SYNTHESIS, CHARACTERIZATION, CYTOTOXICITY AND ANTIMICROBIAL STUDIES OF SCHIFF BASE DERIVED FROM 2-AMINOBENZOIC ACID AND BENZALDEHYDE AND ITS Cu(II) AND Zn(II) COMPLEXES

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ABSTRACT

A Schiff base ligand has been synthesized by the condensation of 2-aminobenzoic acid and benzaldehyde. Metal complexes of the Schiff base were prepared by the reaction of the Schiff base and metal (II) chlorides of copper and zinc in ethanol. The compounds were characterized by solubility test, melting point/decomposition temperature, molar conductance, magnetic susceptibility, FT-IR, metal-ligand ratio determination and UV-Visible Spectrophotometry. The Schiff base and metal(II) complexes were all soluble in DMSO, DMF, methanol, ethanol but insoluble in water and n-hexane. The IR spectra of the Schiff base showed a band at 1618cm^{-1} due to *v*(-C=N-) stretching vibration of the azomethine. This band shifted to lower frequencies of 1551cm^{-1} and 1543cm^{-1} respectively in the spectra of the metal (II) complexes. Conductivity measurement values indicated the non-electrolytic nature of the metal complexes. Job's method of continuous variation suggests 1:2 Metal-ligand ratio. The *in-vitro* antibacterial activity carried out on *Staphylococcus aureus, Streptococcus pnemoniae*, *E. coli* and *Klebsiela pnemoniae*revealed that the metal complexes exhibit higher activity than the Schiff base. The result for the antifungal screening revealed that the Schiff base and Zn (II) complex showed appreciable activity against *Aspergillus fumigatus* and *Aspergillus flavus*, the Schiff base and Cu (II) complex were found to be inactive at all concentration against *Candida albicans*. The cytotoxic results showed that the Schiff base and Zn (II) complex were highly-toxic with LC_{50} values of 63.191µ/mg and 76.684µ/mg while Cu (II) complex was non-toxic with LC_{50} of 1175.79µ/mg.

Keywords: Schiff base, 2-aminobenzoic acid, benzaldehyde, antimicrobial studies, cytotoxicity

INTRODUCTION

Schiff bases are condensation products of primary amines with carbonyl compounds. They were first reported in 1864 by a German chemist, Hugo Schiff. Schiff base are compounds carrying imine or azomethine (-C=N-) functional group. Schiff base played a vital role in the development of coordination chemistry and were involved as key point in the development of inorganic biochemistry and optical materials (Anu *et al.,* 2013). The field of Schiff base complexes has been fast developing on account of the wide variety of possible structures for the ligands depending upon the aldehydes and amines. Schiff bases are considered as very important class of organic compounds, which have wide applications in many biological aspects. Transition Metal complexes of Schiff bases are thoroughly studied and also have applications in catalysis and organic synthesis (Mounika *et al.,* 2010).

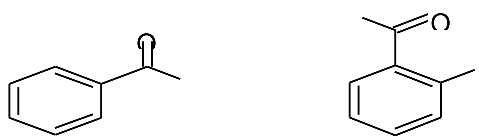
Schiff bases have shown a broad range of biological applications, including antibacterial, antifungal, antimalarial, antiproliferative, antiviral, anti-inflammatory and antipyretic properties (Da silva*et al*., 2011). Schiff bases have found wide applications in the field of coordination chemistry and these are widely employed as ligands in preparing coordination complexes (AlShemary *et al.,* 2017). They show a significant biological activity and have a great ability of forming stable complexes with transition metals ions due to the presence of oxygen and nitrogen which are excellent donor atoms (Abu-Dief and Mohamed, 2015). The aim of this research work is to synthesize Schiff base from 2-aminobenzoic acid and benzaldehyde and its metal (II) complexes of Cu (II), and Zn (II). In addition we also report the antimicrobial and cytotoxicity activities of the Schiff base and the complexes.

MATERIALS AND METHODS

Preparation of Schiff Base

The Schiff base was prepared by mixing ethanolic solution of 2-aminobenzoic acid (4.11g, 0.03 Mole) and benzaldehyde (3.06 cm^3, 0.03 Mole). The mixture was refluxed with stirring for 4 hours. The resulting solution was concentrated to half volume and then put in a crushed ice; yellow-brown precipitate was formed. The precipitate was collected by filtration, washed several times with distilled water and n- hexane, then finally dried in dessicator over (P_2O_5) (Devendra *et al.,* 2011).

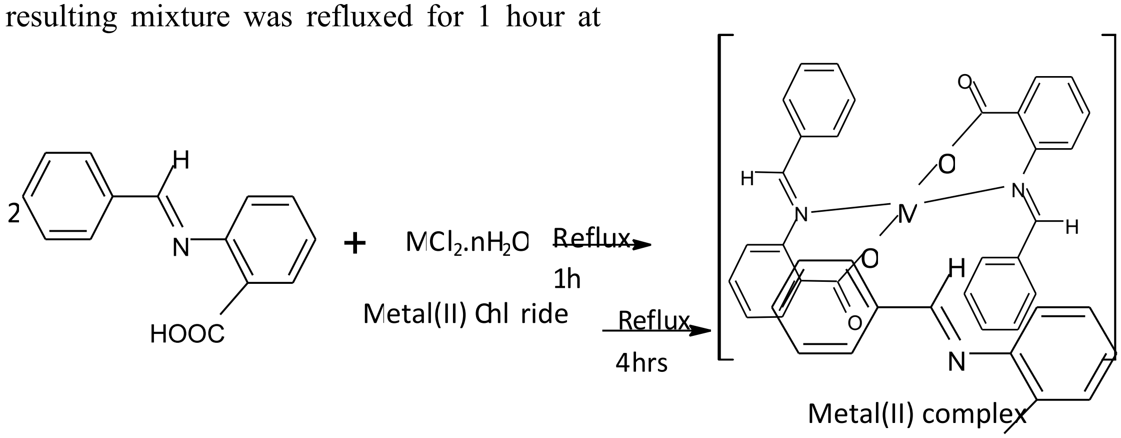
HO
NH_2
H +
B



Reaction scheme 1: Formation of the Schiff base

Preparation of the Metal Complexes

The metal complexes were synthesized by mixing an ethanolic solution of the Schiff base (0.02 Mole., 2.25g) with an ethanolic solution of the metal (II) chlorides of Copper and Zinc (0.01 Mole., 0.85g, 0.68g). The resulting mixture was refluxed for 1 hour at 80°C. Then it was cooled to room temperature, the coloured metal complex that precipitated out was filtered, washed several times with distilled water and n-Hexane and finally dried over P_2O_5 in a desiccator (Devendra*et al,* 2011).



Reaction scheme 2: Preparation of Metal(II) Complex

RESULTS AND DISCUSSION

The results obtained from different analysis are presented in the tables below.

Table 1: Physical Properties of the Schiff base Ligand and its Metal (II) Complexes.

Compound	Colour	% Yield	M.P (^{0}C)	D. Temp (^{0}C)
Ligand	Yellowish brown	79	114	-
$[CuL_2].3H_2O$	Dark-green	58	-	164
$[ZnL_2].2H_2O$	Orange	56	-	162

A new Schiff base 2-(Benzylidene-amino)-benzoic acid was synthesized by the condensation of 2-aminobenzoic acid and benzaldehyde in ethanol. A yellowish-brown crystal was formed with a percentage - yield of 79% and melting point of 114^{0}C (Table 1). The Cu (II) and Zn (II) complexes synthesized were found to be of different colours with percentage composition of 58% and 56%. The decomposition temperatures of the metal complexes were 162^{0}C and 164^{0}C respectively.

Table 2: Solubility Test of Schiff base and its Metal (II) Complexes

Compounds	D.water	MeOH	EtOH	$CHCl_3$	Acetone	CCl_4	nhexane	P.ether	DMF	DMSO
Ligand	IS	S	S	S	S	S	IS	S	S	S
$[CuL_2].3H_2O$	IS	S	S	SS	S	IS	IS	IS	S	S
$[ZnL_2].2H_2O$	IS	S	S	IS	S	IS	IS	SS	S	S

L=$C_{14}H_{11}NO_2$, MeOH=Methanol, EtOH=Ethanol, D.Water=Distilled water, $CHCl_3$=Chloroform, P.Ether=Petroleum ether, S=Soluble, SS=Slightly soluble, IS=Insoluble, DMF=Dimethylformamide, DMSO=Dimethylsulfoxide, CCl_4= Carbontetrachloride

The solubility test carried out on the Schiff base showed that the Schiff base was soluble in methanol, ethanol, DMSO, DMF, carbon tetrachloride, acetone, diethylether, and chloroform but insoluble only in water and n-hexane. However, the metal complexes were soluble in ethanol, methanol, DMSO, DMF, and acetone but insoluble in water, n-hexane and carbon tetrachloride. Cu (II) was slightly soluble in chloroform while Zn (II) was insoluble but in petroleum ether the Cu (II) complex was insoluble while Zn (II) was slightly soluble. (Table 2). The infrared spectral data of the Schiff base ligand and its metal complexes were listed in table 3.

Table 3: IR Spectra of the Schiff base and its Metal (II) Complexes

Compounds	V(C=N) cm^{-1}	V(M=N) cm^{-1}	V(M-O) cm^{-1}	V(C=O) cm^{-1}	V(OH) cm^{-1}
Ligands	1618	-	-	1703	3376
$[CuL_2].3H_2O$	1551	564	441	1689	3275
$[ZnL_2].2H_2O$	1543	653	415	1674	3212

The spectral of the Schiff base showed a strong absorption band at 1618cm^{-1} due to v(C=N) stretching ,indicating that condensation has taken place (Hossain *et al.,* 2016). The band was shifted to lower frequency of 1543cm^{-1} and 1551cm^{-1} in the spectra of the metal (II) complexes respectively. The appearance of the new bands of 564cm^{-1} and 653cm^{-1} as well as

415cm^{-1} and 441cm^{-1} in the spectra of metal (II) complexes were attributed to v(M-N) and v(M-O) groups, clearly revealed an involvement of the ligand in co-ordination with metal ions (Ali, 2014). The broad band at 3376cm^{-1} in the spectra of the Schiff base is due to v(OH). The bands at 3275cm^{-1} and 3212cm^{-1} in the spectra of the metal (II) complexes indicating the presence of co-ordinated water molecules (Aliyu *et al.,* 2011).

Table 4: Conductivity Measurement Data of 10^{-3} M Metal (II) Complexes in DMSO

Complexes	Electrical Conductivity (ohm-1 cm-1) ×10-6	Molar Conductance ($ohm^{-1}cm^2mol^{-1}$)
$[CuL_2].3H_2O$	44.40	14.80
$[ZnL_2].2H_2O$	14.06	4.70

L=C14H11NO

The molar conductance of each of the metal (II) complex was measured in DMSO and the values obtained were in the range of 4.70-31.10 $ohm^{-1}cm^2mol^{-1}$ which are relatively low, indicating the non-electronic nature of the metal complexes (Table 4) (Mukhtar et al., 2018).

Table 5: Magnetic Susceptibility Data of Metal(II) Schiff base Complexes

Complexes	Mass Susceptibility, X_g (erg. G^{-2} g^{-1})	Molar Susceptibility, X_m(erg.G^{-2} mol^1)	μ_{eff} (B.M)	Magnetic property
$[CuL_2].3H_2O$	$2.70x10^{-6}$	$1.37x10^{-3}$	5.70	Paramagnetic
$[ZnL_2].2H_2O$	1.99×10^{-5}	1.01×10^{-3}	4.92	Diamagnetic

L=C14H11NO2

The observed magnetic susceptibility measurement of Cu (II) complex and Zn (II) at room temperature are shown in table 5. The values shows that Cu (II) complex was paramagnetic while that of Zn (II) complex was diamagnetic, showing the absence of unpaired electrons (Taghreed, 2016). The magnetic moments of the complexes determined were within the range expected for tetrahedral complexes (Golcu *et al.,* 2005).

Table 6: Emperical Formula of the Complexes

Compound	% of Metal	%of Ligand	% of Water	Metal:Ligand ratio	Empirical Formular
Cu(II) complex	13.25	73.75	13	1:2	$[CuL_2].3H_2O$
Zn(II) complex	11.71	81.29	7	1:2	$[ZnL_2].2H_2O$

The metal-ligand ratio determination was done by using Job's method of continuous variation (UV-Visible). The result revealed that the metal-ligand ratio was 1:2. Gravimetric analysis was used for the determination of the percentage of metal (II) ions in the complexes.

Table 7: Antibacterial Activity of the Schiff base and its Metal (II) Complexes.

Isolates	Compounds	Zone of inhibition/ Standard: Concentration (µg/ml)			Ciproflaxacin 500mg
		60	30	15	
Staphylococcus aureaus	Ligand	6	6	6	31
	$[CuL_2].3H_2O$	8	7	6	
	$[ZnL_2].2H_2O$	13	12	11	
Streptococcus pnemoniae	Ligand	6	6	6	28
	$[CuL_2].3H_2O$	6	6	6	
	$[ZnL_2].2H_2O$	14	6	6	
Eschericia coli	Ligand	6	6	6	22
	$[CuL_2].3H_2O$	7	6	6	
	$[ZnL_2].2H_2O$	11	10	8	
Klebsiellapnemoniae	Ligand	15	9	6	26
	$[CuL_2].3H_2O$	7	6	6	
	$[ZnL_2].2H_2O$				

The in-vitro antibacterial activity of the Schiff base ligand and its respective metal (II) complexes have been carried out against four bacterial isolates, (*Staphylococcus aureus*, *Streptococcus pnemoniae, Eschericia coli* and *klebsiellapnemoniae*) using well diffusion method by using DMSO as solvent (Table 7). The results indicated that the metal complexes exhibit higher antibacterial activity than the Schiff base, this is probably due to chelation in the metal complexes (Al-Shemary *et al.,* 2017). The Schiff base failed to show activity at all concentrations against all bacterial isolates except against *Klebsiellapnemoniae* which exhibited activity.

Table 8: Antifungal Activity of the Schiff base and its Metal (II) Complexes

Isolates	Compound	Zone of Inhibition/ Standard: Concentration (µg/ml)			Ketoconazole 200mg
		60	30	15	
Aspergillusfumigatus	Ligand	20	17	8	31
	$[CuL_2].3H_2O$	8	6	6	
	$[ZnL_2].2H_2O$	12	11	6	
Aspergillusflavus	Ligand	11	9	6	29
	$[CuL_2].3H_2O$	12	6	6	
	$[ZnL_2].2H_2O$	12	10	7	
Candida albican	Ligand	6	6	6	30
	$[CuL_2].3H_2O$	6	6	6	
	$[ZnL_2].2H_2O$	13	11	6	

Antifungal studies were carried out by well diffusion technique on potato dextrose agar against *Aspergillus fumigatus, Aspergillus flavus* and *Candida albicans*(Table 8). The data showed that the Schiff base and the corresponding metal (II) complexes shows an appreciable activity against *Aspergillus fumigatus* and *Aspergillus flavus* isolates. However, the Schiff base and Cu (II) complex were found to be inactive at all concentration against *Candida albicans.*

Table 9: Cytotoxicity Test of the Schiff base Ligand and its Metal (II) Complexes against Brine Shrimp Larvae.

				death		
Ligand	1000	3	0,0,0	30	100	63.191
	100	3	5,6,5	14	46.67	
	10	3	8,8,8	6	20	
$[CuL_2].3H_2O$	1000	3	4,4,6	16	53.33	771.495
	100	3	7,7,8	8	26.67	
	10	3	9,9,9	3	10.00	
$[ZnL_2].2H_2O$	1000	3	3,2,3	22	73.33	161.834
	100	3	5,5,6	14	46.67	
	10	3	8.9,9	4	13.33	

The cytotoxicity assay of the Schiff base and its corresponding metal complexes have been conducted against brine shrimp larvae and the results are presented in table 10. By Clarkson's toxicity criterion assessment of samples, the results revealed that the Schiff base and Zn (II) complex are highly-toxic with LC50 values of 63.191µ/mg and 76.684µ/mg while Cu (II) complex was non-toxic with LC50 of 1175.79µ/mg. The higher the concentration, the higher the toxicity, (Clarkson *et al.,* 2004)

CONCLUSION

Novel Schiff base ligand has been synthesized by condensation of 2-aminobenzoic acid and benzaldehyde. The metal (II) complexes of Copper and Zinc have beensynthesized and characterized. The metal-ligand ratio inthe complexes is 1:2. All the complexes are nonelectrolytes in DMSO solvent. The spectral data show that the Schiff base act as bidendatecoordinating throughnitrogen atom of the azomethine. Cu (II) complex is paramagnetic while Zn(II) complex is diamagnetic. The antimicrobial studies revealed that the metal complexes showed better activity when compared to that of the ligand. The Cytotoxicity test shows that the Schiff base and Zn (II) complexes are toxic while Cu (II) complex is non-toxic.

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