Caliphate Journal of Science & Technology (CaJoST)

ISSN: 2705-313X (**PRINT**); 2705-3121 (**ONLINE**) **Research Article**

Article Info

Received: 23rd April 2021

Revised: 10th August 2021

Accepted: 16th August 2021

[1]Department of Industrial Chemistry, Federal University Lokoja, Kogi State, Nigeria,
[2]Department of Chemistry, Federal University Lokoja, Kogi State, Nigeria,
[3]Department of Chemistry, University of Benin, Benin City Edo State Nigeria,
[4]Department of Biochemistry, Federal University Lokoja, Kogi State, Nigeria,

***Corresponding author's email:**

khandora2000@gmail.com,

muluh.khan@fulokoja.edu.ng

Cite this: *CaJoST,* 2021, 2, 139-151

Antimicrobial Activity of 2-((2-Hydroxybenzylidene) Amino) Nicotinic Acid and Its Cobalt (II) Complexes Synthesized from O-Phenylenediamine and 5-Nitrosalisaldehyde

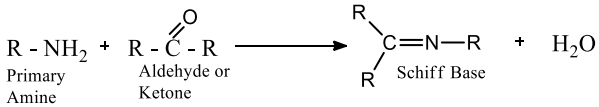
Izuagbe G. Osigbemhe[1], *Muluh. E. Khan[2], Amowie. P. Oviawe[3] and Glen. Elkanah[4]

Compounds and Cobalt (II) complex of 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid obtained from o-phenylenediamine and 5-nitrosalicaldehyde were prepared and characterized using AAS, UV-Visible, IR, ^{1}HNMR, ^{13}CNMR, and GCMS. The synthesized complex was screened against some microbes in order to establish its potential antimicrobial activity using some known drugs as references. The results obtained indicated that, the Schiff Base exhibited antimicrobial action against all the tested microbes except *Candidas albicans* isolate, which exhibited zero diameter zone of inhibition including *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aeruginosa,* and *Proteus mirabilis.* It was also found that the synthesized Schiff Base exhibited two digits purity range, implying that it was relatively stable. The biological activity of the metal complex of the Schiff Base was found to be comparably more suiting than that of the synthesized Schiff Base.

Keywords: Synthesis, characterization, Schiff Base, Cobalt (ii) complexes, and antimicrobial activity.

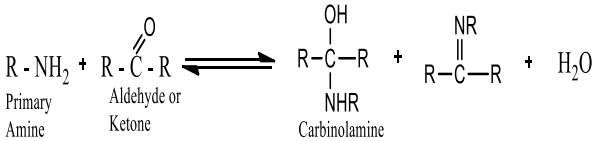
1. Introduction

Schiff bases are nitrogen analogue of carbonyl compounds in which the C=O group is replaced by a C=N-R group. They are most often formed by the condensation of an aldehyde or ketone with a primary amine; **Scheme 1** (Loudon, 2002).



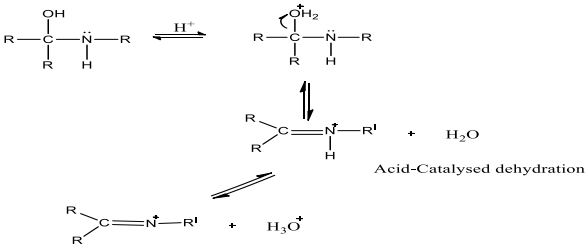
where R may be an alkyl or an aryl group.
Scheme 1: Reaction of aldehyde / ketone to give a Schiff Base**.**

Schiff bases of aryl substituents are stable, polymerizable and more readily synthesized, (Gupta and Sutar, 2008, Cozzi, 2004). According to Siji *et al*., (2011), the formation of a Schiff base is reversible and generally takes place under acid or base catalysis or upon heating,



Scheme 2: Equation of primary amine and aldehyde / ketone to form a carbaminalamine

The mechanism of Schiff base formation is another variation on the theme of nucleophilic addition to the carbonyl group. In this case, the nucleophile is the amine. In the first part of the mechanism, the amine reacts with the aldehyde or ketone to give an unstable addition compound called carbinolamine. The carbinolamine loses water by either acid or base catalyzed pathways. Since carbinolamine is an alcohol, it undergoes acid catalyzed dehydration.



Scheme 3: A schematic representation of the carlbinoalamine formation

The dehydration of the carbinolamine is the rate-determining step of Schiff base formation and this is why the reaction is catalyzed by acids.
According to Oviawe and Elemikhe, (2012) Schiff bases appear to be important intermediates in a

number of enzymatic reaction involving interaction of the amino group of an enzyme, usually that of a lysine residue, with a carbonyl group of the substrate (Mutagh, 2007). The study of the biological role of metal ions has a long history in chemistry and medicine, in pharmacology and in toxicology, but it is only recently that the extent and variety of metal ion involvement has been appreciated. For example, among the transition metals, the elements, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Mo have been used in therapy or claimed to be of therapeutic value (Eman *et al,* 2008).

Interaction of various metal ions with antibiotics may enhance or suppress their antimicrobial activity, the pharmacological activity of antibiotics, after complexation with metals, is enhanced as compared to that of the free ligands (Rehman *et al.*, 2008). Many of the well-known antibiotics, penicillin, streptomycin, bacitracin and tetracycline are chelating agents and their action is improved by the presence of small amount of metal ions, (Licker, 2004). Binding of metallo-elements with polydentate ligands to form ring structure, where the metal atom is part of the ring, is called chelation and antibiotics like Streptomycin, Cycloserine, Ampicillin, Isoniazid and others are also known to have chelating properties, (Eswaran *et al.*, 2009).

According to Tolulope *et al*. (2017), the resistance of microorganism to classical antimicrobial compound poses a challenge to effective management and treatment of some diseases. Consequently, the use of Schiff bases has given hope in overcoming fungi resistant microorganism. Also, Afanas *et al*., (1989) stated that the biological activity of a Schiff base can be altered through coordination to a metal ion. Such alteration has been found to enhance the biological activities of most Schiff bases and expands their pharmaceutical applications. Therefore, the aim of this study is to synthesize, characterize, and coordinate the Schiff base synthesized from the coupling of 2-{[(2-hydroxy-5-nitrophenyl) methylidene] amino} nicotinic acid and its Cobalt (II) complex and then test their efficacies on some disease causative microbes. The synthesis of some target Schiff bases and possibility of altering their biological activity via coordination to metal ions has been extensively studied and this present work is an extension of such studies and is considering the synthesis and biological evaluation (antibacterial and antifungal activity) of Cobalt (II) complex of the above Schiff base derived from aromatic/hetero-aromatic carboxyaldehyde and (un)-substituted hetero aromatic amines. A detailed literature survey revealed that the synthesis of the Schiff base reported in this research has not been reported.

2. Materials and Methods

2.1 Reagents

2-aminonicotinic acid (2-aminopyridine-3-carboxylic acid); Salicylaldehyde; H_2SO_4, Ethanol, Methanol, Nutrient agar. All the reagents used, are of analytical grade from Sigma-Aldrich, Merck, Germany and were used without further purification.

2.2 Organisms

Bacteria: *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aeruginosa,* and *Proteus mirabilis.* Apart from *Bacillus subtilis, Staphylococcus aureus*, which are gram positive, others are gram negative.

Fungi- *Candida albicans, Penicillium notatum* and *Aspergillus niger*.

The microorganisms used were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH). All organisms were checked for purity at Pax Herbal Clinic and Research Laboratories, Ewu, Edo State and were maintained at 4^{o}C in slants of Nutrient Agar and Sabourand Dextrose Agar (SDA) slants for bacteria and fungi respectively.

2.3 Equipment/Apparatus

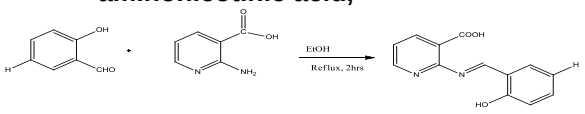
Gas chromatography Mass spectrometry (GCMS); Thermal Scientific DSQ II Focus Instrument Model; Fourier Transform Nuclear Magnetic Resonance Spectrometer (FTNMR) – Bruker 400MH_z machine; Ultra-violet spectra were recorded on a Hitachi U-2000 double beam Spectrophotometer; Infra-red spectra (KBr Discs) were recorded on a Hitachi Model 200-50 IR Spectrophotometer. Melting points were taken on a Gellenkamp apparatus and are uncorrected. All instrumental determinations were carried out in Durham University, Chemistry Department, United Kingdom.

2.4 Syntheses of Schiff Base, 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid.

Equi-molar portions of 2-aminonicotinic acid (0.01 mol) with 0.01 mol salicylaldehyde were mixed together in ethanol (30-40 mL) containing a few drops of conc. H_2SO_4 at a pH of 3.5 to 4.5. The resultant mixture was then heated under reflux for 2 hours at 70^{O}C and filtered hot by suction filtration. The product of reaction was allowed to crystallize from filtrate, left at room temperature of 25^{o}C for 2 days. The crystals formed were re-crystallized in hot ethanol and

dried in a desiccator over $CaCl_2$ vacuum and theirs yields calculated.

2.5 Coupling of salicylaldehyde and 2-aminonicotinic acid,



2.6 Synthesis of Cobalt Complex of 2-{[(2-hydroxyphenyl) methylidene]amino}nicotinic acid

The metal complex of Co (II) was prepared by reaction of equimolar (0.01 mol) of each metal salt with a corresponding (0.01 mol) of the Schiff base ligand. The various 0.01 mol of the metal salts were each refluxed with 0.01 mol of the ligand in ethanol as medium for 2 hours. They were all filtered and washed with ethanol after which they were allowed to stand for 24 days. The resulting crystals were then dried and the melting point determined. The synthesized complex was coloured.

2.7 Preparation of Inoculum

A loop-full of the test organism was taken from their respective agar slants and sub-cultured into test-tubes containing Mueller Hinton broth for bacteria and Saboraud Dextrose liquid for fungi. The test-tubes were incubated for 18 hours at 37^{o}C for bacteria and for 48 hours at 30^{o}C for the fungi. The obtained microorganisms in the broth were standardized using normal saline to obtain a population density of 100 cfu/mL for the bacteria. For the fungi, fungal spores were harvested after visible notices of growth and suspension was standardized. (Tor-Anyiin *et al*., 2016)

2.8 Preparation of the Media

38 g of Mueller Hinton Agar, 52g of SDA were weighed independently into different conical flasks; 1000 mL of distilled water was added and capped with a cotton wool. The media were boiled to dissolution and then sterilized at 121^{o}C at 15 minutes. The media were then allowed to cool to 45^{o}C and 20 mL of the sterilized medium was poured into sterile Petri-Dishes and allowed to cool and solidify. The plates were labelled with the test microorganism (each plate with a test microbe). The microbes were spread evenly over the surface of the medium with the aid of a glass spreader. The plates were dried at 37^{o}C for 30 minutes respectively.

2.9 Antimicrobial Assay

The synthesized compounds (the Schiff base/complex) were assayed for their antimicrobial activity using the disc diffusion technique by Kirby-Bauer (Friedrich,& Elaine, 2002). Whatman filter paper (No. 1) was cut into sizes of 6 mm diameter with office perforator and sterilized at 105^{o}C for 1 hour. The sterile discs were impregnated with 20 uL of 100 mg/mL of the synthesized Schiff base and dried in the oven at 60^{o}C for about 15-30 minutes. Mueller Hinton agar plates were seeded with standardized broth culture of test organisms containing 100 cfu/mL equivalent to 0.5 Mcfarland standards (NCCLS) and the prepared discs containing 2 mg of the compound were placed on the plates. They were then incubated at 37^{o}C for 24 hours and observed for diameters of zones of inhibition. The zones diameters were measured with a transparent ruler and the result recorded in millimetres (mm), in duplicates. Sterilized discs were soaked in 100% DMSO as controls and 2 mg/mL of Ampicillin-Cloxacillin (Ampiclox) for bacterial isolates and ketoconazole for fungi, (Nna *et al*., 2019)

2.10 Minimum Inhibitory Concentration – Broth Dilution Method

The minimum inhibitory concentration of the compound was carried out using Macro Broth Dilution Technique (Boron and Fingold, 1990). 9 mL of each broth was dispersed into separate test-tubes and was sterilized at 121^{o}C for 15 minutes and then allowed to cool. Dilutions of the compound were made from the stock concentration to obtain 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 mg/mL. The standardized inoculum (0.1 mL) of the microbes was inoculated into different concentrations of the compound in the broth. The test tubes of the broth were incubated at 37^{o}C for 24 hours and 30^{o}C for 1-7 days for bacteria and fungi respectively and observed for turbidity. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

2.11 Minimum Bactericidal/Fungicidal Concentration – Macro Broth Dilution Method

Fresh Muller Hinton agar media were prepared, sterilized at 121^{o}C for 15 minutes and was poured into Sterile Petri-Dishes and left to cool and solidify.

The contents of the MIC tubes were then sub-cultured onto the media and incubated at 37^{o}C for 24hours and 30^{o}C for 1-3 days for bacteria and fungi respectively. It was then observed for colony growth. The MBC/MFC was the plate with the lowest concentration of extract and without colony growth, (Khan *et al.,* 2019).

3. Results and Discussion

3.1 Characterisation of the Schiff Base 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid and it's Cobalt (II) Complex

The yield of 75% as yellow powder; m.p. 122-123^{o}C,

Infrared (KBr, cm^{-1}) Values,

(OH, Carboxylic acid)	1735
(OH, Phenol),	3285.85
(C=O, carboxylic acid),	1383.7
(HC=N)	1631
(C=N, pyridine),	1610

^{1}H NMR parameters

(DMSO-d6, , ppm); 7.40 (ddd, ^{1}H, j=7.71, 7.68, 2.14 H_3, phenyl C_5-H), 7.15 (dd, ^{1}H, j =7.71,7.67, 2.11 H_3, phenyl C_4-H), 7.40 (dd, ^{1}H, j = 7.71, 7.67, 2.11 H_3, phenyl C_4-H), 7.40 (dd, IH, d=7.84, 5.25H_3, pyridine C_5-H), 8.89 (d, ^{1}H, j=5.25H_3, phenyl C_6-H), 8.31(d, ^{1}H, j=7.84HZ, Pyridine C_4-H), 8.72 (d, 1H, j=5.25HZ, Pyridine C_6-H), 8.67 (S,^{l}H,CH=N), 10.22 (S,^{l}H,OH), 11.31 (S,^{l}H, COOH).

3.2 Cobalt (II) 2-{[(2-hydroxyphenyl) methylidene] amino} nicotinic acid

Its yield was found to be 68% pink crystals; m.p. (decomposed) 208-210^{0}C.

IR (KBr, cm^{-1}), values

1735	(OH, Carboxylic acid)
3285.85	(OH, Phenol),
1383.7	(C=O, carboxylic acid),
1631	(HC=N),
1610	(C=N, pyridine);

^{1}H NMR parameters

(DMSO-d_6, δ, ppm); 7.40 (ddd, ^{1}H, j=7.71, 7.68, 2.14 H_3, phenyl C_5-H), 7.15 (dd, 1H, j =7.71,7.67, 2.11 H_3, phenyl C_4-H), 7.40 (dd, 1H, j = 7.71, 7.67, 2.11 H_3, phenyl C_4-H), 7.40 (dd, IH, d=7.84, 5.25H_3, pyridine C_5-H), 8.89 (d, 1H, j=5.25H_3, phenyl C_6-H), 8.31(d, 1H, j=7.84H_Z, Pyridine C_4-H), 8.72 (d, 1H, j=5.25H_Z, Pyridine C_6-H), 8.67 (S,IH,CH=N), 10.22 (S,IH,OH), 11.31 (S,IH, COOH), 530 (M-N), 460 (M-O).

3.3 The IR Spectra of Schiff base due to 2-aminonicotinic acid and the Salicylaldehyde

The IR spectra of these Schiff bases showed bands resulting from the OH stretching of the phenol and carboxyl function in the 3282-3286cm^{-1} and 1735-1741cm^{-1} regions respectively, whereas the carboxyl (C=O) stretching were observed in the 1382-1383cm^{1} regions. The azomethine (HC=N) stretching were observed in the 1630-1635cm^{-1} region, and the pyridine (C=N) stretching appeared at 1610cm^{-1} in all the structures synthesized with this combination of amines and aldehydes.

3.4 The ^{1}H-NMR spectra due to 2-amino nicotinic acid and their Salicylaldehydes

The ^{1}H-NMR spectrum of 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid displayed OH protons of the phenol at chemical shift, δ, 10.22 ppm and the carboxyl OH proton at δ 11.32ppm as a singlet. The ^{1}H-NMR spectrum of 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid demonstrated the phenyl C_5-H and C_4-H as a doublet of double of double doublet at δ 6.96 and δ 7.21 respectively. The phenyl C_3-H and C_6-H, however, appeared as double doublet at δ 7.11 and δ 7.85, respectively.

3.5 The ^{13}C-NMR spectra of Schiff base ligands nicotinic acid and the Salicylaldehyde

The ^{13}C-NMR spectrum of 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid displayed a peaks at δ 165,δ 158,δ 147,δ 143,δ 110, and δ 108. The carbonyl in carboxylic group experiencing a de-shielding effect occurs at the downfield of δ 165. The imine group was found at δ 158 while the benzene carbon occurred at δ 108 - δ 143.

3.6 The GC-MS fragmentation of 2-{[(2-hydroxyphenyl)-methylidene]amino} nicotinic acid

The GC-MS showed the mass ion at 242 and major fragment at 132, 93, 84, 78, 57, 45 (base peak), 44, 43 and 32.

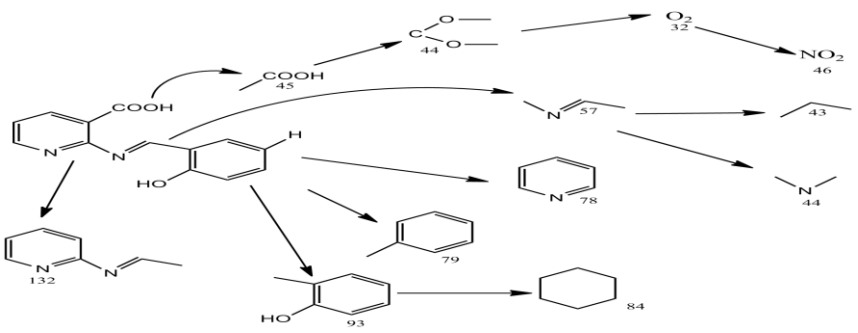


Figure 4: **The GS-MS fragmentation of 2-{[(2 hydroxyphenyl)-methylidene]amino}nicotinic acid**

Table 1: Result of *in vitro* anti-bacteria activities of Schiff bases

Compounds	Diameter zone of inhibition (mm)						
	B. subtilis	*E. coli*	*E. aerogenes*	*K. pneumonia*	*P. Aeruginosa*	*S. aureus*	*P. mirabilis*
2-{[(2-hydroxyphenyl)methylidene]amino}nicotinic acid	8	10	9	10	8	0	0
Ampiclox (Standard)	19	0	0	0	17	19	0
DMSO	0	0	0	0	0	0	0

Table 2: Result of i*n vitro* anti-fungi activities of Schiff base

Compounds	Diameter zone of inhibition (mm)		
	Aspergillus niger	*Candida albicans*	*Penicillium notatum*
2-{[(2-hydroxyphenyl)methylidene]amino}nicotinic acid	0	0	0
Ampiclox	0	0	0
Ketokonazole	0	0	9
DMSO	0	0	0

Table 3: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC)

Compounds	Minimum Inhibitory concentration (MIC) and Minimum Bactericidal (MBC mg/ml)													
	B. subtilis		*E. coli*		*E. aerogenes*		*K. pneumonia*		*P. Aeruginosa*		*S. aureus*		*P. mirabilis*	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
2-{[(2-hydroxyphenyl)methylidene]amino}nicotinic acid	1.2	1.5	1.8	2.1	1.5	1.8	1.5	1.8	1.2	1.5	0	0	0	0

(mg/mL) of Schiff bases

The MIC/MBC values were determined as mg/ml of active compound in medium.

Table 4: Results of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Schiff bases

Compounds	Diameter of zone of inhibition (mm)					
	A. niger		*C. albicans*		*P. notatum*	
	MIC	MBC	MIC	MBC	MIC	MBC
2-{[(2-hydroxyphenyl)methylidene]amino}nicotinic acid	0	0	0	0	0	0

The MIC/MBC values were determined as mg/ml of active compound in medium.

3.7 Biological Activity of Schiff Bases

In this study, 2-{[(2-hydroxyphenyl)methylidene]amino}nicotinic acid and its Cobalt (II) complex, were investigated on seven bacteria and three fungi isolates respectively. The results of *in vitro* antimicrobial activities were presented in Tables 1-4. Diameter zones of inhibition were observed 24 hours after incubation at a constant temperature of 37ºC for bacteria and 30ºC at 2-5 days of incubation for fungi. Diameter zone of inhibition obtained indicated that most of the Schiff bases were active against the bacteria and fungi isolates even better when compared with standard (Ampicillin-cloxacillin for bacteria and ketoconazole for fungal infections).

According to the results of the antimicrobial activity screening, the Schiff base 2-{[(2-hydroxyphenyl)methylidene] amino} nicotinic acid was also active against most of the test bacterial

isolates better in comparison with standard drugs though bacteria isolates of *S. aureus* and *P. aeruginosa* were not susceptible to 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid, may be due to the fact that the microbes had developed resistance before the introduction of the Schiff base.

3.8 Biological Activity of Metal Complexes

The antimicrobial activity of the metal complex was also compared with those of standard drugs Ampicilin-Cloxacillin and Ketoconazole. The overall results of diameter zone of inhibition obtained indicated that the metal complex was active against the bacteria and fungi isolate comparably, more active than the standard drugs used. This result was in correspondence with the findings of Fasina and Ogundele (2014) that reported antibacterial activity of some transition metal complexes of Schiff base derived from o-phenylenediamine and 5-nitrosalicaldehyde. In their work the Schiff bases were more active than the metal complexes against all bacterial strains with the activity recorded for the complexes varying with metal ion present. The activity of the complexes obtained appears to be dependent on the geometry of the metal complex. The variation in the activity of different metal complexes against different microorganisms depends on the impermeability of the microbes' cell or differences in the ribosome in the microbial cells (Gajendra *et al.,* 2010).

3.9 MIC and MBC/MFC of Schiff bases

The antimicrobial properties of the Schiff bases were further investigated by macro-dilution to determine their minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration for Bacterial isolates and Minimum Fungicidal Concentration (MFC) applicable to fungi isolates. The MIC of Schiff bases: 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid were good.2-{[(2-hydroxy-5-methoxyphenyl)methylidene]-amino} nicotinic acid Schiff base MIC and MBC recorded of 1.8 and 2.1 for *E. Coli* respectively, MIC 1.2 and MBC 1.5 for *B. subtilis,* MIC 1.5 and MBC 1.8 for *P. aeruginosa*.

4. Conclusion

The high affinity of the Schiff bases for chelation towards transition metals has been taken advantage of in synthesizing the complexes earlier mentioned. Based on the UV-Visible, IR, ^{1}HNMR, ^{13}CNMR and GCMS data of the Schiff bases and complexes, and structures have been proposed for the compounds. From the results on zone of inhibition, it was established that most of the Schiff bases and complexes had better activity than Ampicillin-cloxacillin for bacteria and ketoconazole for fungal infections. Schiff base 2-{[(2-hydroxy-5-nitrophenyl) methylidene] amino} nicotinic acid notably exhibited high and good MIC and MBC recorded of 1.8 and 2.1 for *E. coli* respectively, MIC 1.2 and MBC 1.5 for *B. subtilis,* MIC 1.5 and MBC 1.8 for *P. aeruginosa*.

Acknowledgements

The authors of this work appreciate Researchers of University of Benin, Benin City, Edo State Nigeria, who assisted in some aspects of the synthesis and analysis of the Schiff bases, the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH), at Pax Herbal Clinic and Research Laboratories, Ewu, Edo State, for checking for purity of the organisms and Chemistry Department, Durham University, United Kingdom for helping in the characterization of the molecules.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

References

Afanas, B., Dorozkhko, A.L.,Brodskii, A.V., Kostyuk, V.A. Potapovitch, A.I. (1989): *Biochemical pharmacology*, 38(38): 178-1739.

Boron, J.E. and Fingold, S.M.(1990). Method for testing Antimicrobial effectiveness. In: Bailey Scotts Diagnostic Microbiology, 8th edition. Mosby, CV Missouri, 6(8):23-28

Cozzi, P.G. (2004): Mental-Salen Schiff Base Complexes in Catalysis Practical Aspects. *Chemical Society Review*, 7(33): 410-421.

Eman, AA,E.; Khaled, H.H; Safaa, K.H. and Nabil, S.Y. (2008): Synthesis, Characterization and Biological activity of some transition metal Complexes with shiff bases derived from 2-formylindole, salicylaldehyde, N[amino rhodamine. *Australian Journal of Basic and Applied Sciences*. 2 (2): 210-220.

Eswaran, S.; Adhikari, A.V.; and Shetty, S. (2009): Synthesis and Antimicrobial Activity of Novel Quinoline Derivatives Carrying 1,2,4-Triazole Moiety, *European Journal of Medicinal Chemistry*, Vol. 44, No.11, pp 4637-4647.

Fasina, T.M. and Ogundele, O. (2014): Synthesis, characterization and antimicrobial activity of some transition metal complexes off Schiff base derived from o-phenylenediamine and 5-

nitrosalicyaldehyde, *Der pharma Chemica, 6*(4): 18-22

Friedrich, B. and Elaine, W.E. (2002): "Benzaldehyde" in Ullmann's *Encyclopedia of Industrial chemistry*. Wiley-VCH, Weinheim 8 (23): 463

Gajendra, K., Dharmendra, K.,Singh, C.P., Amit, K. and Rana, V.B.(2010) "Synthesis,Physical Characterization and Biological Evaluation of Schiff Base Cr(III), Mn(III), Fe(III) Complexes" *European journal of Chemistry, 7(3)*: 813-820

Gupta, K.C.; and Sutar, A.K. (2008): Catalytic Activities of Schiff Base Transition Metal Complexes, *Coordinator Reviews*, 12(252): 1420-1450.

Khan M. E, Ugbede, O. A, Tor-Anyiin T. A (2019):Screening of *Cassia sieberiana (Fabaceae*) Leaf Extract for *in-vitro*anti Microbial and Anti-ulcer Activities. *Progress in Chemical and Biochemical Research,* Vol. 2(3) Pp. 143-149

Licker, M.J. (2004): Mc-Graw Hill concise Encyclopedia of Chemistry. Mc Graw Hill Inc New york.

Loudon,G.M. 2002, *'Organic Chemistry'*, Addison-Wesley, California, Ed. 4 5(8): 74.

Mutagh, J. (2007) *General Practise*. Mc Graw Hill Australia.

Prince Joe Nna, Terrumun Amom Tor-Anyiin and Khan, Emmanuel Muluh, (2019):

Stigmasterol and -sitosterol from the Root of Mangifera indica and Their Biological Activities against Some Pathogens. *Journal of Complementary and Alternative Medical Research;* 7(4): 1-10.

Oviawe, A.P.; Elemikhe, E.E.(2012): Synthesis, Spectroscopic Characterisation and Antimicriobial Screening of some Schiff Base Ligands and their Divalent Metal Complexes of Fe,Co,Ni,Cu and Zn *Journal of Chemical Society of Nigeri*a. 34(10):152-156

Rehman, W.; Saman, F.; and Ahmed, I. (2008): Synthesis, Characterization, and biological study of some biological potent Schiff Base transition metal complexes. *Russ.* 34(9), 678-682.

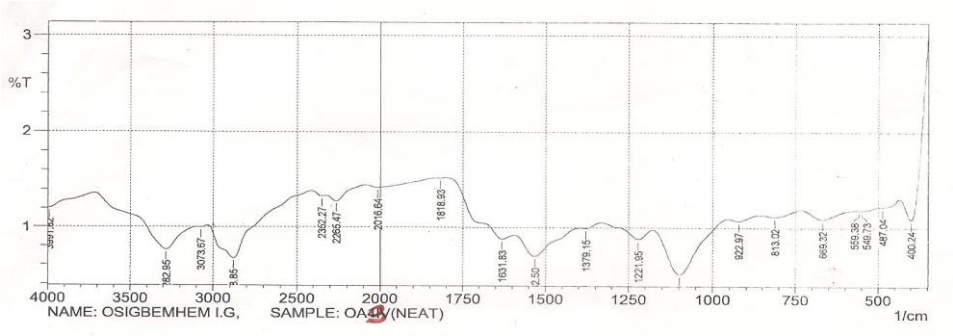
Siji, V.L.; Sudarsanakumar, and Suma, S. (2011): Synthesis, Spectroscopic Characterization, and Antimicrobial Activity of Cobalt (II) Complexes of Acetone-N(4) Phenylsemicarbarzone; Crystal Structure of $[Co(HL)_2 (MeOH)_2] (NO_3)_2$. *Transition metal Chemistry,* 4(36): 417-424.

Tolulope, F., Dueke-Eze, M., Cordella, U. & Nneoma, I. F. (2017). Synthesis, spectroscopic and antimicrobial properties of Co(II), Ni(II) and Cu(II) complexes of)E)-N-(2-hydroxy-5nitrobenzylidene) isonicotinohydrazide. Journal of Applied Environmental Management, 21, 1, pp. 120-125.

Tor-Anyiin TA, Igoli JO, Anyam JN. Studies on Dacryodes edulis III: Isolation and characterization of Gallic acid from methanolic extract of raw (Untreated) seed of Dacryodes edulis and its antimicrobial properties. Journal of Chemical Society of Nigeria. 2016;40(1):6-9.

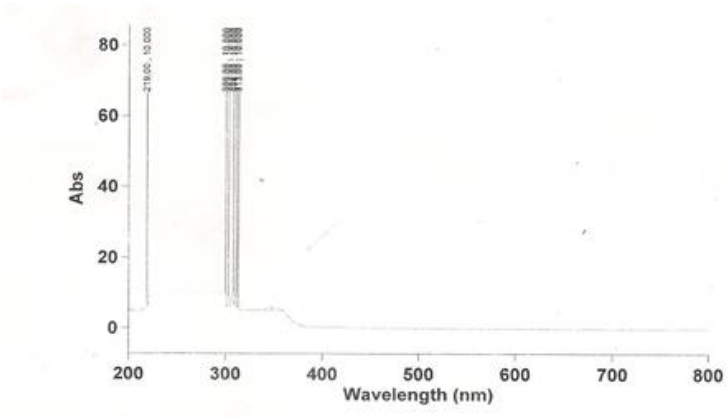
Appendix I

IR SPECTRA: 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



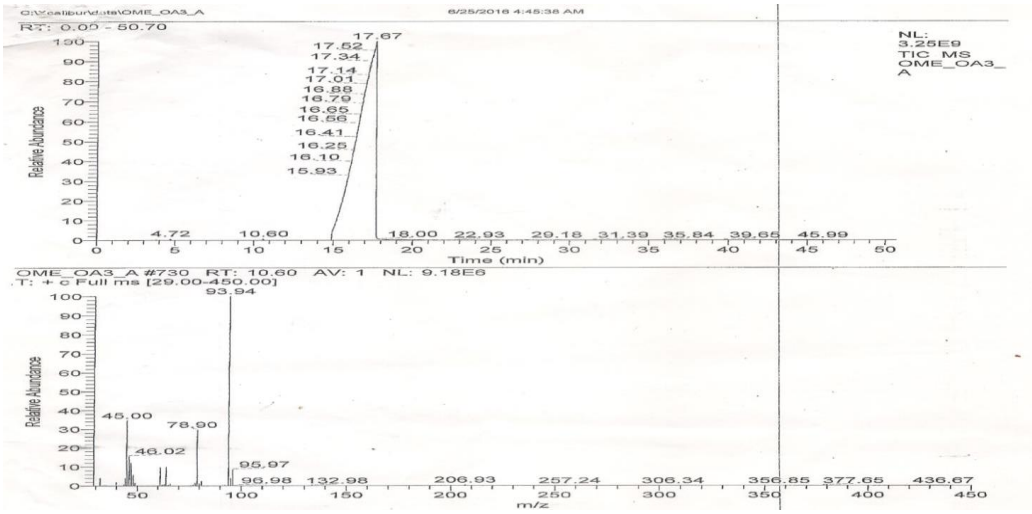
Appendix 2

UV SPECTRA: 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



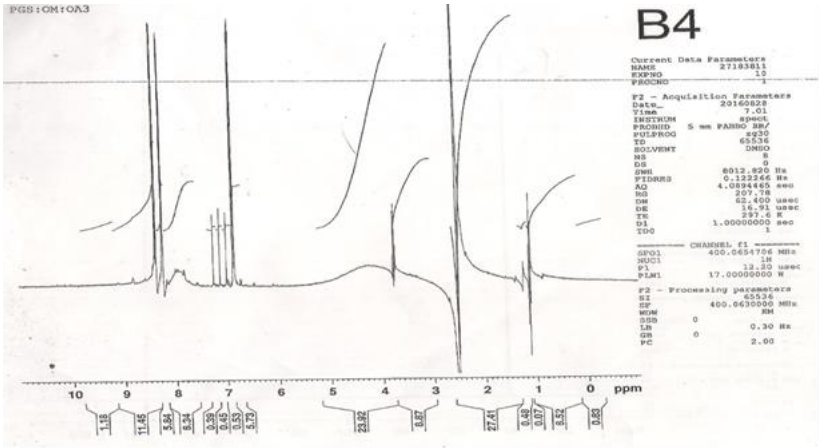
Appendix 3

GC-MS SPECTRA: 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



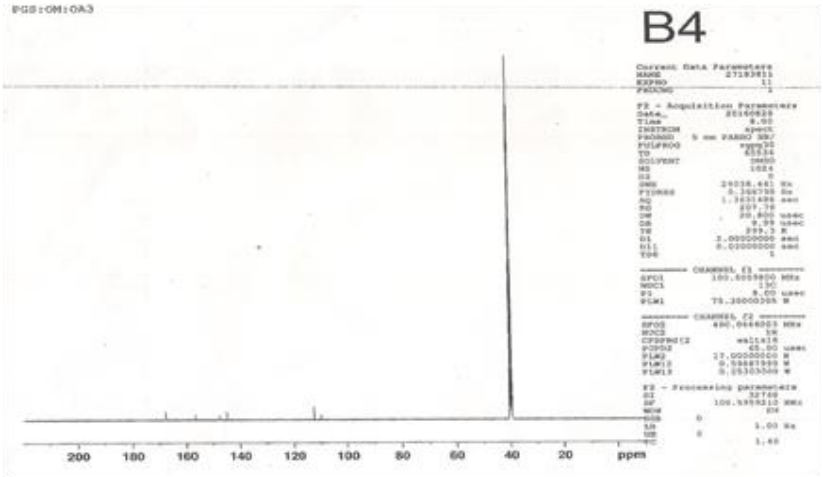
Appendix 4

^{1}H-NMR SPECTRA: 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



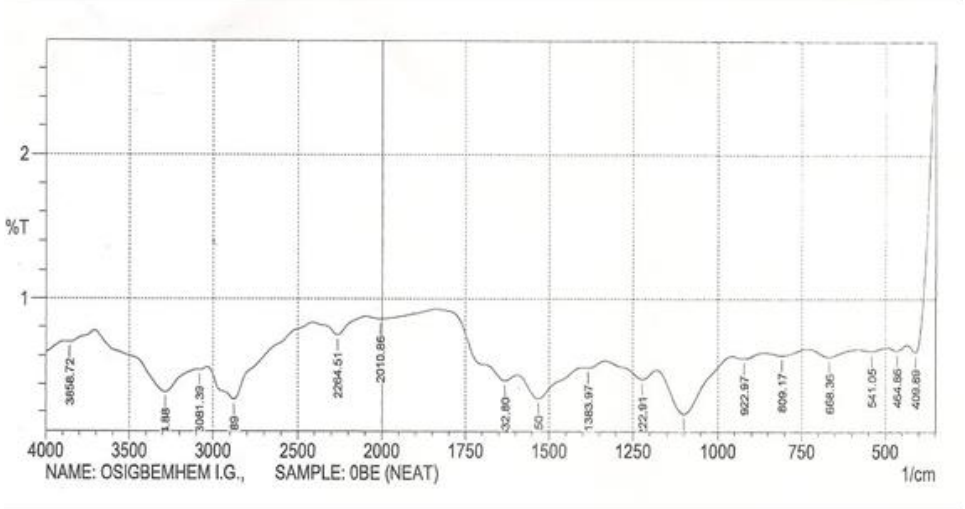
Appendix 5

^{1}C-NMR SPECTRA: 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



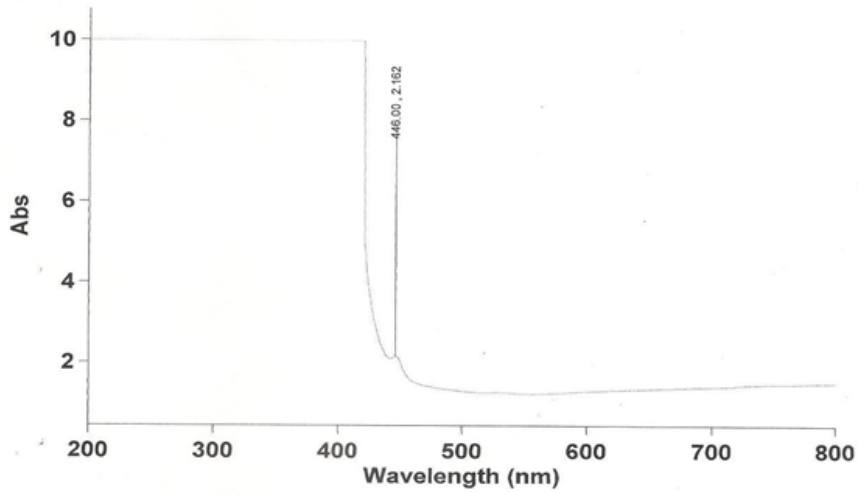
Appendix 6

IR SPECTRA: Cobalt (II) 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



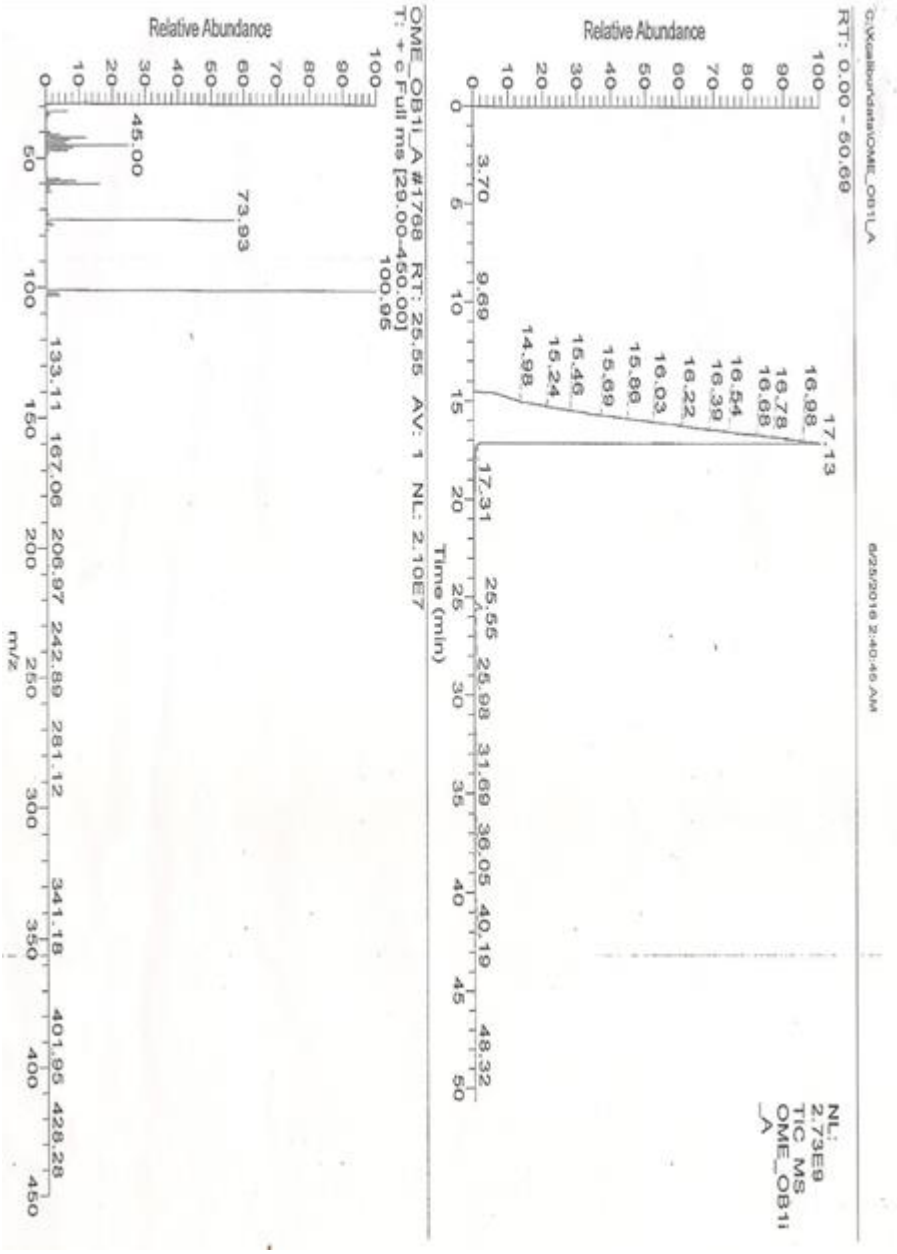
Appendix 7

UV SPECTRA: Cobalt (II) 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



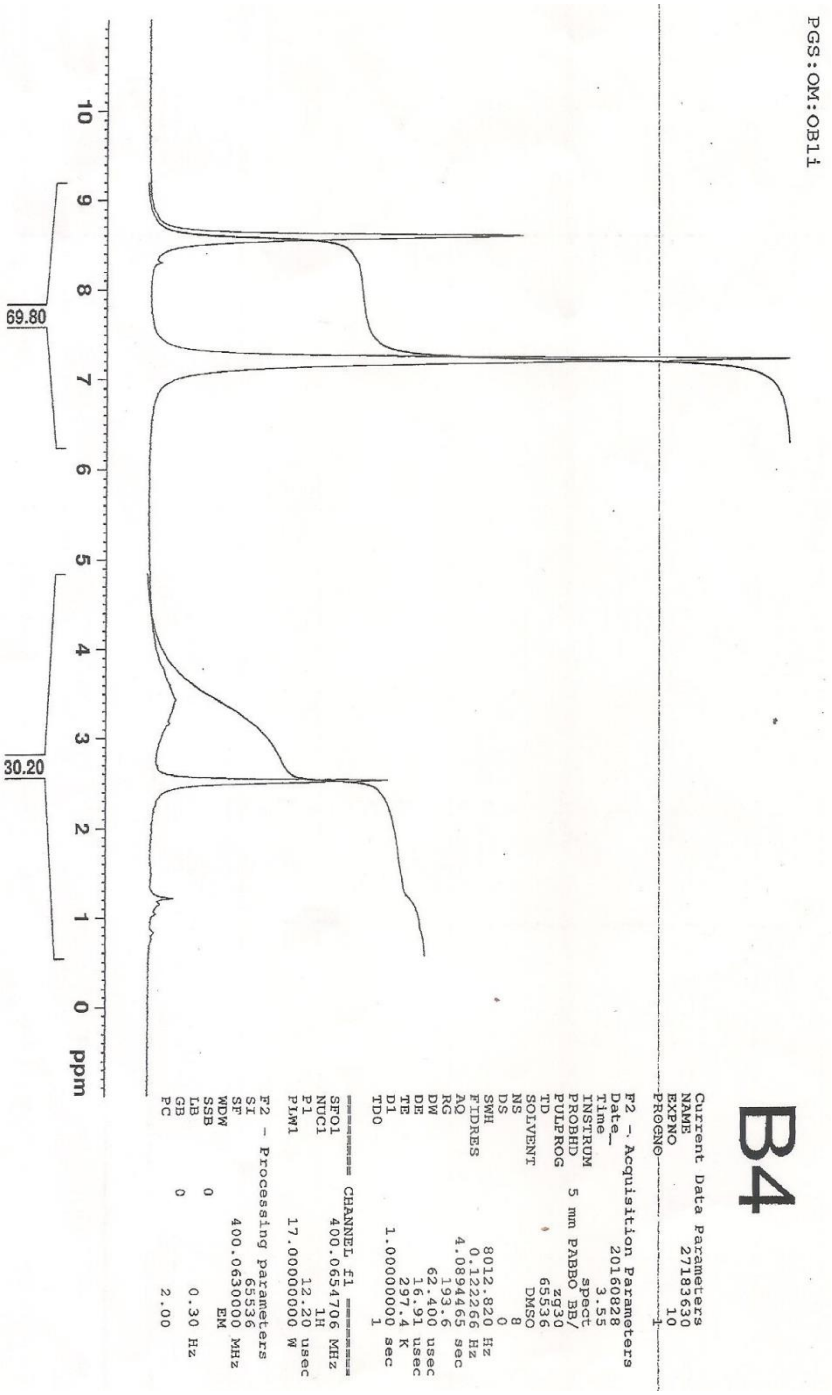
Appendix 8

GC-MS SPECTRA: Cobalt (II) 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



Appendix 9

^{1}H-NMR SPECTRA: Cobalt (II) 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid



Appendix 10

^{13}C-NMR SPECTRA: Cobalt (II) 2-{[(2-hydroxyphenyl)methylidene]amino} nicotinic acid