Synthesis, Characterization, Cytotoxicity and Antimicrobial Studies of 2-(Benzylidene-Amino)-Benzoic Acid and Its Metal (II) Complexes

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Abstract

A Schiff base 2-(Benzylidene-amino)-benzoic acid has been synthesized by the condensation 2-aminobenzoic acid and benzaldehyde and its Mn (II), Co (II) and, Ni (II) complexes were prepared. The compounds were characterized based on solubility, melting point/decomposition temperature, molar conductance, magnetic susceptibility, Fourier transform Infrared Spectroscopy (FT-IR), gravimetric analysis, and UV-Visible Spectrophotometry. The FT-IR spectra of the Schiff base have shown a band at 1618 cm^{-1} which is assigned to (-C=N-) stretching vibration of the azomethine. This band was observed at lower frequencies in the spectra of the metal (II) complexes in the range of 1536-1588 cm^{-1} which indicated complexation. Conductivity measurement indicates the non-electrolytic nature of the metal complexes. The Schiff base failed to show activity at all concentration against *Staphylococcus aureus*, *Streptococcus pnemoniae* and *E. coli* but active against *Klebsiela pnemoniae* while the metal (II) complexes were active against all the bacterial isolates. The antifungal study revealed that the Schiff base and the metal (II) complexes show appreciable activity against *Aspergillus fumigatus* and *Aspergillus flavus*, the Schiff base was found to be inactive at all concentration against *Candida albicans*. The cytotoxicity test revealed that the Schiff base was highly-toxic, Mn (II) complex was below-toxic while the Metal (II) complexes of Ni and Co were found to be moderately toxic.

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

1.0 Introduction

Schiff bases are the products yielded from condensation reaction of primary amines and carbonyl compounds. They were first discovered in 1864 by a German chemist and Nobel prize winner Hugo Schiff. When an aldehyde or ketone is condensed with a primary amine, a Schiff base is produced. It is a compound containing azomethine group, R-C=N- (Abdulkarim *et al.*,2023). The field of Schiff base complexes is fast developing on account of the wide variety of possible structures for the ligands, depending upon the aldehydes and amines. Schiff bases are considered vital class of organic compounds, which have wide applications in many biological aspects. Transition Metal complexes of Schiff bases are thoroughly studied and have applications in catalysis and organic synthesis. Schiff bases have shown broad range of biological applications, including antibacterial, antifungal, antimalarial, anti-proliferative, antiviral, anti-inflammatory and antipyretic properties (Tasneem *et al*,.2020). Ligands used as Schiff base possessed remarkable features such as ease of formation and versatility, and therefore, they have played important role in the development of coordination chemistry. They readily form stable complexes with most of the transition metals. Schiff bases may be bidentate, tridentate, tetradentate or polydentate ligands capable of forming very stable complexes with transition metals (Siraj and Sadiq, 2016).

The Schiff bases form metal complexes with d-block metals and these complexes have been known to act as highly efficient catalysts in various syntheses and other useful reactions. Many Schiff base complexes of ruthenium and palladium are used as catalyst in the syntheses of quality polymers (Sadia *et al.*, 2018). Schiff base complexes are gaining popularity among the various industries including polymers, dyes and pharmaceutical, food and agrochemical (Rani *et al*., 2015).

This work was aimed to synthesis transition metal complexes of Mn (II), Co (II), Ni (II) using Schiff base synthesized from condensation of 2-aminobenzoic acid and benzaldehyde. The complexes were characterized by solubility test, melting points, molar conductance, magnetic susceptibility FT-IR analysis. Cytotoxicity assay of the Schiff base and its corresponding metal complexes have been conducted for anti-bacterial and fungal application.

2.0 Materials and Methods

Materials

Reagents used in this work were of analytical grade purity and were used without further purification. The reagents were purchased from Sigma Aldrich, Germany. All glass wares used in this research work were well washed with detergent, rinsed with distilled water, and dried in an oven at 110 ºC. All weighing were carried out on an electric metler balance model H3OAR, melting point and decomposition temperature were determined using Stuart SMP10 melting point apparatus. Molar conductivity was determined using Jen Way 4010 model. IR spectral analysis was carried out using FTIR Cary 630 (Agilent Technology) model in the range of 4000 – 400 cm^{-1}. The bacterial and fungal isolates were obtained from the Department of Microbiology, Faculty of Life Sciences, Bayero University Kano.

Methods

Preparation of Schiff Base

The Schiff base was prepared by mixing ethanolic solution of 2-aminobenzoic acid (4.11 g, 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. A yellow-brown precipitate was formed, the precipitate was collected by filtration, washed several times with distilled water and n- hexane, then finally dried in desiccator over (P_2O_5).

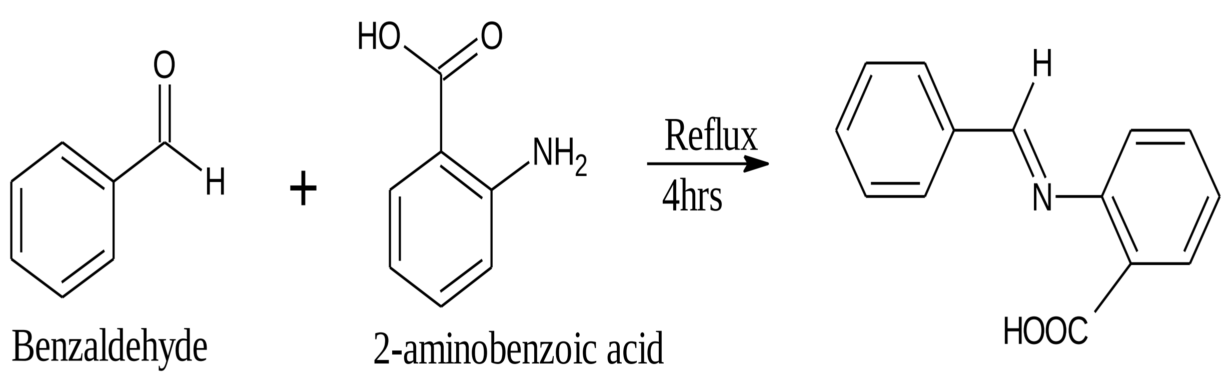

Fig. 1: Formation of the Schiff base (Nasiru and Muhammad, 2020)

Preparation of the metal complexes

The metal complexes were synthesized by mixing an ethanolic solution of the Schiff base (0.02 Mole, 2.25 g) with an ethanolic solution of the Metal (II) chloride (0.01 Mole., 0.99 g, 1.19 g and 1.19 g of Manganese, nickel and cobalt chlorides respectively). The resulting mixture was refluxed for 1 hour at 80 ^{0}C. On cooling, the metal complex compounds that precipitated out was filtered, washed several times with distilled water and n-Hexane finally dried over P_2O_5 in a desiccator.

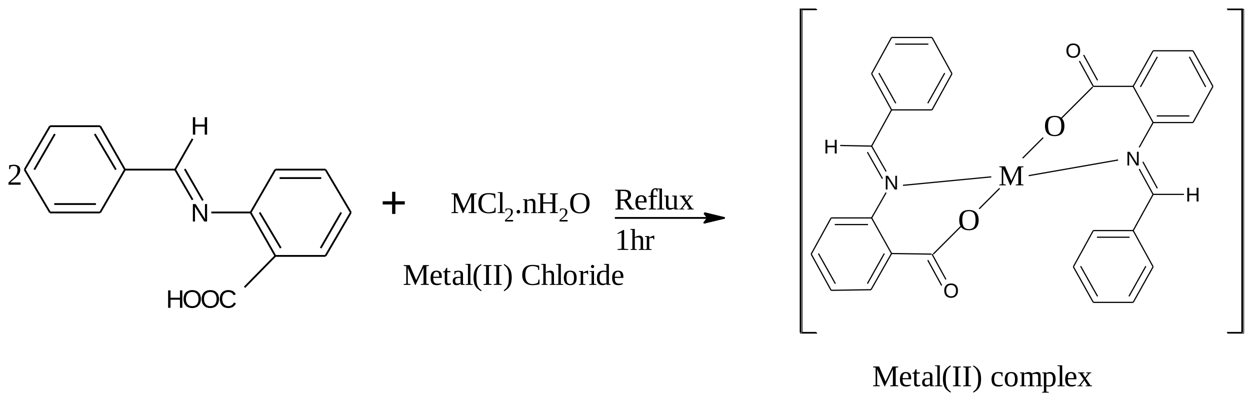

Fig. 2: Preparation of Metal (II) Complex (Nasiru and Muhammad, 2020)

Solubility Test

The solubility test of the Schiff base and the metal complexes was carried out in water, ethanol, methanol, dimethylsulphoxide (DMSO), dimethylformamide (DMF), n- hexane, diethyl ether, chloroform, acetone and tetrachloromethane (CCl_4). 0.3 g of the Schiff base and metal(II) complexes were each added in 5 cm^3 of the solvents mentioned above, their solubility was observed.

Brine Shrimp Lethality Test

Screening of Schiff base ligand and metal complexes against brine shrimp larvae was carried out according to the method described by Sani *et al*., (2021). Brine shrimp eggs were hatched for 48 hours. The stock solutions were prepared by dissolving 20 mg of each of the Schiff base ligand and metal complexes in 2 cm^3 of methanol. Two drops of DMSO was used in each vial to moisten the compounds followed by addition of 2 ml of sea water. From the stock solution, aliquots of 500 µl, 50 µl and 5 µl were pipetted and added into separate vials and allowed to dry overnight. Usually three (3) vials for each concentration (1000, 100, 10) were prepared making a total of nine (9) vials per sample. Ten (10) shrimps were transferred to each vial using a Pasteur pipette and the volume of the liquid in each vial was adjusted to 5 cm^3 using sea water. After 24 hours, the numbers of surviving shrimps were counted and the LC_{50} was determined at 95% confidence interval using regression analysis.

Anti-bacterial Studies

The *in-vitro* antibacterial activity of the Schiff base ligand and its metal complexes were assayed by using bacterial isolates of *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Escherichia coli*. The suspension of each microorganism was smeared on the surface of the solidified Muller-Hinton Agar (MHA) already poured into petri dishes. The Schiff base and the metal complexes were separately dissolved in DMSO so as to have three distinct concentrations (60 µg/disc, 30 µg/disc and 15 µg/disc) through serial dilution and placed on the surface of the culture media, incubated at 37 ^oC for 24 hours. Activities were determined by measuring (mm) the diameter of the zone of inhibition and compared with a standard drug (Ciprofloxacin) (Abdulkarim *et al.*, 2023).

Anti-fungal Studies

The *in-vitro* antifungal activity of the Schiff base ligand and that of its metal complexes were tested against three pathogenic fungi; *Aspergillus flavus, Candida albicans and Aspergillus fumigatus,* using disc diffusion method. Ketoconazole was used as standard fungicide and DMSO was used as a negative control (Abdulkarim *et al.*, 2023). The fungal suspension was smeared on the solidified Potato Dextrose Agar (PDA) already poured into petri dishes. The Schiff base and the metal complexes were separately dissolved in DMSO to have three different concentrations (60 µg/disc, 30 µg/disc and 15 µg/disc) per well. They were placed on the surface of the culture media and allowed to stand at room temperature for 48 hours. Activities were determined by measuring (mm) the diameter of the zone of inhibition and compared with the standard.

3.0 Results and Discussion

A 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 114ºC (Table 1). The Mn (II), Co (II) and Ni (II) complexes were synthesized and found to be of different colours with percentage composition of 61%, 56% and 58%. The decomposition temperature of the metal complexes ranges from 155 -164ºC (Table 1).

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	-
$[MnL_2].3H_2O$	Brown	57	-	160
$[CoL_2].2H_2O$	Green	56	-	155
$[NiL_2].2H_2O$	Sea Green	61	-	157

Key: M.P = Melting Point and D. Temp = Decomposition Temperature

Solubility test carried out on the Schiff base showed that the Schiff base was soluble in methanol, ethanol, DMSO, DMF, carbon tetrachloride, acetone, diethyl ether, 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, carbon tetrachloride and slightly soluble in diethyl ether and chloroform (Table 2). This may be due to the high dielectric constant of the solvent. Similar findings were reported by Alkasim (2020) for the metal (II) complexes synthesized using a Schiff base derived from 2-Aminobenzenethiol and 4-Nitrobenzaldehyde (Alkasim, 2020).

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

Solvents	Ligand	$[MnL_2].3H_2O$	$[CoL_2].3H_2O$	$[NiL_2].3H_2O$
Water	IS	IS	IS	IS
Methanol	S	S	S	S
Ethanol	S	S	S	S
n-hexane	IS	IS	IS	IS
CCl_4	S	IS	IS	IS
Acetone	S	SS	SS	IS
Chloroform	S	SS	SS	IS
Diethylether	S	SS	S	SS
DMF	S	S	S	S
DMSO	S	S	S	S

Key: Soluble, SS = Slightly soluble, IS = Insoluble, DMF = Dimethylformamide, DMSO Dimethylsulfoxide L= Ligand,

The infrared spectral data of the Schiff base ligand and its metal complexes were listed in table 3. The spectral of the Schiff base showed strong absorption band at 1618 cm^{-1} due to ν(C=N) stretching, indicating that condensation has taken place (Bahir and Siraj, 2021). The band was shifted to lower frequency in the range of 1536-1588 cm^{-1} in the spectra of the metal (II) complexes. The appearance of the new bands in the range of 564-661 cm^{-1} and 415-478 cm^{-1} in the spectra of metal (II) complexes were attributed to ν(M-N) and ν(M-O) groups (Sulaiman *et al*,.2023).

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

Compound	v(C=N) cm^{-1}	v(M-N) cm^{-1}	v(M-O) cm^{-1}
Ligand	1618	-	-
$[MnL_2].3H_2O$	1588	633	478
$[CoL_2].2H_2O$	1536	605	437
$[NiL_2].3H_2O$	1540	661	419

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 $ohm^{-1}\ cm^2\ mol^{-1}$ which are relatively low, indicating the non-electronic nature of the metal complexes (Table 4) (Mukhtar *et al*,.2018).

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

Complexes	Electrical Conductivity ($ohm^{-1}\ cm^{-1}$)$\times10^{-6}$	Molar Conductance ($ohm^{-1}cm^2mol^{-1}$)
$[MnL_2].3H_2O$	47.40	15.80
$[CoL_2].2H_2O$	63.30.	21.10
$[NiL_2].3H_2O$	93.30	31.10

The susceptibility measurement of Mn (II), Co (II) and Ni (II) indicated that they are all paramagnetic (Table 5). The finding was in agreement with the previous report on the transition metal (II) complexes of Co, Ni, Cu, Zn and Cd synthesized with Schiff base derived from Saccharine (Tagreed, 2016).

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
$[MnL_2].3H_2O$	$2.70x10^{-6}$	$1.37x10^{-3}$	5.70	Paramagnetic
$[CoL_2].2H_2O$	1.99×10^{-5}	1.01×10^{-3}	4.92	Paramagnetic
$[NiL_2].3H_2O$	9.62×10^{-6}	4.88×10^{-3}	3.41	Paramagnetic

Key: B.M = Bohr Magneton

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 Klebsiella pnemoniae*) using well diffusion method by taking 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. The Schiff base failed to show activity at all concentrations against all bacterial isolates except against *Klebsiella pnemoniae* which exhibited activity. The Azomethine group in the Schiff base compound has been shown to be responsible for their antimicrobial activities (Ado *et al*., 2021). The observed result agreed well with the findings published by Sani *et al*. (2021).

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

Isolates	Compounds	Zone of inhibition μg/ml 60	30	15	Standard: Ciproplaxacin 500 mg
Staphylococcus aureaus	Ligand	6	6	6	31
	$[MnL_2].3H_2O$	13	11	8	
	$[CoL_2].2H_2O$	12	11	9	
	$[NiL_2].3H_2O$	16	8	7	
Streptococcus pnemoniae	Ligand	6	6	6	28
	$[MnL_2].3H_2O$	8	6	6	
	$[CoL_2].2H_2O$	9	7	6	
	$[NiL_2].3H_2O$	9	6	6	
Eschericia coli	Ligand	6	6	6	22
	$[MnL_2].3H_2O$	8	6	6	
	$[CoL_2].2H_2O$	14	12	6	
	$[NiL_2].3H_2O$	7	7	6	
Klebsiella pnemoniae	Ligand	15	9	6	26
	$[MnL_2].3H_2O$	12	10	6	
	$[CoL_2].2H_2O$	15	11	9	
	$[NiL_2].3H_2O$	13	10	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 revealed that the Schiff base and the corresponding metal (II) complexes shows an appreciable activity against all the isolates. However, the Schiff base was found to be inactive at all concentration against *Candida albicans.* The result is in good agreement with the finding reported by Ado *et al* (2021).

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

Isolates	Compound	Zone of Inhibition μg/ml 60	30	15	Standard: Ketoconazole 200mg
Aspergillus fumigates	Ligand	20	17	8	33
	$[MnL_2].3H_2O$	14	10	8	
	$[CoL_2].2H_2O$	20	17	12	
	$[NiL_2].3H_2O$	19	15	12	
Aspergillus flavus	Ligand	11	9	6	29
	$[MnL_2].3H_2O$	13	9	7	
	$[CoL_2].2H_2O$	13	10	8	
	$[NiL_2].3H_2O$	16	11	10	
Candida albican	Ligand	6	6	6	30
	$[MnL_2].3H_2O$	14	12	8	
	$[CoL_2].2H_2O$	21	15	13	
	$[NiL_2].3H_2O$	16	12	10	

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 9. The LC_{50} values estimated for the ligand, Mn (II), Co (II) and Ni (II) were in the range of 63.191- 771.495 µg/ml, by Clarkson's toxicity criterion assessment of samples, the results revealed that the Schiff base was highly-toxic, Mn (II) was found to be below-toxic while Co (II) and Ni (II) complexes were moderately-toxic. The higher the concentration, the higher the toxicity. The results obtained were almost similar to the result reported by Sani *et al.*, (2021).

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

Compound	Conc.µg/ml	Replica	Survivors	Total death	%Mortality	LC_{50}
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	
$[MnL_2].3H_2O$	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	
$[CoL_2].2H_2O$	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	
$[NiL_2].3H_2O$	1000	3	3,2,3	22	73.33	203.539
	100	3	7,5,5	13	43.33	
	10	3	9,10,9	2	6.67	

4.0 Conclusion

The Schiff base and its metal complexes of Mn (II), Co (II) and Ni (II) have been synthesized and studied by various analytical techniques. All the complexes are non-electrolytes in DMSO solvent. The spectral data showed that the Schiff base act as bidendate ligand coordinating through nitrogen atom of the azomethine. The Schiff base complexes are paramagnetic. 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 the metal complexes are toxic.

Reference

Ado, I., Na'aliya, J., Sani, S., and Haleelu, M.M (2021). Synthesis, Spectroscopic and Antimicrobial Studies of Co(II), Ni(II), Cu(II) and Zn(II) Complexes Derived from Benzoic Acid Bidentate Schiff Base Ligand. *J. Appl. Sci. Environ. Manage*. Vol. 25 (9) 1599-1603.

Abdulkarim, A., Sani, U. and Safiyanu, A. L. (2021). Synthesis, characterization, cytotoxicity and antimicrobial studies of Schiff base derived from 2-benzoylbenzoic acid and 4- nitoaniline and its metal (II) complexes. *FUDMA Journal of Sciences,* 7(6): 200-205.

Alkasim, A. U. (2020) Synthesis, Characterization and Antimicrobial Activities of Schiff Base derived from 2-Aminobenzenethiol and 4-Nitrobenzaldehyde and their Metal (II) Complexes. (masters thesis, Bayero University, Kano)

Bashir, A. & Siraj, I.T. (2021). Synthesis, Characterization and Antimicrobial Studies of Schiff Base Derived from the Reaction of 2-Thiophenecarboxaldehyde and Ethylenediamine and its Metal (II) Complexes. *ChemSearch Journal* 12(1): 143 – 148.

Mukhtar, H., Sani, U. Shagal M.H., and Joseph, J.T. (2018). Synthesis, characterization and antimicrobial studies of Schiff base Derived from 2,4-dinitrophenyl hydrazine and its metal (II) Complexes. *IOSR Journal of Applied Chemistry*, 11(9): 49-53.

Nasiru, P. Y. and Muhammad, S. M. (2020). Synthesis, characterization and antibacterial activity of Schiff base and its metal (II) complexes derived from 3-aminophenol and benzaldehyde. *International Journal of Research and Scientific Innovation (IJRSI) | Volume VII,* ISSN 2321–2705.

Rani, A., Manoj, K., Rajshree, K., Hardeep, S. T. (2015). Schiff bases as an antimicrobial agent: A review *Journal of Biological and Chemical Science*, ***2(1)****:* 62-91.

Sadia, A. D., Farhana, A., Saddam Hossain, M.D., Nuruzzaman Khan, M.D., Zakaria, C. M. Kudrat, E. Z. and Mohsin, A. (2018). A short review on chemistry of Schiff base metal complexes and their catalytic application. *International journal of chemical studies, 6(3):* 2859-2866.

Sani, U., Aisha S.D., Samaila, A., and Dailami, S. A. (2021). Synthesis, characterization, cytotoxicity and antimicrobial studies of metal (II) complexes of Schiff base derived from 2- benzoylbenzoic acid and 1,3-diaminopropane. *Bima journal of science and technology,* 5(1): 225-237.

Siraj, I.T. and Sadiq, I.A. (2016). Synthesis, Characterization and Antimicrobial Activities of a Schiff Base Derived from Phenylalanine and Acetylacetone and its Mn(II), Ni(II) and Cu(II) Complexes. *ChemSearch Journal* 7(2): 34 – 39.

Suleiman, A. K., Sadi A. H., Badamasi, H. and Ahmadu, M. (2023). Synthesis, Characterization and Antimicrobial Studies of Schiff Base Derived from 2-Amino Phenol and O-Anisaldehyde and Its Co (II), Cu (II) and Zn (II) Complexes. *Dutse Journal of Pure and Applied Science*. (9) 301-310.

Taghreed, M. M, (2016). Synthesis, characterization and antimicrobial activity of some transition complexes with new Schiff base derived from Saccharine. *International Research Journal of Pure and Applied Chemistry,* 11(2): 1-5.

Tasneem, I. H., Musa, A. A., Ismail, A. A., Awad, S. I., Mohamed, A., Hassan, A. A. and Asim, N. (2020). Synthesis, characterization and biological activity of a Schiff base derived from o-amino benzoic acid and its Co(II), Cd(II) and Ni(II) complexes. *European Journal of Chemistry,* 11 (1) (2020) 15-20