Schiff Base Derived from 2, 6 – Diaminopyridine and Benzaldehyde and its Co(II), Ni(II) and Cu(II) Complexes: Synthesis, Characterization and Antimicrobial Screening

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ABSTRACT

In this study, a Schiff base was successfully synthesized from 2, 6 – diaminopyridine and benzaldehyde using 1:2 molar ratio by condensation. The Schiff base was used to prepare transition metal complexes with Co(II), Ni(II) and Cu(II) salts. The ligand and metal complexes were characterized using FTIR, melting point/decomposition temperature determination, solubility test, conductivity measurements, magnetic susceptibility, UV-Vis spectroscopy and CHN microanalysis. The antimicrobial activities of the synthesized compounds were tested using agar well diffusion method, against different strains of bacterial and fungal isolates. The synthesized compounds were soluble in DMF and DMSO. Evidence from the IR study indicated that the characteristics band attributed to aldehyde stretching at 1700 cm^{-1} disappeared on the final Schiff base and the new absorption band at 1596 cm^{-1} appeared due to the ν(C=N) stretching. The band shifted in the spectra of the complexes between 1603 – 1614 cm^{-1}. Complexes were non-electrolytic, non-ionic and of octahedral geometry from results of molar conductivity and magnetic susceptibility respectively. The elemental microanalysis of the Schiff base is consistent with the calculated results from the molecular formula of the proposed structure of the synthesized compounds. The complexes have the formulas $[CoL_2Cl_2]H_2O$, $[NiL_2Cl_2]H_2O$ and $[CuL_2Cl_2]$. The antimicrobial results indicated that, most of the metal(II) complexes exhibited higher antimicrobial action than the free Schiff base ligand and nickel(II) complex was the most prominent against the gram-positive bacteria (*Staphylococcus aureus*) and the fungal isolate (*Aspergillus flavus*).

Keywords: Antimicrobial activity, Azomethine, characterization, Schiff base, transition metal complexes,

INTRODUCTION

The escalation in drug resistant to clinically used anti-infective agents reveal that there is a pressing necessity in the quest for novel antimicrobial compounds to treat numerous resistant infections with diverse mechanisms of action [1]. As a result, Schiff bases, compounds containing azomethine, are studied extensively due to their ease of preparation and vast application [2]. The azomethine functional group present in the Schiff base is a group that has a carbon-nitrogen double bond (>C=N-) with the nitrogen bonded to either an alkyl or aryl group [3]. The formation of a Schiff base from an aldehyde or ketone is a reversible reaction which usually involves two types of reactions, namely, addition, followed by elimination and most commonly takes place in the presence of an acid or base catalyst, or by heating. First, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The dehydration of carbinolamine to form the Schiff base is catalyzed by an acid or base [4]. The bonding tendency of Schiff bases are dependent upon factors such as the nature of atoms like nitrogen (N), oxygen (O) and sulphur (S) which act as coordination sites, electronegativity and steric factors.

The molecular structure of the Schiff base can influence biological activities. The structural study of these compounds may reveal a reasonable explanation for the structure-bioactivity relationships. In this context, the biological activity of a determined substance depends on multiple variables, most importantly, on the compound structure. The structure of the compound itself determines other properties including a rapport for different targets in the biological systems, biological media stability, and cellular uptake, among others [5]. The imine group from Schiff base has been shown to be critical towards biological activities.

Schiff bases and their metal complexes has spiked the interest and curiosity of numerous chemists searching for promising chemotherapeutic agents for disease control [2, 6]. Schiff bases obtained by the condensation of amino and carbonyl compounds and their complexes are intrinsic class of compounds that coordinate to metal ions via the azomethine nitrogen and have been extensively recognized for its wide range of medicinal applications which include antimicrobial, antimycobacterial, antiallergenic, antihypertensive, anticonvulsant, anticancer, antioxidant, anti-inflammatory, protein kinase C inhibitors and mushroom tyrosinase inhibition activities [1].

Schiff bases with heterocyclic backbone and their metal complexes are believed to imitate the exemplary metals ingrained in enzymes and metalloproteins. The bio-potential of the metal complexes are liable to be modified by properties such as the pattern of ligands, choice of metals, bio potential of ligands, bio potential of metals, as well as their relative ratios [7]. Heterocyclic compounds such as pyridine, 2, 6 – diaminopyridine, and related molecules are good ligands because of the presence of at least one ring nitrogen atom with a localized pair of electrons. Heterocyclic compounds are the most abundant in nature and were required for a wide range of applications in biology including antibacterial, antifungal, anticancer, antioxidant, anti-inflammatory activity, and also in analytical processes. These heterocycle-containing compounds have important properties in the fields of material science and biological systems [8].

Many applications have been reported by various authors, which proved that Schiff bases and their complexes serves as important compounds [9]. Different strains of bacteria which include both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) were tested against newly synthesized Schiff bases of 2 – hydroxy – 3 - methoxybenzaldehyde with 2, 6 - diaminopyridine (L_1) and N phenyl benzene - 1, 4 - diamine (L_2) respectively. The results indicated that, the highest inhibition of growth occurred on L_1 Schiff base against *Staphylococcus aureus* especially at highest concentration, but no significant activity was observed against *Escherichia coli* when compared to Ciprofloxacin which was the standard used, while L_2 Schiff base showed moderate activity against both *Escherichia coli* and *Staphylococcus aureus* [10].

The synthesis of two novel Schiff bases namely 3 - [(5 – methylbenzothiazol – 2 - ylimino) methyl] – naphthalene – 2 - ol and 3 - [(5 – fluorobenzothiazol – 2 - ylimino) methyl] – naphthalene 2 - ol from the reaction of 2 - hydroxynaphthaldehyde with 2 – amino – 6 - methylbenzothiazole and 2 – amino – 6 - fluorobenzothiazole respectively and their Ni(II) and Cu(II) complexes have also been reported. The antioxidant activities of the complexes were determined using Ferric Reducing Ability of Plasma (FRAP) and 2,2 – Diphenyl – 1 – picrylhydrazyl (DPPH) methods. The results showed that the copper(II) complexes demonstrated the best antioxidant potentials compared to the other compounds [3].

The DNA binding mode of a ligand prepared by reaction of 2 – hydroxy – 1 - naphthaldehyde with 4 -aminoacetophenone was used with 2, 6 – diaminopyridine to synthesize mixed ligand complexes with Cu(II), Fe(II) and Pd(II) were studied using plasmid DNA and

Phosphate-Buffered Saline buffer and the results revealed that all the compounds exhibited DNA - binding properties and high negative docking scores supported these findings. The copper(II) complex exhibited the highest activity in both the DNA binding studies and docking experiments. The experiment was repeated in a H_2O_2 environment and the results displayed an increase in DNA - binding potential which strengthens hope that the complexes synthesized possessed anti-cancer properties [11].

Furthermore, a Schiff base and its copper(II), nickel(II), cobalt(II), vanadyl(II) and zinc(II) complexes were synthesized from the condensation of 2, 6 – diaminopyridine with benzalidene – curcuminyl – 4 – iminoantipyrine. The ligand and its copper(II) complex were analyzed against the growth of breast cancer human cell line (MCF-7). In the results, it was seen that the copper(II) complex was more sensitive towards the breast cancer cell lines than the ligand [12]. Also, two Schiff bases L_1 and L_2 derived from the condensation of 2, 6 - diaminopyridine with 2 – mercapto – 3 – formyl quinoline and 2 – hydroxy – 3 – formyl quinoline respectively and their corresponding Co(II), Ni(II), Cu(II) and Zn(II) complexes were synthesized. The Schiff bases and their complexes were screened for analgesic activity by tail-flick method in rats and acetic acid induced writhing method in mice. The results indicated that there was a little increase in the reaction time to flick the tail in all the treated groups. Even though analgesic activity was not appreciable, the copper(II) complexes of both ligands showed comparatively better analgesic activity than the other compounds. The Zn(II) complexes displayed zero analgesic activity in this method [13]. In the same study, the compounds were analyzed for their anti-inflammatory and antipyretic tendencies with nimesulide used as the standard. The results of the anti-inflammatory activity of the compounds and the standard against carrageenan-induced edema displayed that all the % reduction values of the compounds were significantly lower than those of nimesulide which showed the highest activity. However, cobalt(II), nickel(II) and copper(II) complexes of both ligands exhibited good anti-inflammatory effect with the copper(II) complexes showing the highest activity. On the other hand, the results of the anti-pyretic activity of the synthesized compounds showed that all compounds with the exception of the ligands and the zinc(II) complex of L_1 showed significant reduction in the rectal temperature at all the time intervals compared to control. The Cu(II) complex of L_2 displayed better percentage of reduction in pyrexia among all the synthesized compounds while nimesulide showed highest antipyretic activity [13].

Following previous studies, this work reports the synthesis, characterization and antimicrobial evaluation of Schiff base ligand derived from the condensation of 2,6 – diaminopyridine with benzaldehyde and their corresponding Co(II), Ni(II) and Cu(II) complexes.

MATERIALS AND METHODS

The compounds, 2,6 – diaminopyridine and benzaldehyde, were both obtained from Sigma Aldrich; Reagents were also obtained from Sigma Aldrich and were used without further purification. Cobalt(II) chloride and nickel(II) chloride were obtained from Sigma Aldrich. Copper(II) chloride was obtained from Merck. Bacterial isolates (*Staphylococcus aureus* and *Escherichia coli*) and fungal isolates (*Candida albican* and *Aspergillus flavus*) were obtained at the Department of Microbiology, Bayero University, Kano. FT-IR spectra were obtained from Agilent Technologies spectrophotometer. Melting point and decomposition temperatures were recorded in open glass capillary tubes using Gallen Kamp melting point apparatus. Molar conductance was determined using Jenway 4010 conductivity meter. Magnetic susceptibility tests were carried out using the magnetic susceptibility balance MKI. UV spectra were conducted on a Perkin Elmer Lambda – 35 UV-vis spectrophotometer. Elemental microanalysis of the compounds for C, H and N were determined using Thermal Scientific Flash Smart elemental analyzer.

Synthetic procedure for Schiff base ligand

The reactants, 2, 6 – diaminopyridine (10 mmol) and benzaldehyde (20 mmol) were measured in 1:2 stoichiometric molar ratio and dissolved in 20 ml each of ethanol resulting in a dark green solution and clear solution respectively. The ethanol solution of the benzaldehyde was then added slowly to a stirring ethanolic solution of 2, 6 – diaminopyridine to give a very dark green mixture. Three drops of acetic acid were added after which the resulting solution was refluxed in a paraffin oil bath for 6 hours and left to stand at room temperature overnight. The precipitate was filtered, washed with diethyl ether, air dried at room temperature and then dried in a desiccator over phosphorus pentaoxide to give a yellowish green solid [14].

Synthetic procedure for divalent metal complexes

A general procedure was used in the syntheses of the metal(II) complexes. Each complex was prepared by dissolving 5 mmol of the metal chloride salt in 20 ml of ethanol. The solution obtained was added into a stirring hot ethanol solution of 10 mmol of the prepared Schiff base which changed color within a few minutes. The resulting mixture was then refluxed for 4 hours and allowed to cool. The precipitated solids obtained were filtered off, thoroughly washed with ethanol, then with diethyl ether, air dried at room temperature and then completely dried in a desiccator over phosphorus pentaoxide [14].

Antimicrobial sensitivity

The biological activity of the Schiff base and metal(II) complexes was studied on two different types of bacteria and fungi.

Antibacterial activity

The antibacterial activity of the synthesized compounds against Gram positive bacterial pathogen *Staphylococcus aureus* and Gram-negative *Escherichia coli* were evaluated using agar well diffusion method as reported by Yushau [15] using ciprofloxacin as the control. Four concentrations of the compounds in DMSO (4000 µg/ml, 2000 µg/ml, 1000 µg/ml and 500 µg/ml) were prepared using serial dilution method. Muller Hinton agar media was sterilized and after solidification, wells were cut on it using cork borer. The test bacterial pathogens were swabbed onto the surface of the agar plates and the wells were impregnated with 0.1 ml of the test samples. The plates were incubated at 30 °C for 24 h.

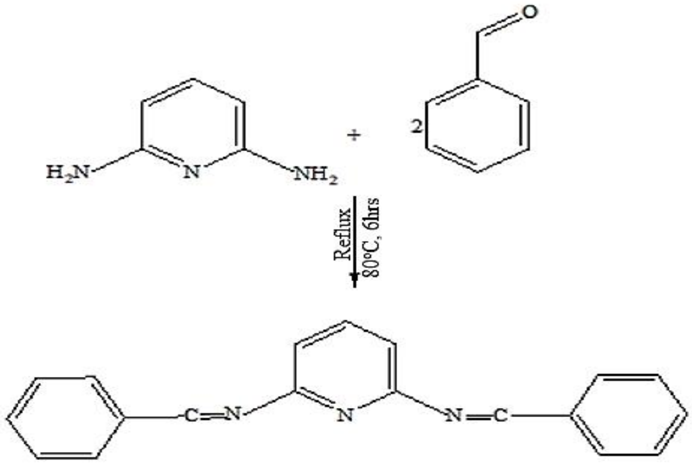
Antifungal activity

Antifungal activity of the Schiff base and its complexes against fungal pathogens (*Candida albican* and *Aspergillus flavus*) were conducted. Similar procedure [15] was also carried out here in Sabouraud dextrose agar media and with ketoconazole as the control. Four concentrations of the compounds in DMSO (4000 µg/ml, 2000 µg/ml, 1000 µg/ml and 500 µg/ml) were prepared using serial dilution method. Sabouraud dextrose agar media was sterilized and after solidification, wells were cut on it with a cork borer. The test fungal isolates were swabbed onto the surface of the agar plates and the wells were infused with 0.1 ml of the test samples. The plates were incubated at 30 °C for 72 h.

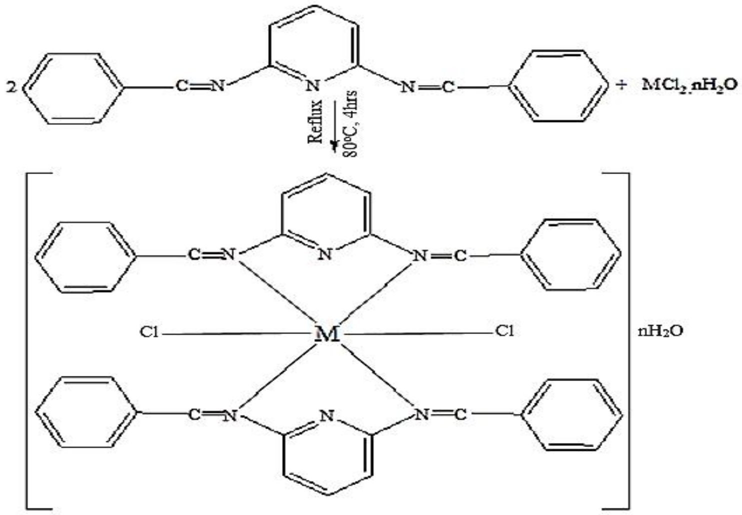
The diameters of the zone of inhibition were measured in millimeters. Each antibacterial and antifungal assay was performed in triplicate and mean values were reported.

RESULTS AND DISCUSSION

The Schiff base was prepared by refluxing 2, 6 – diaminopyridine and benzaldehyde in a 1:2 molar ratios respectively (Scheme 1). Its metal complexes were also obtained by refluxing with Co(II), Ni(II) and Cu(II) chloride salts in 2:1 ratio of Schiff base to metal salt as shown in Scheme 2.



Scheme 1: Preparation of the Schiff base



n = 0 for Cu(II); n = 1 for Co(II) and Ni(II)

Scheme 2: Preparation of Metal(II) Complexes

The Schiff base was synthesized as a yellowish green solid with good percentage yield of 84.91% which melted at 201 °C. The complexes synthesized were colored light green for nickel(II) complexes, black for cobalt(II) complex and dark green for copper(II) complex with good yield ranging from 73.15 – 85.16 and different decomposition temperatures between 252 - 272 °C as presented in Table 1. The molar conductance has proven to be a very crucial tool in the investigation of electrolytic nature of inorganic compounds. Complexes that show low molar conductivity values indicate that they are non-electrolytic in nature while complexes with high values are said to be electrolytes. The molar conductivity values of the synthesized complexes in DMSO were in the range of 10.20 - 31.80 ohm^{-1}cm^{2}mol^{-1} signifying the non-electrolytic character of the complexes. Moreover, the results of the micro analysis for the Schiff base and its complexes reveal that the calculated and theoretical results are in accordance with the expected metal to ligand ratio of 1:2 (Table 1).

The Schiff base and complexes were further characterized by carrying out solubility test to determine their natures in ethanol, methanol, DMSO, DMF, N-hexane, chloroform, distilled water, acetone, diethyl ether and xylene. The results obtained displayed that the compounds were completely soluble in DMSO and DMF; slightly soluble in ethanol, methanol, acetone and chloroform with the a few exceptions; and were completely insoluble in the remaining solvents (Table 2).

Table 1: Physical properties, molar conductance and elemental microanalysis of the synthesized compounds

Compound	Colour	M.P/ Dec temp. (°C)	%Yield	Molar Conduct. (ohm^{-1}cm^{2}mol^{-1})	Found (Calculated) (%)		
					C	H	N
L	Yellowish Green	201	84.91	-	71.41 (71.02)	4.30 (4.70)	13.87 (13.08)
[CoL$_2$Cl$_2$]H$_2$O	Black	272	84.84	10.20	68.34 (68.56)	4.08 (4.54)	12.76 (12.62)
[NiL$_2$Cl$_2$]H$_2$O	Light Green	270	73.15	15.70	68.01 (68.59)	4.89 (4.54)	12.80 (12.63)
[CuL$_2$Cl$_2$]	Dark Green	252	85.16	31.80	65.81 (66.31)	4.54 (4.39)	12.34 (12.21)

Table 2: Solubility test of Schiff base and its metal (II) complexes

Compounds	Ethanol	Methanol	DMF	DMSO	Diethyl Ether	N – Hexane	Chloroform	Acetone	Distilled Water	Xylene
L	SS	SS	S	S	IS	IS	SS	SS	IS	IS
$[CoL_2Cl_2]H_2O$	SS	SS	S	S	IS	IS	IS	IS	IS	IS
$[NiL_2Cl_2]H_2O$	SS	SS	S	S	IS	IS	SS	IS	IS	IS
$[CuL_2Cl_2]$	SS	SS	S	S	IS	IS	SS	SS	IS	IS

L = Ligand = $C_{19}H_{15}N_3$

S = Soluble; SS = Slightly Soluble; IS = Insoluble; DMF = Dimethyl Formamide;

DMSO = Dimethyl Sulfoxide

The FT-IR analysis is carried out to identify coordination sites of ligands, formation and strength of metal-ligand bonds in the complexes, and to study the bonding vibrational modes of ligand in metal complexes. The IR spectra of complexes and ligands have a diagnostic importance to determine the structure of the compounds and to analyze the quality of organic and inorganic samples i.e., the presence or absence of certain functional groups present in a compound [7]. An instrument known as Fourier Transform Infrared spectrometer, which is primarily based on the fundamental principles of molecular spectroscopy was used to identify the presence of certain functional groups in the synthesized compounds. The important FT-IR spectral data of the Schiff base and metal(II) complexes are presented in Table 3. The spectrum of the Schiff base displayed the absence of C=O absorption band at 1700 cm^{-1} and formation of the azomethine bond by the appearance of a new absorption band at 1596 cm^{-1}. This new azomethine bond was found to shift in the spectra of the complexes ranging from 1603 – 1614 cm^{-1} upon complexation with the metal ions. The formation of metal - ligand bonds was indicated further by the appearance of new bands between 585 – 592 cm^{-1} in the spectra of the complexes that were initially absent in the spectrum of the ligand.

Table 3: FTIR Spectral data of Schiff base and its complexes

Compound	ν (C=N) cm^{-1}	ν (M-N) cm^{-1}
L	1596	-
$[CoL_2Cl_2]H_2O$	1614	592
$[NiL_2Cl_2]H_2O$	1611	589
$[CuL_2Cl_2]$	1603	585

L = Ligand = $C_{19}H_{15}N_3$

Magnetic susceptibility measurements for the effective magnetic moment, μ_{eff}, in Bohr Magneton (B.M.) are important in evaluating the magnetism, as well as the possible geometry of complexes. The effective magnetic moment (μ_{eff}) values obtained; 4.63 B. M, 3.96 B. M and 1.62 B. M for cobalt (II), nickel (II) and copper (II) complexes respectively were all within range of the values reported for octahedral geometry around the respective metal ions (Table 4). This is in accordance with a previously reported study [16].

Table 4: Magnetic susceptibility data of the complexes

Complex	Magnetic Susceptibility (cm^3/ g)	Molar Magnetic Susceptibility (cm^3/ mol)	Bohr Magneton (μ_{eff})
$[CoL_2Cl_2]H_2O$	1.3916×10^{-9}	8.7621×10^{-7}	4.63
$[NiL_2Cl_2]H_2O$	9.89×10^{-10}	6.58×10^{-7}	3.96
$[CuL_2Cl_2]$	3.83×10^{-10}	2.502×10^{-7}	1.62

L = Ligand = $C_{19}H_{15}N_3$

UV - visible spectroscopic technique is carried out in order to identify ligand field (d-d transitions) and charge transfer. The UV - visible analysis of the ligand and its complexes were carried out in DMSO (Table 5). The high energy absorption bands at 219.60 and 327.16 nm in the spectrum of the ligand were as a result of the intra – ligand (π - π*) and non – bonding electron (n - π*) electronic transitions respectively. The π - π* electronic transitions in the spectra of the complexes were observed in the range of 215.97 to 231.91 nm. These absorption bands were hypsochromically shifted in the spectra of cobalt(II) and nickel(II) complexes and bathochromically shifted in the spectra of copper(II) complex. The bands in the range of 321.59 to 324.57 nm in the spectra of all the complexes were assigned to n - π* transitions due to the presence of non-bonding electron of the azomethine moiety. The shift of absorption bands to lower or higher wavelength in the spectra of these complexes when compared to the spectrum of the ligand might be attributed to coordination to the metal ions through nitrogen atom of the azomethine group.

Table 5: UV – Visible analysis data of Schiff base and metal(II) complexes

Compounds	Wavelength λ of Electronic Transitions (nm)	
	$\pi - \pi^*$ Transition	$n - \pi^*$ Transition
L	219.60	327.16
$[CoL_2Cl_2]H_2O$	216.86	321.59
$[NiL_2Cl_2]H_2O$	215.97	323.27
$[CuL_2Cl_2]$	231.91	324.57

L = Ligand = $C_{19}H_{15}N_3$

The antimicrobial sensitivity of the synthesized Schiff base and complexes and the parent drugs (used as standard) were investigated using agar well diffusion method. Different strains of bacteria which includes both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) and fungal isolates (*Candida albican* and *Aspergillus flavus*) were tested. The result indicates that, the Schiff base ligand (500 µgml^{-1}) was active against *Staphylococcus aureus* and inactive against *Escherichia coli*. Additionally, the highest zone of inhibition was observed on nickel(II) complex against *Staphylococcus aureus* and on copper(II) complex against *Escherichia coli* especially at highest concentration (4000 µgml^{-1}). The compounds generally show moderate activities against both *Escherichia coli* and *Staphylococcus aureus* compared to the parent drug (Ciprofloxacin). However, some of the compounds (the ligand, Co(II) and Ni(II) complexes) displayed no significant activity against *Escherichia coli* at lower concentrations as presented in Table 6.

The results from the antifungal sensitivity test of the synthesized compounds revealed that cobalt(II) complex and nickel(II) complex demonstrated the best activity towards *Candida albican* at nearly all concentrations while nickel(II) complex showed the highest inhibition growth against *Aspergillus flavus* (Table 7). The compounds generally showed moderate to good antifungal activities against *Aspergillus flavus* and *Candida albican* in comparison to the standard drug (Ketoconazole).

Table 6: Antibacterial sensitivity test showing the inhibition zones (mm) against bacterial isolates

Compound	*Staphylococcus Aureus*				*Escherichia coli*			
	4000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	4000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml
Ciprofloxacin (as standard)	-	-	-	22	-	-	-	30
Ligand	13	11	9	7	15	10	8	-
$[CoL_2Cl_2]H_2O$	14	11	10	8	13	10	8	-
$[NiL_2Cl_2]H_2O$	17	15	12	10	14	9	-	-
$[CuL_2Cl_2]$	14	11	9	8	18	15	12	10

Table 7: Antifungal sensitivity test showing the inhibition zones (mm) against bacterial isolates

Compound	*Candida albican*				*Aspergillus flavus*			
	4000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	4000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml
Ketoconazole (as standard)	-	-	-	27	-	-	-	24
Ligand	16	14	11	9	14	10	8	-
$[CoL_2Cl_2]H_2O$	17	14	11	10	13	11	9	7
$[NiL_2Cl_2]H_2O$	17	15	9	7	16	14	12	10
$[CuL_2Cl_2]$	14	10	-	-	15	12	9	8

Key: mm = Millimeter

- = negligible or no activity

CONCLUSION

This study investigated the biological potentials of Schiff base and its metal(II) complexes synthesized from 2, 6 – diaminopyridine and benzaldehyde and characterized using various physical, analytical and spectroscopic techniques. A yield of approximately 85% was achieved after 6 hours of condensation of the Schiff base which reacted with metal(II) ions to give its metal(II) complexes. The coordination of the azomethine nitrogens of the Schiff base ligand to the metal ions have been studied via FTIR data comparison of the ligand with those of the metal(II) complexes and the bonding of the Schiff base through the (>C=N) nitrogens to the metal ions was confirmed by the appearance of new bands (585 – 592 cm^{-1}) due to the ν(M-N) vibrations in the metal complexes spectra. The UV spectra of the ligand and complexes carried out in DMSO displayed high energy bands attributed to intra ligand and non-bonding electronic transitions. The shift of absorption bands to lower or higher wavelength in the spectra of these complexes in comparison with the spectrum of the ligand is further proof of complexation through the nitrogen atom of the azomethine group. The molar conductivity values of all the complexes in DMSO at room temperature (10.20, 15.70 and 31.80 $ohm^{-1}cm^{2}mol^{-1}$), revealed the non-electrolytic character of the synthesized complexes. The structure of the complexes was determined through magnetic susceptibility which revealed that all the complexes adopt octahedral geometries about the metal center and results of the microanalysis are in accordance with the metal to ligand ratio of 1:2. The *in vitro* antimicrobial evaluation of the synthesized compounds showed moderate to good activity against the pathogens tested, highlighting its potential pharmaceutical application.

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