mol), and the ligand (0.001 mol), were taken in methanolic solution (20 mL each), and stirred for 5 minutes. The mixture was refluxed for 1hr at 80 °C. After cooling and concentration, the content was kept overnight at room temperature, the precipitate formed was filtered, washed with ethanol-water mixture (70:30) and dried over phosphorous pentoxide [8] and [7].

Antibacterial Activity Test

Using disc diffusion method, both the ligands and their metal (II) complexes were screened for *in-vitro* antibacterial activity against gram-positive *Staphylococcus aureus* and *Streptococcus pneumoniae* as well as gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* isolates. The isolates were cultured on Mueller-Hinton Agar (MHA) and dissolved in distilled water and autoclaved at 121 °C for 15 minutes. About 20 mL of the media was poured in a plate and allowed to solidify before inoculation. Different concentrations of the compounds (1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL) were formed using a serial dilution method with a stock of 1 g in 1 mL of distilled water. A 6 mm disc (made from Whatman filter paper) was sterilized in an autoclave and then inserted into the prepared concentrations of the compounds. Ciprofloxacin (50 µg/mL) was used as a positive control. The diameter of the inhibition zones (in mm) was measured using a ruler [9].

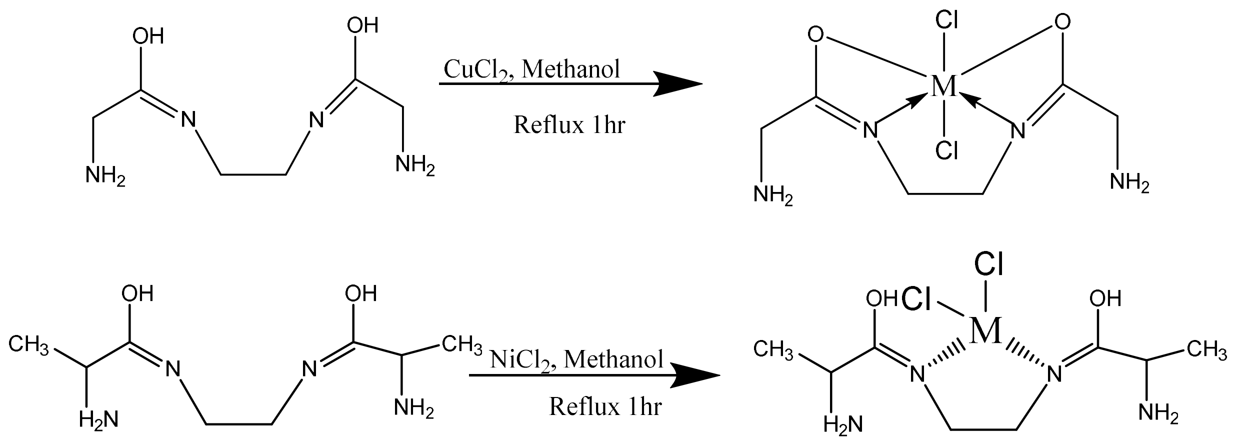

Figure 3: Proposed Structures of the Schiff base Metal Complexes, where M = Ni^{2+} and/or Cu^{2+}

RESULTS AND DISCUSSION

Table 1. The physical properties of the synthesized ligands and their metal complexes

Compounds	Colour	Texture	Yield (%)	M.W. ($gmol^{-1}$)	M.P. (°C)	D.T.(°C)	Molar cond. (ohm^{-1} cm^2mol^{-1})
$C_6H_{14}N_4O_2$ (L_1)	White	Crystalline	64	174.20	208	-	-
$C_6H_{12}NiN_4O_2Cl_2$	Greenish white	Powder	63	301.69	-	246	16
$C_6H_{12}CuN_4O_2Cl_2$	Blue	Powder	67	306.50	-	225	142
$C_8H_{18}N_4O_2$ (L_2)	White	Crystalline	73	202.26	209	-	-
$C_8H_{16}NiN_4O_2Cl_2$	Cream	Crystalline	70	329.69	-	296	15
$C_8H_{16}CuN_4O_2Cl_2$	Violet	Powder	80	334.50	-	260	90

Where: M.W. = molecular weight, M.P. = melting point and D.T. = decomposition temperature.

The sharp values of the melting points and decomposition temperatures (Table 1) indicate the purity of the compounds. It was observed that all the complexes decomposed at higher temperatures when compared with the melting points of their respective ligands. This indicates high thermal stability of the complexes [10]. The compounds give moderately higher yields ranging from 63% - 80%, which is fairly economical. L_1 and L_2-Ni were obtained in crystalline form, signifying their purity. The ligands give white precipitate which were washed and dried. However, the complexes were obtained in different colors, which, is attributable to electronic charge transfer, the result is shown in Table 1. The molar conductivity results of the metal complexes, presented in Table 1 indicate that, all Ni complexes are weak electrolytes with conductance values between 15 and 16 ohm^{-1} cm^{-2}mol^{-1}. L_1-Cu complex shows conductivity value within the range of 1:2 electrolytes (i.e., 120-160 ohm^{-1} cm^{-2}mol^{-1}). L_2-Cu complex, with a value of 90 ohm^{-1} cm^{-2}mol^{-1}, is a 1:1 electrolyte.

The electrolytic nature of compounds corresponds to their solubility in polar solvents.

Table 2. Solubility test of the synthesized ligands and their metal complexes

Compounds	**Deionized Water**	**MeOH**	**EtOH**	**DMSO**	**Acetone**	**Benzene**	**Diethyl ether**	**n-hexane**
Ligand L_1	S	SH	SH	SH	IS	IS	IS	IS
Ni(II) complex	S	SH	SH	SS	IS	IS	IS	IS
Cu(II) complex	S	SS	SH	SS	IS	IS	IS	IS
Ligand L_2	S	SH	SH	SS	IS	IS	IS	IS
Ni(II) complex	S	SS	SS	SS	IS	SS	IS	IS
Cu(II) complex	S	SS	SS	SS	IS	IS	IS	IS

KEY: S = soluble, SS = sparingly soluble, SH = soluble on heating, IS = insoluble

The solubility test indicates that all compounds are predominantly insoluble in non-polar organic solvents, including benzene, diethyl ether, and n-hexane, but soluble in polar solvents such as deionized water, methanol, ethanol, and DMSO, as demonstrated in Table 2. However, the solubility of some compounds was tested on cold and/or heat to further check the effect of temperature on their solubility. By this result, the compounds are likely polar in nature, as polar compounds dissolves in polar solvents, and vice versa [11].

Table 3. Magnetic susceptibility of the metal complexes

Compounds(Complexes)	Mass suscep.(X_g)	Molar suscep. (Xm)	μ_{eff} (B.M.)	Magnetic property	Suggested Geometry
$C_6H_{12}NiN_4O_2Cl_2$	1.39×10^{-9}	3.21×10^{-7}	0.0276	Paramagnetic	Tetrahedral
$C_6H_{12}CuN_4O_2Cl_2$	1.64×10^{-9}	3.87×10^{-7}	0.0314	Paramagnetic	Distorted octahedral
$C_8H_{16}NiN_4O_2Cl_2$	1.60×10^{-9}	4.14×10^{-7}	0.0314	Paramagnetic	Tetrahedral
$C_8H_{16}CuN_4O_2Cl_2$	3.07×10^{-9}	8.09×10^{-7}	0.0439	Paramagnetic	Distorted octahedral

The low μ_{eff} values (0.02-0.04 B.M.) of the complexes (Table 3) suggest weak paramagnetism. All the values are much lower than the expected for typical high-spin Ni (II) and Cu (II) complexes (1.73-2.83 B.M.). This may be attributed to either strong ligand fields which causes spin-pairing, square planar geometries which favour low-spin configurations, or possible antiferromagnetic coupling. The low μ_{eff} values support the tetrahedral geometry in the Ni (II) complexes (Figure 3), typical of low-spin d^8 complexes with N/O donors. The Cu (II) complexes forms distorted octahedral structures (Figure 3) due to the John-Teller distortion in d^9 systems [12].

Table 4. Characteristic FT-IR absorption bands of the Schiff base ligands and their metal complexes

Compounds	υ(C=N) (cm^{-1})	υ(OH)(cm^{-1})	υ(C-O) (cm^{-1})	υ(M-O) (cm^{-1})	υ(M-N) (cm^{-1})
$C_6H_{14}N_4O_2$ (L_1)	1565	3339	-	-	-
$C_6H_{12}NiN_4O_2Cl_2$	1572	3093	-	-	685
$C_6H_{12}CuN_4O_2Cl_2$	1572	-	1118	521	685
$C_8H_{18}N_4O_2$ (L_2)	1587	3071	-	-	-
$C_8H_{16}NiN_4O_2Cl_2$	1580	3071	-	-	767
$C_8H_{16}CuN_4O_2Cl_2$	1610	-	1073	521	857

The IR spectra of the free ligands shows, bands in the frequency regions 1565 and 1587 cm^{-1} for L_1 and L_2, respectively (Table 4). These can be assigned to C=N stretching vibrations, which is a basic feature of azomethine group, typical of Schiff bases [13]. The observed bands shifted to between 1572 and 1610 cm^{-1} frequencies in the respective metal complexes. This indicates the coordination of the Schiff bases with the metal ions. The bands at frequencies 3071 – 3339 cm^{-1} could be assigned to OH bonds, while 685-857 cm^{-1} to M-N bonds in the complexes. C-O bands are observed in L_1-Cu complex and L_2-Cu complex at frequencies 1118 cm^{-1} and 1073 cm^{-1} respectively. Similarly, M-O bands at frequencies 521 cm^{-1} are each observed in L_1-Cu complex and L_2-Cu.(Table 4).

Table 5. UV-Visible Spectral data of the ligands and their metal complexes

Wavelength(nm)	**ABSORBANCES**					
	L_1	**L_1-Ni**	**L_1-Cu**	**L_2**	**L_2-Ni**	**L_2-Cu**
200	2.91	2.81	2.94	2.92	2.77	2.80
250	3.13	3.03	3.22	3.32	3.04	3.11
300	1.18	1.52	2.76	1.75	1.41	2.65
350	0.146	0.398	1.49	0.615	0.280	1.53
400	0.111	0.343	0.927	0.351	0.230	0.854
450	0.102	0.261	0.745	0.230	0.205	0.791
500	0.114	0.246	0.884	0.196	0.210	2.42
550	0.0271	0.143	1.64	0.0891	0.131	3.54
600	0.123	0.139	3.11	0.124	0.137	3.67
650	0.164	0.164	3.75	0.164	0.164	3.71
700	0.164	0.164	3.81	0.164	0.164	3.73

The free ligands (L_1 and L_2) show absorption maxima between 200-300 nm. This is attributed to π→π* transitions from conjugated C=N and C=O bonds observed in the 200-250 nm range [14]. Also, n→π* transitions from lone pair electrons on oxygen and nitrogen atoms was observed in the wavelength range of 250-300 nm [15]. Both transitions are typical of Schiff bases.

On the other hand, upon complexation with Ni (II) and Cu (II), new absorption bands appear in the 400-700 nm, corresponding to d→d transitions and LMCT bands [14]. Whereas Ni (II) complexes display weak bands (500-700 nm), which is typical of tetrahedral or a distorted square-planar geometry [16]. Cu (II) complexes exhibit broad absorptions between 400-700 nm, suggesting octahedral or distorted octehedral geometry [17]. However, in both complexes, strong LMCT bands within 400-500 nm are observed, indicating electron donation from the ligand to the metal center [18].

Table 6a: Antibacterial activity of the Schiff base ligands and their metal complexes against gram positive bacteria

COMPOUNDS	**CONCENTRATIONS (µg/mL) & ZONES OF INHIBITIONS** (ZI)									
	Staphylococcus aureus (**µg/mL** / mm)					*Streptococcus pneumoniae(***µg/mL** / mm)				
	1000	**500**	**250**	**125**	**50**	**1000**	**500**	**250**	**125**	**50**
L_1	8.4	ND	ND	ND	-	9.1	7.3	ND	ND	-
L_1-Ni	15.6	12.7	10.0	8.3	-	15.2	12.4	10.2	9.3	-
L_1-Cu	12.9	10.4	8.1	7.7	-	14.6	10.2	9.4	7.5	-
L_2	7.4	ND	ND	ND	-	8.6	ND	ND	ND	-
L_2-Ni	13.4	10.1	8.6	7.2	-	13.7	10.3	8.1	7.3	-
L_2-Cu	11.3	9.6	8.1	ND	-	10.4	8.1	7.7	7.0	-
Ciprofloxacin	-	-	-	-	16.4	-	-	-	-	15.1

ND = Not detected

Table 6b: Antibacterial activity of the Schiff base ligands and their metal complexes against gram negative bacteria

COMPOUNDS	**CONCENTRATIONS (µg/mL) & ZONES OF INHIBITIONS** (mm)									
	Escherichia coli (**µg/mL** / mm)					*Pseudomonas aeruginosa* (**µg/mL** / mm)				
	1000	**500**	**250**	**125**	**50**	**1000**	**500**	**250**	**125**	**50**
L_1	9.4	7.2	ND	ND	-	8.9	ND	ND	ND	-
L_1-Ni	12.2	10.3	8.4	ND	-	11.7	9.9	8.7	7.2	-
L_1-Cu	14.9	11.5	9.9	9.3	-	14.9	12.4	10.6	7.9	-
L_2	7.3	ND	ND	ND	-	11.1	9.9	ND	ND	-
L_2-Ni	8.7	7.1	ND	ND	-	10.2	8.3	7.3	ND	-
L_2-Cu	11.7	9.8	8.1	7.0	-	14.9	12.4	10.6	7.9	-
Ciprofloxacin	-	-	-	-	18.1	-	-	-	-	21.3

ND = Not detected

Both the ligands as well as their corresponding Ni (II) and Cu (II) metal complexes were subjected to antibacterial screening. The results (Tables 6a and 6b), show that in all the bacterial strains, the ligands, show activity only at higher concentrations (500-1000 μg/mL). However, the metal complexes inhibit enhanced activity at various concentrations. This is due to the fact that chelation makes ligand a potent bacterial agent by reducing the polarity of the metal ion, which further increases the delocalization of π-electrons over the entire chelate ring thereby enhancing the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms [19]. From the results, Ni (II) complex of L_1 has the highest zone of inhibition of 15.6 mm, while Cu (II) complex of L_2 has the lowest (11.3mm) against *S. aureus* at the same concentration of 1000 μg/ml. Similarly, Ni (II) complex of L_1 has the highest zone of inhibition of 15.2 mm, while Cu (II) complex of L_2 has the lowest (10.4 mm) against *S. pneumoniae* at the same concentration of 1000 μg/ml (Table 6a). On the other hand, Cu (II) complex of L_1 shows the highest zone of inhibition of 14.9 mm, while Ni (II) complex of L_2 shows the lowest (8.7 mm) against *E. coli* at the same concentration of 1000 μg/ml. Whereas Ni (II) complex of L_2 shows the lowest zone of inhibition of 10.2 mm against *P. aeruginosa* , both Cu (II) complexes of L_1 and L_2 shows the same highest zone of inhibition (14.9 mm) against *P. aeruginosa* at the same concentration of 1000 μg/ml (Table 6b). The enhanced activity of the complexes was ascribed to the presence of metal ions in the lattice which makes it stronger and effective antibacterial agent [20]. Compared with L_2 complexes, the L_1 complexes inhibit higher activity in all the isolates.

CONCLUSION

The paper reported the successful synthesis and characterization of two Schiff base ligands from the condensations of ethylenediamine with glycine and alanine as well as their Ni (II) and Cu (II) complexes. Although, with activities below that of the reference drug, the antibacterial activity results indicated that, the Schiff base ligands and their metal (II) complexes are promising antibacterial agents. Compared with L_2 complexes, the L_1 complexes inhibit higher activity in all the isolates.

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CONFLICT OF INTEREST

Authors declared that there is no conflict of interest with regard to this publication.

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ETHICAL STATEMENT

This work required no ethical statement.

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