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Synthesis, characterization and antimicrobial studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes of a Schiff base derived from 3-nitrobenzaldehyde with Arginine

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Abstract

The Schiff base was synthesized by condensation of 3-nitrobenzaldehyde and Arginine and its metal (II) complexes were prepared from the divalent chloride salts of Cobalt, Nickel, Copper and Zinc. The Schiff base and its complexes were characterized on the basis of solubility, melting/decomposition temperature, molar conductance, magnetic susceptibility, Job analysis, FT-IR and elemental analysis. The complexes were non-electrolytes in dimethyl sulphoxide and the FT-IR spectra of the Schiff base showed band at 1622 cm^{-1} assignable to υ(C=N). The band shifted to the lower frequencies in the spectra of the complexes at the ranges; 1607 - 1618cm^{-1} and this also suggests coordination through azomithine nitrogen and carboxylate oxygen. The free Schiff base and its complexes have been tested *in vitro* for their antibacterial as well as antifungal activity. The results revealed the compounds were found to be active against some selected bacterial and fungal isolates with an enhanced activity in the complexes.

Keywords: Synthesis, characterization, antimicrobial, Schiff base

Introduction

Schiff base constitute one of the classes of ligands in coordination chemistry due to their suitable synthetic preparation and high versatility. These prospects influence their ability to form stable complexes with many of transition metal ions [1].Schiff base complexes have been a topic of much interest due to increasing application in the field of catalysis, electrochemistry, bioinorganic, separation process, metallic deactivators and environmental chemistry [2].

Schiff bases and their metal complexes have shown to be developing leads for both synthetic and structural research due to their relatively simple synthesis and structural diversity [3]. It has also been reported that metal complexes were more biologically active as compared to non-coordinated Schiff bases [4,5,6]. Chelation to the metal ion can be useful in developing cytotoxic drugs, radioactive agents in imaging studies and radio immunotherapy (RIT) [7].

Schiff bases derived from an amino and carbonyl compound are significant class of ligands that coordinate to metal ions via azomethine nitrogen and have been studied widely [8]. In azomethine derivatives, the C=N linkage is important for biological activity; several azomethines were reported to have outstanding antibacterial, antifungal, anticancer and diuretic activities [9]. They also have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal and agrochemical [10]. With the increasing incidence of deep mycosis, there has been developing emphasis on the screening of new and more effective antimicrobial drugs with low toxicity. Schiff base complexes are considered to be among the most important stereochemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety [11]. A considerable number of Schiff base complexes have potential biological interest, being used as more or less successful models of biological compounds [12]. Not only have they played a seminal role in the development of modern coordination chemistry, but also they can also be found at key points in the development of inorganic biochemistry, catalysis and optical materials [13].

Schiff bases of transition metal complexes have been reported and found to be important for their biological activity [14]. They have been used as analytical reagent [15], and catalytic reagent [16]. Many Schiff bases and their complexes are known to show encouraging antifungal, antibacterial antiviral including anti HIV and anticancer activities [17]. Furthermore, some Schiff bases containing N and O donor atoms are also efficient anti-corrosion agents and serve different analytical purposes in organic and inorganic chemistry fields, consequently their vast applications in industries [18, 19].

Amino acid based Schiff bases are very effective metal chelators and their metal complexes are models for a numerous important biological systems [20]. They are crucial intermediates in variety of metabolic reactions including amino acids such as: decarboxylation, transamination, recemization and C-C bond cleavage, which are catalyzed by enzymes [21]. Metal Schiff base complexes obtained from amino acids play a vital part as the compounds for modelling more complicated PLP – amino acid Schiff base. Pyridoxal-5-phosphate (PLP), are crucial to interaction in a variety of metabolic reactions involving amino acids, which are catalyzed by enzymes that requires PLP as a cofactor. The reduced Schiff base containing different amino acids derivatives are excellent multidentate ligands for generating interesting multidimensional network structures, the additional reactive groups on the amino acid side chain of the ligands can lead to the formation of unexpected and unusual structures [22].

Considering the growing interest in metal complexes derived from amino acids, this research work focuses on widening this area of interest and therefore the article concentrates on the synthesis of some metal (II) complexes from 3-nitrobenzaldehyde and Arginine (Amino acid) and their antimicrobial applications.

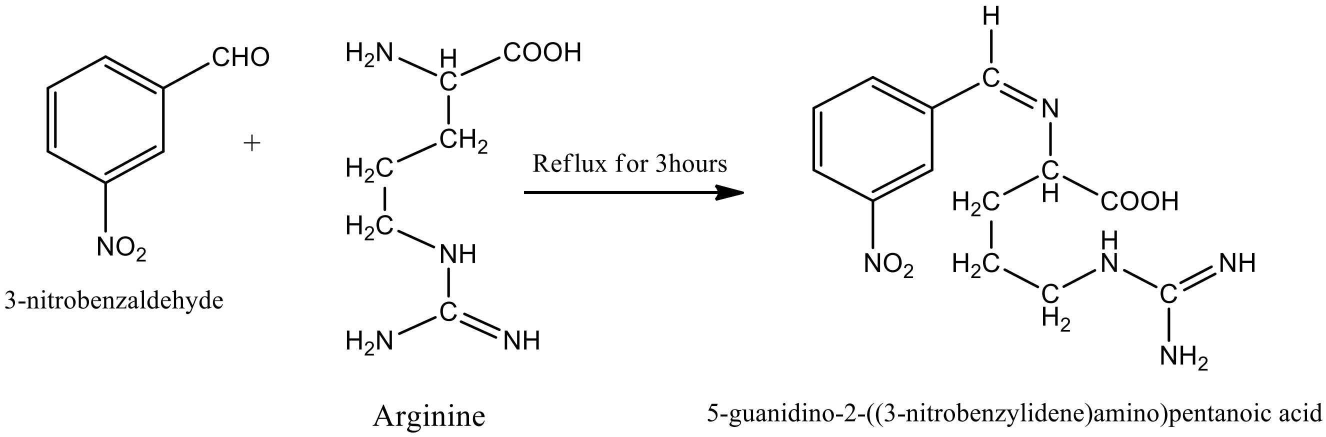
Experimental

Materials

All reagents and chemicals used were of analar grade and were used without further purification. All weighings were carried out with B154 balance. Melting point and decomposition temperatures were carried out on Stuart SMP 10 melting point apparatus. Determination of water of crystallization was done using drying oven model DHO-9053A. Infrared Spectral data were recorded using Agilent Technologies FT-IR spectrophotometer carry 630. The elemental analysis, CHN was carried out at Shahtra India using CHN model S. No. 11064047 elemental analysersteme GmbH.The electrical conductivity measurements were also carried out using conductivity meter model DDS 307, Jenway. The magnetic susceptibility measurement was carried out using magnetic susceptibility balance Sherwood Scientific MKI at room temperature.

Synthesis of Schiff Base (L)

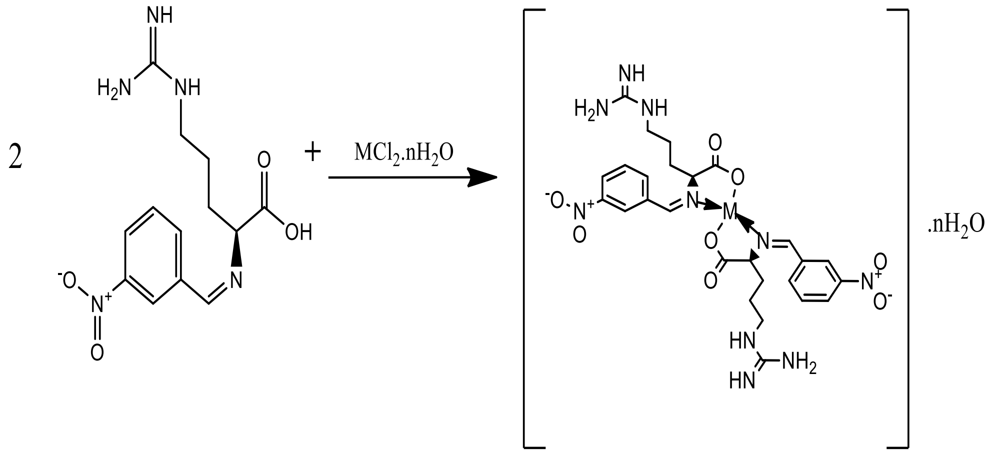
A homogenous mixture (30 cm^3) of an aqueous solution of Arginine (0.01 mol) was added under constant stirring to an ethanolic solution (30 cm^3) of 3-nitrobenzaldehyde (0.02 mol). The mixture was refluxed for 3hours. Excess solvent was removed by slow evaporation and the light yellow crystalline product obtained was dried over P_2O_5 in a dessicator.



Scheme 1: Reaction for the formation of the Schiff base (L)

Synthesis of Metal (II) Complexes

The newly synthesized Schiff base (L) (0.02mol) and appropriate metal chlorides (MCl_2.nH_2O, M=Co, Ni, Zn and Cu) (0.01mol) were dissolved separately in ethanol (30 cm^3) and mixed under constant stirring magnetically for 5hours at 80-85°C. The solid product obtained was filtered, washed with ethanol and dried in a desiccator over P_2O_5.



Where M = Co(II), Ni(II), Cu(II) and Zn(II)

Scheme 2: Reaction for the formation of Metal (II) Complexes

Determination of Melting/Decomposition Temperature: 50 milligrams of the Schiff base and its complexes were placed in a dry capillary tube and mounted onto the melting point apparatus. The temperature was read from the apparatus at which the samples start to melt/decompose.

Solubility test of Schiff base and its Metals (II) Complexes

The solubility of the Schiff base and the metals (II) complexes were determined by taking approximately 0.1g of the sample in a clean dried test tube followed by few drops of solvents used and shaken.

Molar conductance measurement of the Metals (II) Complexes

A 10^{-3}M solution of each of the complex compound was prepared using Dimethylsulphoxide as solvent, and the electrode of the conductivity meter was inserted into the solution and the values of specific conductance were recorded, and the corresponding molar conductance values were correspondingly also calculated [23]. All the measurements were carried out at room temperature and knowing the cell constant, the specific conductance was calculated from the relation.

Specific conductance = cell constant × observed conductance

Furthermore, the molar conductance was calculated using equation;

$$\text{Molar conductance} = \frac{1000K}{C} \quad \text{(Equation 1)}$$

Where:

C = the molar conductance

K = the specific conductance [24]

Fourier Transform Infrared Spectroscopy Analysis (FT-IR)

FT-IR analysis was carried out at Bayero University Kano, Nigeria using Agilent Technologies FT-IR spectrophotometer carry 630. A few milligrams of the samples were placed on the diamond head of the FT-IR spectrophotometer and the frequencies were observed and recorded.

Determination of metal ions in the metal (II) complexes

0.05g of each of the metal(II) complexes was accurately weighed and heated with a mixture of 3 cm^3 concentrated nitric acid (HNO_3) and 1.41gcm^{-3} of hydrogen peroxide (H_2O_2) to dryness, then 3 cm^3 of 5% hydrochloric acid solution (HCl) was added to dissolve the residue until a clear solution was obtained. The solution was allowed to cool and diluted to 100 cm^3 with distilled water. The calibration solutions were made by further diluting 10cm^3 of the stock solutions with distilled water to the mark of 100 cm^3volumetric flask. The concentrations of the metal ions were then measured against the blank solution using Atomic Absorption Spectrophotometer (AAS) [24]

Magnetic susceptibility measurement of the metal (II) complexes

The magnetic susceptibility of the complexes were measured using Gouy balance in which the balance was switched on and allowed to stabilized for 30minutes until 000 reading was displayed. Then the empty glass tube was weighed and its weight was recorded as Wo (in grams) and then was inserted into the machine and the initial reading was taken as (Ro). Each of the synthesized complex compounds was carefully packed in the same tube to a height of 1.5 to 2.5cm, giving the sample height in the tube which was recorded as L (cm). The actual mass of the sample (M) was obtained from final weight (W_1) of the tube containing the sample minus the weight of the empty tube (Wo). The machine was then zeroed and the packed sample tube was inserted into the machine and the reading also taken as (R_1) [25]. All recordings were done at room temperature and the magnetic susceptibility (Xg) was calculated using relation;

$$Xg = \frac{C(L)(R_1 - R_0)}{1\,x\,10^9(m)}\ erg.G^{-2}g^{-1}$$

(Equation 2)

Where:

Xg = Gram Magnetic Susceptibility of the complex

C = a constant of proportionality of the balance which was set during calibration as 1

L = Sample length in the tube in cm (whose range should be set between 1.5cm – 2.5cm, minimum value is 1.5cm)

R_0 = Initial reading of empty glass tube

R_1 = Final reading of the glass tube with sample

W_0 = Initial mass of empty glass tube

W_1 = Final mass of the glass tube with sample

M = Mass of the sample in tube in grams and $M = W_1 – W_0$

Determination of Ligand to Metal ratio by Job's Method (UV-Vis)

The ligand to metal ratio in the complexes was determined using UV-Visible spectrophotometer. The solution of the ligand (0.003M) and the solution of metal ions (0.003M) were mixed systematically, forming nine solution mixtures containing various volumes of the two solutions. The absorbance of the nine solution mixtures was taken at a wavelength of maximum absorbance [26]. The results obtained were shown in table 8. The number of coordinated ligand(s) per metal ion **(n)** was calculated using the expression below;

$$\mathbf{\tilde{n} = \frac{X\mathit{i}}{1 - X\mathit{i}}} \qquad \text{(Equation 3)}$$

Key: **ñ** = average number of coordinated ligand per metal ion

$X\mathit{i}$ = Mole fraction

Antimicrobial activity of Schiff bases and its metal (II) Complexes

Antimicrobial sensitivity test of Schiff base and complexes against bacterial and fungal isolates using Agar well diffusion method collected from Aminu Kano Teaching Hospital, Kano – Nigeria with standard antibiotic controls (Gentamycin and Ketoconazole).

Antibacterial Activity

The Schiff base and its complexes were screened for their antibacterial activity against gram positive and gram negative bacteria namely, *Staphylococcus aureus, Escherichia coli and Streptococcus pneumoniae*. The agar well-diffusion method was used in these assays at three (3) different concentrations (2000 µg/ml, 1000 µg/mland 500 µg/ml) and Gentamycin was used as control. The zone of inhibitions were measured and recorded as shown in table 8.

Antifungal Activity

The Schiff base and its complexes were dissolved separately in DMSO for the concentrations of 2000 µg/ml, 1000 µg/ml, 500 µg/ml. The samples were placed on the surface of the inoculated culture media (potatoes dextrose agar) and incubated at room temperature for 48hrs. The *in vitro* antifungal activity against *Candida albican, Aspergillus flavos and Aspergillus nigar* of the Schiff base and its metal (II) complexes were carried out by agar well diffusion method and Ketoconazole was used as control. The zone of inhibitions was measured and recorded as shown in table 9.

Results and Discussions

All the synthesized compounds are coloured and the different colours were due to charge transfer and various electronic transition of electrons between energy levels whose spacing correspond to the wave lengths available in the visible region [27]. The colours exhibited are ultimately related to the magnitude of the spacing between energy level which relies on the factors such as geometry of the complexes, the nature of ligands and the oxidation state of the central metal atom [28]. The decomposition temperature of metal complexes ranges 210-258°C as shown in table 1. The higher decomposition temperatures of the complexes indicate possible coordination of various metals used and high thermal stabilities of the compounds [29].

Table 1: Physical properties of Schiff base (L) and its Metal (II) Complexes

Compound	Molecular Formula	Molecular weight (g/mol)	% Yield	Colour	M.P(^oC)	Decomposition Temp (^oC)
L	$C_{13}H_{17}N_5O_4$	307.13	80	Yellow	187	
$[Co(L)_2].H_2O$	$CoC_{26}H_{32}N_{10}O_8$	673.193	74	Purple		258
$[Ni(L)_2].H_2O$	$NiC_{26}H_{32}N_{10}O_8$	672.953	69	Green		217
$[Cu(L)_2].H_2O$	$CuC_{26}H_{32}N_{10}O_8$	677.806	65	Dark green		225
$[Zn(L)_2].H_2O$	$ZnC_{26}H_{32}N_{10}O_8$	679.64	61	Light Yellow		210

Key: M.P – Melting Point

L: $C_{13}H_{17}N_5O_4$

The solubility test shows the Schiff base and its metal(II) complexes are insoluble in most common organic solvents and completely soluble in Dimethylsulphoxide and Dimethylformamide (table 2) which can be attributed to the high dielectric constant of the solvents.

Table 2: Solubility test of the Schiff base (L) and its Metal (II) Complexes

Compound	Acetone	Chloroform	Ethanol	DMSO	DMF	Methanol	Benzene	Hexane
L	IS	IS	S	S	S	S	IS	IS
$[Co(L)_2].H_2O$	IS	IS	SS	S	S	SS	IS	IS
$[Ni(L)_2].H_2O$	IS	IS	SS	S	S	SS	IS	IS
$[Cu(L)_2].H_2O$	IS	IS	SS	S	S	SS	IS	IS
$[Zn(L)_2].H_2O$	IS	IS	SS	S	S	SS	IS	IS

Key: S = soluble, SS=slightly soluble and IS = Insoluble

L: $C_{13}H_{17}N_5O_4$

The molar conductance measurement carried out in 10^{-3}M Dimethylsulphoxide solution were found in the range of 9 – 16 $ohm^{-1} mol^{-1} cm^2$ as in table 3. The results indicate non-ionic nature of the complexes and suggest that no anions are present outside the coordination sphere. The non-ionic nature of these complexes was similar with the conductance values of transition metal complexes of 2-nitrobenzaldehyde with glycine [30].

Table 3: Molar conductance measurement of the Metal (II) Complexes in 10^{-3} DMSO solution

Compound	Electrical conductivity ($ohm^{-1}cm^{-1}$)	Molar conductivity ($ohm^{-1}cm^2 mol^{-1}$)
$[Co(L)_2].H_2O$	16.0×10^{-6}	16.0
$[Ni(L)_2].H_2O$	10.0×10^{-6}	10.0
$[Cu(L)_2].H_2O$	13.0×10^{-6}	13.0
$[Zn(L)_2].H_2O$	9.0×10^{-6}	9.0

L: $C_{13}H_{17}N_5O_4$

The FT-IR spectra of the Schiff base showed band at 1622 cm^{-1} assignable to υ(C=N) which is a characteristic of the azomithine group [30]. The band shifted to the lower frequencies in the spectra of the complexes at the ranges; 1607 - 1618cm^{-1} as in table 4. The shift in the spectra of the complexes indicates possible coordination of the Schiff bases to the central metal ions [29]. The absorption peaks assignable to υ(M-O) falls within the range 515-538cm^{-1} and υ(M-N) falls within the range 637–678 cm^{-1} for the metal complexes. This also suggests coordination through O and N atoms and confirms the formation of the complexes. [31, 32]

The metal percentage compositions were also determined using Atomic Absorption Spectrophotometer and the results found agreed with the theoretical values as shown in table 5.

The magnetic susceptibility measurement of the complexes at room temperature revealed paramagnetic in all the complexes with the exception of Zinc complex that recorded negative value (diamagnetic). The molar magnetic susceptibility (X_M) of Co(II), Ni(II) and Cu(II) complexes shows values ($1.70 \times 10^{-4} – 6.12 \times 10^{-4}$) while Zn(II) complex showed at -2.0×10^{-4}as shown in table 6.

Table 4: Infrared spectral data of the Schiff base and its Metal (II) Complexes

Compound	ν(OH)	ν(C=N)	ν(M-O)	ν(M-N)	ν(C=O)	ν(CO_2)
L	3312	1622			1644	1376
$[Co(L)_2].H_2O$	3156	1610	523	678	1658	1353
$[Ni(L)_2].H_2O$	3249	1611	530	637	1640	1354
$[Cu(L)_2].H_2O$	3242	1607	538	657	1633	1346
$[Zn(L)_2].H_2O$	3160	1618	515	676	1678	1350

L: $C_{13}H_{17}N_5O_4$

Table 5: Concentration of metal ions in the Metal (II) Complexes

Compound	Calibration curve equation	Absorbance (y)	Concentration (x) (mg/l)	% of Metal
$[Co(L)_2].H_2O$	y = 0.012x	0.057	47.50	9.50
$[Ni(L)_2].H_2O$	y = 0.013x	0.065	50.00	10.00
$[Cu(L)_2].H_2O$	y = 0.113x	0.59	52.21	10.44
$[Zn(L)_2].H_2O$	y = 0.016x	0.075	46.88	9.38

L: $C_{13}H_{17}N_5O_4$

Table 6: Magnetic Susceptibility data the Metal (II) Complexes

Compound	Mass Susceptibility Xg ($ergG^{-2}g^{-1}$)	Magnetic Susceptibility Xm ($ergG^{-2}mol^{-1}$)	Effective Magnetic Moment, μ_{eff} (BM)
$[Co(L)_2].H_2O$	3.7×10^{-6}	2.55×10^{-3}	1.73
$[Ni(L)_2]. H_2O$	9.1×10^{-7}	6.12×10^{-4}	2.83

[Cu(L)$_2$].H$_2$O	2.51 x 10^{-6}	1.70 x 10^{-4}	1.73
[Zn(L)$_2$].H$_2$O	-2.95 x 10^{-7}	-2.0 x 10^{-4}	Diamagnetic

L: $C_{13}H_{17}N_5O_4$

The elemental analysis (CHN) was carried out at Shahtra India using a CHNS mode S/No. 11064047 Elemental analysersteme, GmbH. The analysis revealed the experimental values for Hydrogen, Carbon and Nitrogen compositions of the Schiff base and its metal complexes. The values are in close agreement with the values calculated (theoretical) from molecular formula assigned to the compound. The composition assigned to the Schiff base and its complexes may therefore be formulated as presented in table 7.

Table 7: Elemental Analysis of the Schiff base and its Metal (II) Complexes

Compound	**Experimental (calculated) (%)**		
	C	**H**	**N**
Schiff base	50.59(50.81)	5.21(5.54)	22.54(22.79)
[Co(L)$_2$].H$_2$O	42.42(42.61)	4.60(4.64)	18.99(19.12)
[Ni(L)$_2$].H$_2$O	42.78(42.64)	4.63(4.65)	18.89(19.13)
[Cu(L)$_2$].H$_2$O	42.22(42.09)	4.56(4.59)	18.62(18.88)
[Zn(L)$_2$].H$_2$O	41.69(41.88)	4.51(4.56)	18.58(18.79)

L: $C_{13}H_{17}N_5O_4$

The Job's method of continuous variation was used for the estimation of the ligand to metal ratio. The plot of absorbance against mole fraction in each case at maximum absorbance corresponding to the ligand mole fraction suggests 1:2 metal-ligand ratios in the complexes as shown in table 8 and figures 1-4.

Table 8: Job's method data

Mole fractions, (X)	0.94	0.81	0.69	0.56	0.44	0.31	0.19	0.06

S/N	COMPOUND	Absorbance at λmax							
1	$[Co(L)_2].H_2O$	0.63	1.82	2.98	2.64	2.22	1.83	1.44	0.67
2	$[Ni(L)_2].H_2O$	0.46	1.68	2.89	2.51	2.08	1.90	1.62	0.72
3	$[Cu(L)_2].H_2O$	0.68	1.88	3.08	2.62	2.41	1.83	1.12	0.59
4	$[Zn(L)_2].H_2O$	1.05	1.89	2.75	2.47	2.33	1.77	1.44	0.85

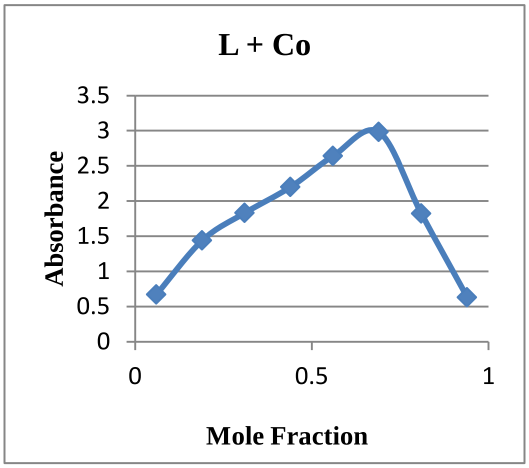


Figure 1: Job's plot of Co (II) Complex

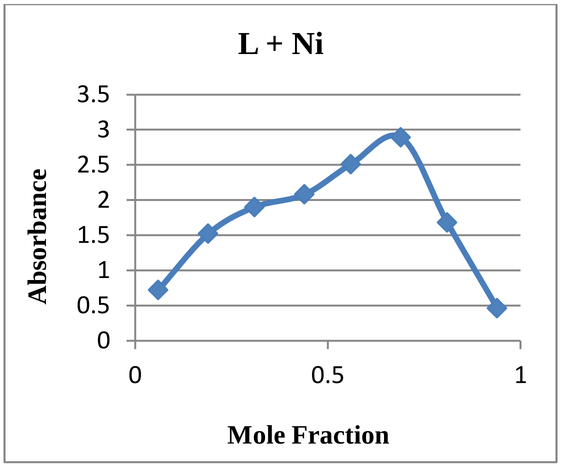


Figure 2: Job's plot of Ni (II) Complex

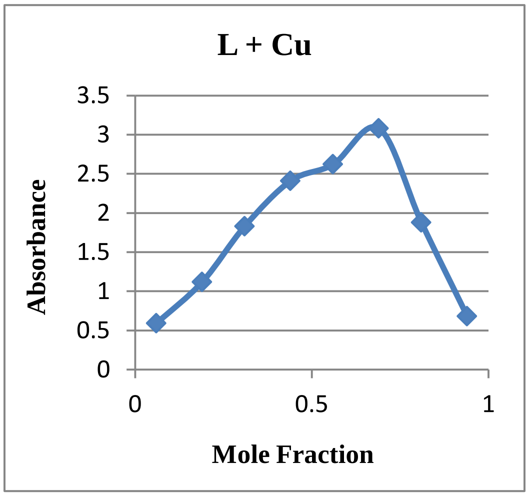


Figure 3: Job's plot of Cu (II) Complex

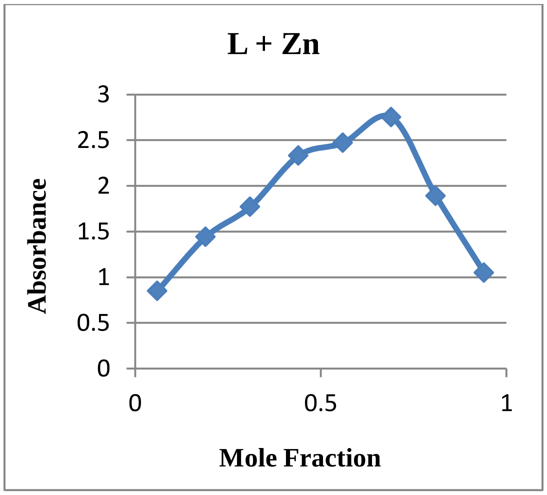


Figure 4: Job's plot of Zn (II) Complex

The antibacterial assay was carried on three bacterial isolates namely *Escherichia coli*, *Streptococcus pneumonia* and *Staphylococus aureus* using gentamycin as control and the zones of inhibition were measured in millimetre (mm). The Schiff base and its metal (II) complexes have significant effect on the three bacterial isolates. A comparable study of the Schiff base and its metal (II) complexes indicate that the metal (II) complexes exhibit higher anti-bacterial activity than free Schiff base but lower anti-bacterial activity compared to the control, as recorded on tables 8. The results obtained compiled with the reported literatures. [33, 34] The enhanced activity in the complexes was as a result of complexation which makes them more powerful and effective bactericidal agents. Generally the anti-bacterial activity increases as the concentrations increases. [35]

Table 9: Antibacterial Sensitivity data of the Schiff base and its Metal (II) Complexes

SN	Compound	Concentration (μg/ml)/Zone of inhibition (mm)												
		Clinical Isolate				Clinical Isolate				Clinical Isolate				Antibacterial Control
		Staph. aureus	2000	1000	500	*Escherichia coli*	2000	1000	500	*Streptococcus pneumonia*	2000	1000	500	Gentamycin (1000μg/ml)
1	L_2		14	12	9		16	15	11		13	12	10	32
2	$[Co(L)_2].H_2O$		22	18	12		17	15	14		17	14	11	
3	$[Ni(L)_2].H_2O$		19	16	13		20	17	14		16	15	11	40
4	$[Cu(L)_2].H_2O$		20	17	-		-	-	-		18	15	12	
5	$[Zn(L)_2].H_2O$		18	14	8		20	16	13		15	14	12	30

L: $C_{13}H_{17}N_5O_4$

The anti-fungal activity was also carried out on three different fungal isolates viz; *Candida albicans, Aspergillus flavos, Aspergillus nigar* using ketoconazole as control. The results (table 9) indicate that the complexes showed an appreciable activity against the entire base and its metal complexes has shown a lesser activity as compared to the control (Ketoconazole). Copper complex shows the highest activity with zone of inhibition of 18 mm and 19 mm against *Candida albican* and *Aspergillus flavos* respectively when compared to Co(II), Ni(II) and Zn(II) complexes. The presence of bulky substituents in the structure of the compounds may be responsible for the enhancement of biological activity [36]. Generally metal complexes are expected to be more effective than the metal free ligands due to chelation, which reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups of the ligand and possible π-electrons delocalization over the aromatic rings [37].

Table 10: Antifungal Sensitivity data of the Schiff Base and its Metal (II) Complexes

SN	Compound	Concentration (μg/ml)/Zone of inhibition (mm)												
		Clinical Isolate				Clinical Isolate				Clinical Isolate				Antifungal Control
		Candida Albican	2000	1000	500	*Aspergillus Flavos*	2000	1000	500	*Aspergillus Niger*	2000	1000	500	Ketoconazole (1000μg/ml)
1	L		-	-	-		13	11	8		-	-	-	33
2	$[Co(L)_2].H_2O$		16	14	11		18	16	12		14	11	10	
3	$[Ni(L)_2].H_2O$		15	13	10		16	14	9		-	-	-	40
4	$[Cu(L)_2].H_2O$		18	8	-		19	12	8		11	9	7	
5	$[Zn(L)_2]. H_2O$		12	10	8		14	12	9		15	7	11	29

L: $C_{13}H_{17}N_5O_4$

Conclusion

The Schiff base was successfully synthesized by condensation of 3-nitrobenzaldehyde and Arginine and it coordinates with the metal ions through the Nitrogen and Oxygen atoms.The compounds were characterized on the basis of solubility, melting/decomposition temperature, molar conductance, magnetic susceptibility, FT-IR and elemental analysis. The results obtained agreed with those obtained from similar work in the literature. The Schiff base coordinates with the transition metal ions to give square planer neutral compounds. Moreover, the antibacterial and antifungal data revealed the compounds were found to be active against some selected bacterial and fungal isolates with an enhanced activity in the complexes.

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