FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 5 No. 2, June, 2021, pp 477-488
DOI: https://doi.org/10.33003/fjs-2021-0502-653

SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION AND BIOLOGICAL STUDIES OF 2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid AND ITS Iron (II) COMPLEXES

[1]Osigbemhe, I. G., *[2]Khan, M. E., [1]Amos, O. and [2]Kabiru, H. D.

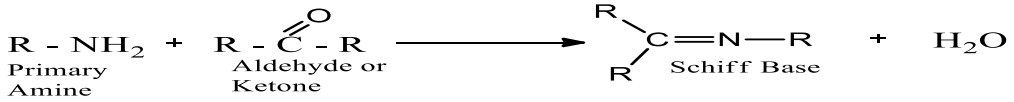
[1]Department of Industrial Chemistry, Federal University Lokoja, Kogi State, Nigeria,
[2]Department of Chemistry, Federal University Lokoja, Kogi State, Nigeria,
*corresponding author`s e-mails; khandora2000@gmail.com, muluh.khan@fulokoja.edu.ng

ABSTRACT
Iron (II) complexes of 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid derived from o-phenylenediamine and 5- methoxysalicaldehyde were synthesized and characterized by elemental analysis, using UV-Visible, IR, ^{1}HNMR, ^{13}CNMR, They were screened against known disease causative microbes to establish their effectiveness and efficacies as antimicrobial agents compared with national standards drugs, ampiclox and ketoconazole. Results showed that, the Schiff base exhibits antimicrobial action against all the bacteria and most of the fungi with exception of *Candidas. albicans* which exhibited naught diameter zone of inhibition. It was also found that the synthesized Schiff base exhibited two digits purity range, implying that it is relatively stable. The metal complex was found to be more susceptible in overall biological activity than the Schiff base synthesized and studied due to their structural stability in relationship to its activity. This has opened another drug window for the remedy to human diseases caused by these microbes

Keywords: Synthesis, characterization, Schiff base, iron complex, antimicrobial activity.

INTRODUCTION

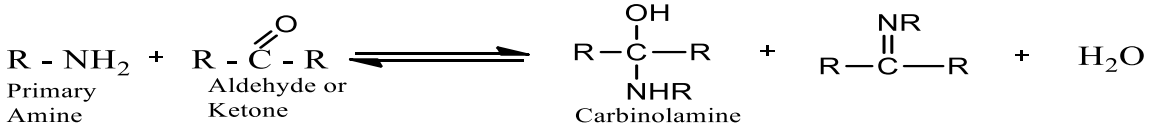
Schiff bases are nitrogen analog of an aldehyde or ketone in which the C=O group is replaced by a C=N-R group. Usually formed by the condensation of a carbonyl compound with a primary amine; Scheme 1 (Loudon, 2002), **Scheme 1:**



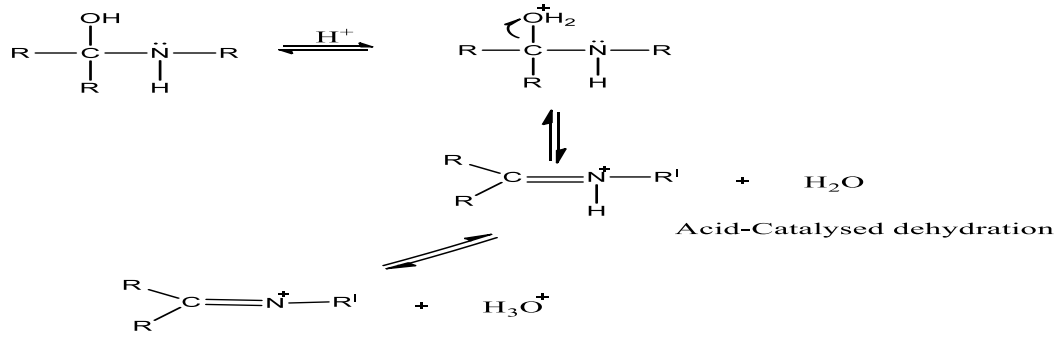
where R may be an alkyl / aryl group.

The stability of Schiff Bases depends on the substituents (Gupta and Sutar, 2008 &Cozzi, 2004) and as reported by Siji *et al*., (2011), the formation of a Schiff base from ldehydes or ketones is a reversible reaction and generally takes place under acid or base catalysis or upon heating.

Scheme 2:



It is driven to the completion by separation of the product or removal of water or both. The carbinolamine loses water by either acid or base catalyzed pathways. Since carbinolamine is an alcohol, it undergoes acid catalyzed dehydration;**Scheme 3:**



Dehydration of carbinolamine proceeds in two steps through an anionic intermediate and the Schiff base formation is a sequence of two types of reactions, addition followed by elimination (Sharma *et al.,* 2002).

Certain metallo-elements without which the normal functioning of the living organism is inconceivable include; Na, Mg, K and Ca and amongst the transition elements include; V, Cr, Mn, Fe, Co, Ni, Cu and Zn. These elements are present in trace and ultra-trace quantities and play a vital role at the molecular level in a living organism and form Schiff base complexes.

Mmetal-Schiff base complexes have been known since the mid-nineteenth century (Hitoshi *et al*., 1997) and even before the general preparation of Schiff bases ligands themselves (Kumar *et al.,* 2009). They have occupied a central place in the development of coordination chemistry, (Eman *et al.*, 2008).

Lots of Schiff Bases have been prepared, (Gajendar *et al.*, 2010, Kotz *et al.*, 2009), but there was no comprehensive, systematic study until the preparative work of Pfeiffer and associates (Pfeiffer *et al.*, 1931, Pfeiffer *et al*., 1942) who reported a series of complexes derived from Schiff bases of salicylaldehyde and its substitutional products.

According to Oviawe and Elemikhe, (2012) Schiff bases appear to be important intermediates in several enzymatic reaction involving the intereactions of the amino group of an enzyme, usually that of a lysine residue, with a carbonyl group of the substrate (Mutagh, 2007). The biological role of metal ions study has a long history in chemistry and medicine, in pharmacology and itoxicology, but it`s only recently that the extent and variety of metal ion involvement have been appreciated, (Eman *et al*, 2008).

Their chemistry is essentially that of the complexed ion, irrespective of whether more polar ions such as Na^+ or K^+ or more covalent species such as Au (III) or Pt (II) are being considered. Properties such as the effective size and solubility of a metal ion *in-vivo* are a function of ligand and solvent present as well as the metal ions themselves and 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 amounts of metal ions (Licker, 2004).

The chelating properties of antibiotics may be used in metal transport across the membrane or to attach the antibiotics to a passive/specific site from which it can interfere with the growth of bacteria (Eswaran *et al.*, 2009). Their activity appears to results from their ability to chelate metal, (Sharma, 2002).Metal chelates during chemical synthesis can be varied in size, charge distribution, stereochemistry redox potentials and other physical properties, (Vogel *et al*., 2008).

Taking cognizance of the above, it`s worthwhile to synthesize the Schiff base and study its biological behavior via coordination to metal ions with the expectation that these studies may result in achieving new targets in synthesizing/ designing metal-based compounds that could fight more aggressively against such bacterial/fungal strains, which becomes resistant to certain presently available and commonly used antimicrobial agents; Thus, this study deals with the synthesis and biological evaluation (antibacterial and antifungal activity) of Iron (II) complexes of the above Schiff base derived from aromatic/hetero-aromatic carboxyaldehyde and (un)-substituted heteroaromatic amines. This Schiff base had not been investigated further, particularly in combination with the above choice metal.

MATERIALS AND METHOD

Reagents: 2-aminonicotinic acid (2-aminopyridine-3-carboxylic acid); Salicylaldehyde ; 5-bromosalicylaldehyde (5-bromo-2-hydroxybenzaldehyde); 5-nitrosalicylaldehyde (2-hydroxy-5- nitrobenzaldehyde); 5-methoxysalicylaldehyde (2-hydroxy-5-methoxybenzaldehyde); 2-amino-1,3,4-thiodiazole; Furfuraldehyde; Thiophene-2-carboxaldehyde; Ethanol; Methanol; Nutrient agar.All the reagents were of analytical grade from Sigma-Aldrich, Merck, Germany and were used without further purification.

Organisms

Bacteria: *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aeruginosa,* and *Proteus mirabilis.*

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 4^0C in slants of Nutrient Agar and Sabourand Dextrose Agar (SDA) slants for bacteria and fungi respectively.

Equipment/Apparatus

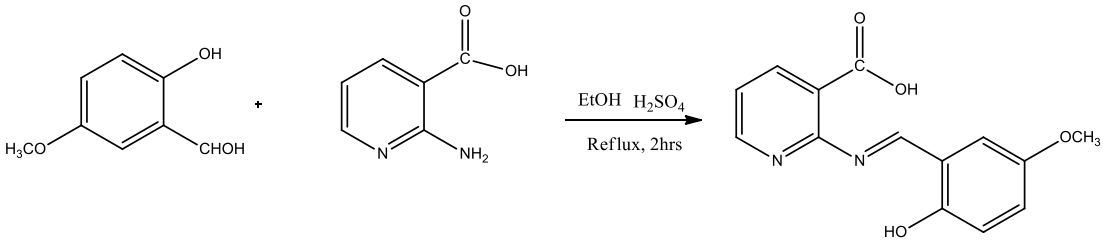
Gas chromatography, Mass spectrometry (GCMS); Thermal Scientific DSQ II Focus Instrument model; Fourier Transform Nuclear Magnetic Resonance Spectrometer (FTNMR) – Bruker 400MH_z machine; Ultra-violet spectra were recorded on a Hitachi U-2000 double beam spectrophotometer; Infra-red spectra (KBr Discs) were recorded on a Hitachi Model 200-50 IR spectrophotometre.

Melting points were taken on a Gellenkamp apparatus and are uncorrected. All instrumental determinations were carried out in Durham University, Chemistry Department, United Kingdom.

Syntheses of Schiff Base, 2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid

Equimolar portions of 2-aminonicotinic acid (0.01mol) with 5-methoxysalicylaldehyde (0.01mol) were mixed together in

ethanol (30-40mL) containing a few drop 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 and filtered hot by suction filtration. The product of reaction was allowed to crystallize from filtrate left at room temperature of 25^0C over two days. The crystals formed were recrystallized hot in ethanol and dried in a desiccator over $CaCl_2$ vacuum and the yield was calculated.



Synthesis of Iron Complex of 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid

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

Antimicrobial Assay

The synthesized compound (Schiff base/complex) was assayed for their antimicrobial activity using the disc diffusion technique by Kirby-Bauer (Isu and Onyeagba, 1998). Whatman filter paper (No. 1) was cut into sizes of 6mm diameter with office perforator and sterilized at 105^0C for 1 hour. The sterile discs were impregnated with 20uL of 100mg/mL of the synthesized Schiff base or complex and dried in oven at 60^0C for about 15-30mins. Mueller Hinton Agar plates were seeded with standardized broth culture of test organisms containing 100cfu/mL equivalent to 0.5 Mcfarland standards (NCCLS) and the prepared discs containing 2mg of the compound were placed on the plates. They were then incubated at 37^0C for 24 hours and observed for clear zones diameters of inhibition against the organisms. The zones diameters were measured with a transparent ruler and the result recorded in millimeters (mm). The assay was done in triplicates. Sterilized disc were soaked in 100% DMSO as negative and 2mg/mL of Ampicillin-Cloxacillin (Ampiclox) for bacterial isolates and ketoconazole for fungi as positive control.

Preparation of Inoculum

A loopful of the test organism was taken from their respective agar slants and subcultured into test-tubes containing Mueller Hinton broth for bacteria and Saboraud Dextrose Liquid for fungi. The test-tubes were incubated for 18hours at 37^0C for bacteria and for 48 hours at 30^0C for fungi. The obtained microorganisms in the broth were standardized using normal saline to obtain a population density of 100cfu/mL for the bacteria. For the fungi, fungal spores were harvested after visible notice of growth and suspension was standardized.

Preparation of the Media

38 g of Mueller Hinton Agar, 52g of SDA were weighed independently into different conical flasks; 1000mL of distilled water was added and capped with cotton wool. The media were boiled to dissolution and then sterilized at 121^0C at 15mins.The media were then allowed to cool to 45^0C and 20mL of the sterilized medium was poured into sterile Petri-dishes and allowed to cool and solidify. The plates were labeled 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 37^0C for 30mins respectively.

Minimum Inhibitory Concentration – Broth Dilution Method

The minimum inhibitory concentration of the compound was carried out using the macro broth dilution technique (Boron and Fingold, 1990). 9mL of each broth was dispersed into separate Test-tubes and was sterilized at 121^0C for 15mins 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 inoculums (0.1mL) of the microbes were inoculated into the different concentrations of the compound in the broth. The test tubes of the broth were incubated at 37^0C for 24 hours and 30^0C 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.

Minimum Bactericidal/Fungicidal Concentration – Macro Broth Dilution Method: Fresh Muller Hinton Agar media were prepared, sterilized at 121^0C for 15mins and waere poured into sterile petri-dishes and left to cool and solidify.

The contents of the MIC tubes (that is the tubes that showed no growth) were then sub-cultured onto the media and incubated at 37^0C for 24 hrs and 30^0C 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.

RESULTS AND DISCUSSION

Chemistry of the Schiff Base 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid

Its yield was found to be 65% as yellow powder; m.p. 136-137^{0}C; IR (KBr, Cm^{-1}): 2876.92 (OH, Carboxylic acid), 3282.95 (OH, Phenol), 1382.01 (C=O, carboxylic acid), 1635.65 (HC=N), 1531.53 (C=N, pyridine); the ^{1}H NMR (DMSO-d_6δ, ppm); 3.79 (S,3H,OCH_3), 7.16 (d, IH, d=7.83, 2.53Hz, phenyl C_4-H), 7.21 (dd, IH, j =7.80, 5.21Hz, phenyl C_4-H), 7.38 (dd, IH, j = 7.80, 5.21Hz, pyridine C_5-H), 7.86 (d, IH, j=2.52Hz, phenyl C_6-H), 8.31 (d, IH, d=7.80Hz, pyridine C_5-H), 8.72 (d, IH, j =5.21, phenyl C_6H), 8.66 (S,IH, CH=N), 10.22 (S,IH, OH), 11.32 (S,IH, COOH).

Iron (II) 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid

Its yield was found to be 70% as brownish crystal; m.p. (decomposed) 180-182^{0}C; IR (KBr, Cm^{-1}): 2876.92 (OH, Carboxylic acid), 3282.95 (OH, Phenol), 1382.01 (C=O, carboxylic acid), 1635.65 (HC=N), 1531.53 (C=N, pyridine); the ^{1}H NMR (DMSO-d_6δ, ppm); 3.79 (S,3H,OCH_3), 7.16 (d, IH, d=7.83, 2.53Hz, phenyl C_4-H), 7.21 (dd, IH, j =7.80, 5.21Hz, phenyl C_4-H), 7.38 (dd, IH, j = 7.80, 5.21Hz, pyridine C_5-H), 7.86 (d, IH, j=2.52Hz, phenyl C_6-H), 8.31 (d, IH, d=7.80Hz, pyridine C_5-H), 8.72 (d, IH, j =5.21, phenyl C_6H), 8.66 (S,IH, CH=N), 10.22 (S,IH, OH), 11.32 (S,IH, COOH), 565(M-N), 460 (M-O).

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) stretchings were observed in the 1382-1383cm^1 regions. The azomethine (HC=N) stretchings 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.

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

In the Schiff bases of 5-bromo, 5-nitro and 5-methoxysalicylaldehyde, the ^{1}H-NMR spectra exhibited the OH protons of the phenol at δ 10.21 – 10.45 and the carboxyl OH protons at δ 11.31 – 11.42 as three separate singlets. The azomethine (HC=N) protons of all the Schiff bases appeared as singlets at δ 8.66 – 8.93. The ^{1}H-NMR spectrum of 5-bromo, 5-nitro, and 5-methoxySalicylaldehyde displayed phenyl C_3-H as a doublet at δ 7.15 and δ 7.16, respectively. The phenyl C_6-H, experiencing a de-shielding effect due to the inductive effect of HC=N function, resonated as a doublet at δ 7.89 and δ 7.86 respectively. The phenyl C_4-H appeared as a double doublet at δ 7.25 and δ 7.21, respectively.

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

The ^{13}C-NMR spectra of 5-bromo-, 5-nitro- and 5-methoxy displayed 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.

The GC-MS fragmentation of 2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid

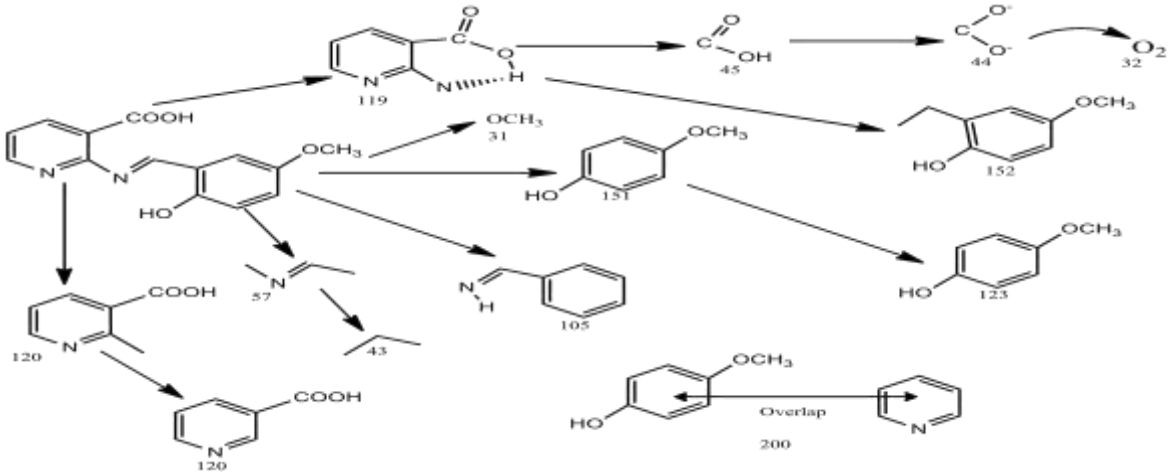


Figure 4: The GC-MS fragmentation of 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid

The GC-MS showed the mass ion at 272.3 and major fragment at 200, 152, 151, 123, 119, 120, 57, 45(base peaks) 43 and 43.

Biological Activities of Ligands and Its Metal Complexes

Preliminary Screening

Table 1: Result of *in vitro* antibacteria activities of schiff bases

Compounds	Dimeter zone of inhibition (mm)						
	B.	*E.*	*E.*	*K. pneumonia*	*P.*	*S.*	*P.*

	subtilis	*coli*	*Aerogenes*		*Aeruginosa*	*aureus*	*mirabilis*
2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino } nicotinic acid	15	20	13	10	9	15	0
AMPICLOX	19	0	0	0	17	19	0
DMSO	0	0	0	0	0	0	0

Table 2: Result of i*n vitro*anti-fungiactivities of Schiff Base

Compounds	Dimeter zone of inhibition (mm)		
	Aspergillus niger	*Candida albicans*	*Penicillium notatum*
2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid	0	0	0
AMPICLOX	0	0	0
KETONAZOLE	0	0	9
DMSO	0	0	0

Table 3: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC) (mg/mL) of Schiff Bases

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*	*MI C*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid	1.2	1.5	0.9	1.2	1.2	1.5	1.5	1.8	1.5	1.5	1.2	1.5	0	0

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	Dimeter zone of inhibition (mm)					
	A. niger		*C. albicans*		*P. notatum*	
	MIC	MBC	MIC	MBC	MIC	MBC
2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid	0	0	0	0	0	0

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

Biological Activity of Schiff Bases

In this study, the Schiff base and itscorresponding metal 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 24h after incubation at a constant temperature of 37ºC for bacteria and 30ºC at 2-5 days of incubation for fungi. Diameter zones of inhibition obtained indicate that the Schiff base was active against the bacteria and fungi isolates even better when compared with standard (Ampicillin-cloxacillin for bacteria and ketoconazole for fungal infections). Thus, the Schiff base 2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid was active against most of the test bacterial isolates better in comparison with standard drugs.

Biological Activity of Metal Complexes

Here, the antimicrobial activities of the metal complexes were also compared with those of the standard drugs Ampicilin-Cloxacillin(Ampiclox) and Ketoconazole. The overall result of diameter zones of inhibition obtained indicate that most metal complexes were active against the bacteria and fungi isolates comparably, more active than the standard drugs used.

This result was in correspondence with the findings of Fasina and Ogundele (2014) that reported the 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 the differences in the ribosome in the microbial cells (Sengupta *et al.,* 1998; Gajendra *et al.,* 2010).

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) for fungi isolates. The MIC of Schiff bases: Schiff base 2-{[(2-hydroxy-5-methoxyphenyl)methylidene]amino} nicotinic acid notably exhibited high but good MIC and MBC results, though fair MIC/MFC result on *P. notatum*.

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, we have been able to characterize the structural units for the compounds responsible for the activity. From the results on zone of inhibition it was established that most of Schiff bases and complexes had better activity than Ampicillin-cloxacillin for bacteria and ketoconazole for fungal infections. *Escherichia coli, Bacillus subtilis, Enterobacter aerogenes* and *P aeruginosa* were most susceptible at minimum concentration of 0.9mg/mL, 1.2mg/mL, 1.2mg/mL and 1.5mg/mL while *Candidas. Albicans* exhibited zero zones of inhibition.

REFERENCE

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.

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.

Hitoshi, T.; Tamoa, N.; Hideyyki, A.; Manabu, F. and Takayuki, M. (1997): Preparation and characterization of novel cyclic teranuclear manganese(III) complexes plyhedron 16:3787.

Kotz, J.C.; Treichel, P.M and Weaver , C.G. (2009): *Bonding and Molecular Structure*.Thomson Books/cole Belmont, California pp 457-466.

Kumar, S. Dhar, D.N. Saxena, P.N. (2009). *Journal of Science and res* 68, 181

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.

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

Pfeiffer, P.; Buchholz, E.; and Baver, O. (1931) *J. Parkl, Chem.,* 129, 163.

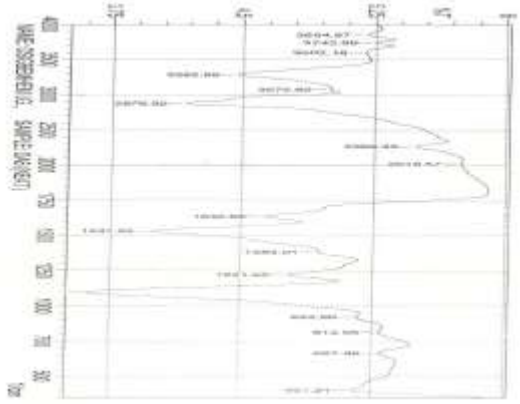
Pfeiffer, P.; Offerman, W.; and Werner, H. (1942), *J. Parkt, Chem.*, 159, 313.

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.

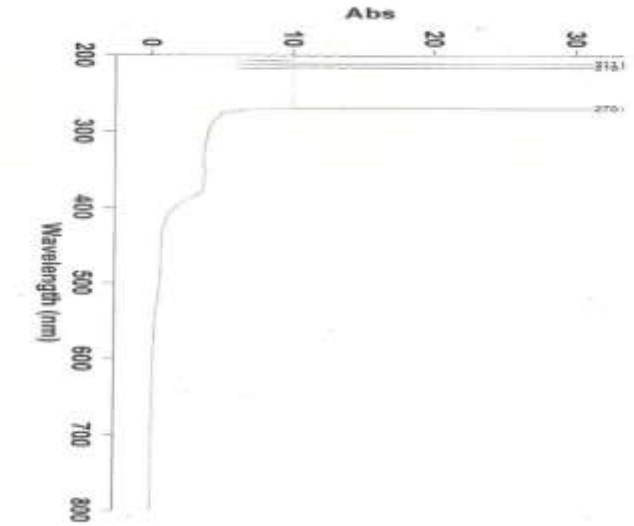
Sharma, K.; Mishra, R.; and Singh, R.V. (2002): Ashian *J.Chem.*, 14, 1453; chem Abstr., 2002, 137,361886t.

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.

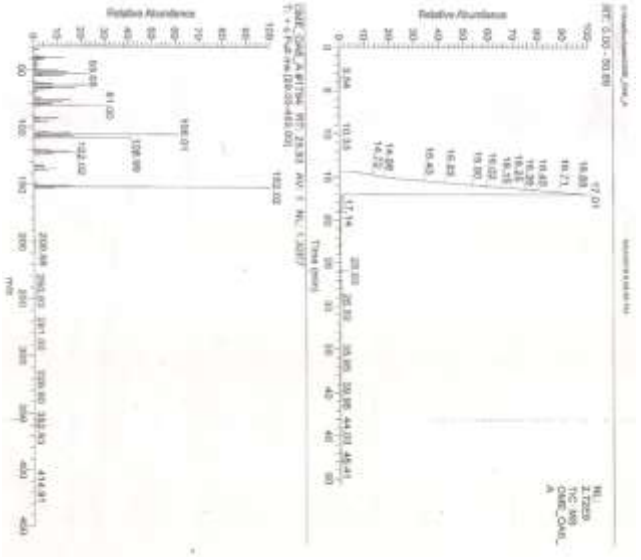
Appendix 1 (IR SPECTRA: 2-{[(2-hydroxy-5-methoxyphenyl)methylidene] amino} nicotinic acid)



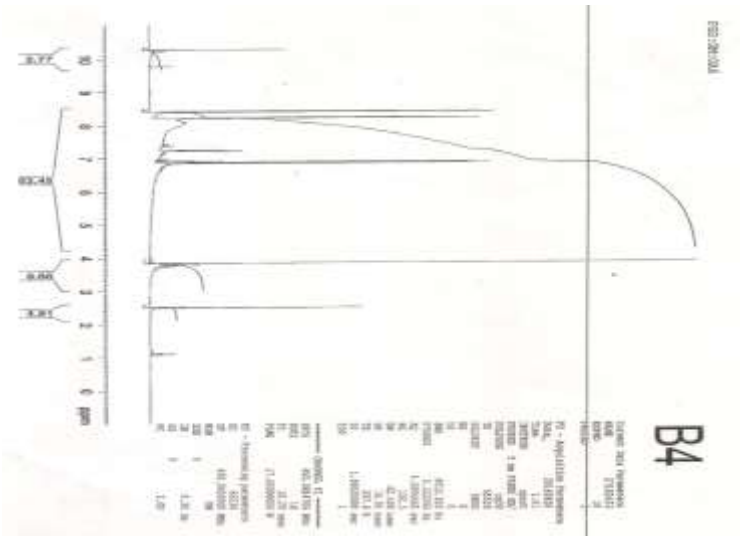
Appendix 2 (UV SPECTRA: 2-{[(2-hydroxy-5-methoxyphenyl)methylidene] amino} nicotinic acid)



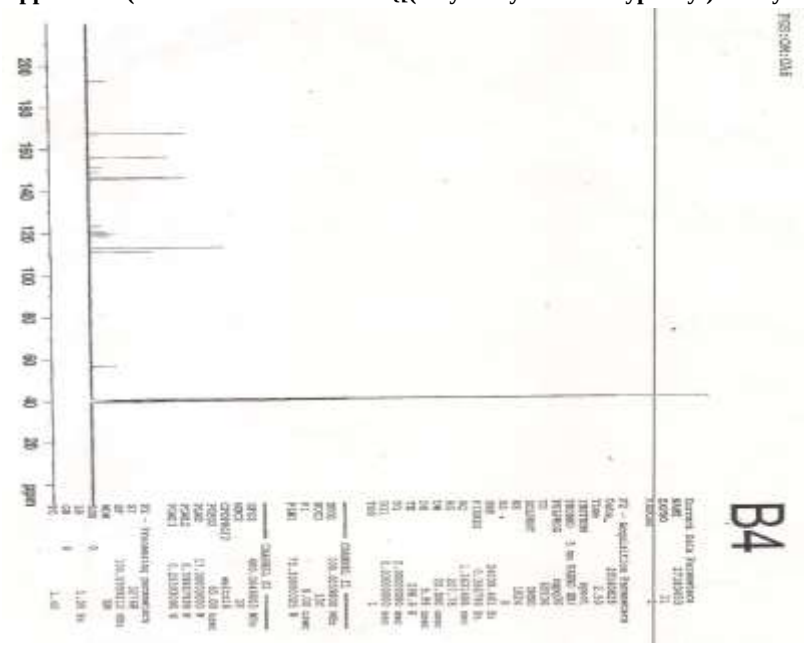
Appendix 3(GC-MS SPECTRA: 2-{[(2-hydroxy-5-methoxyphenyl)methylidene] amino} nicotinic acid)



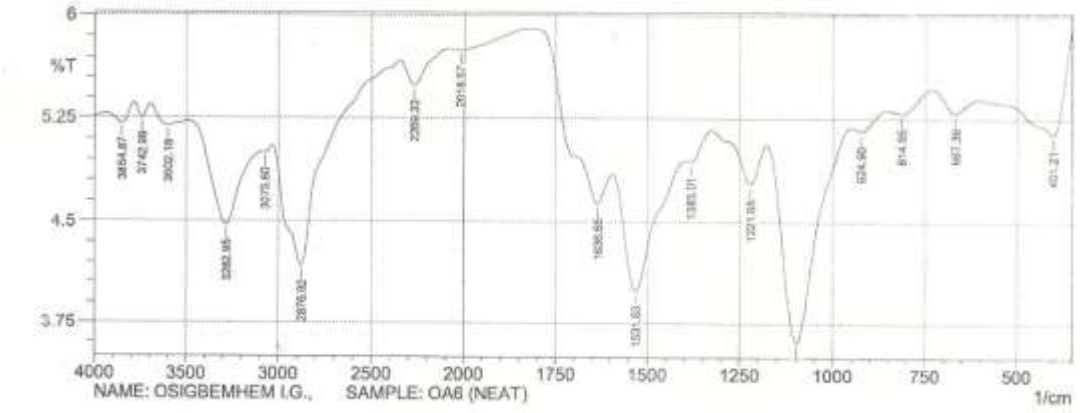
Appendix 4 (^{1}H-NMR SPECTRA: 2-{[(2-hydroxy-5-methoxyphenyl) methylidene] amino} nicotinic acid)



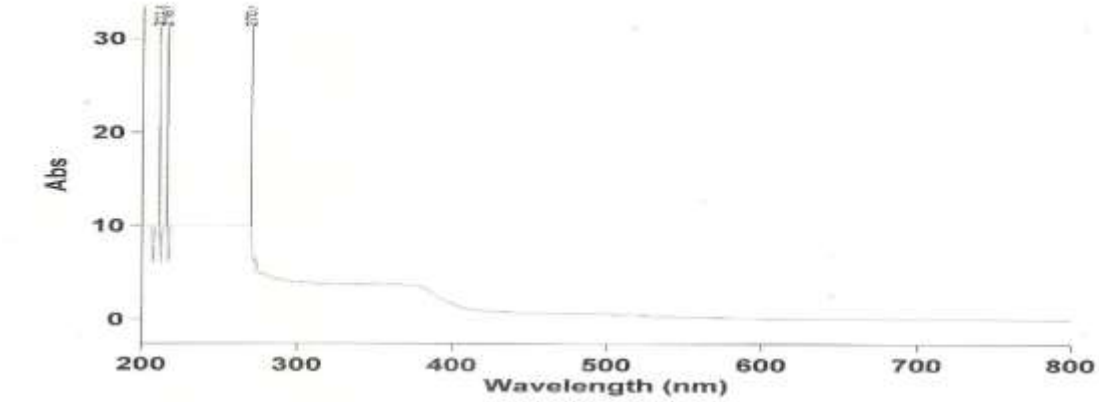
Appendix 5 (^{13}C-NMR SPECTRA: 2-{[(2-hydroxy-5-methoxyphenyl) methylidene] amino} nicotinic acid)



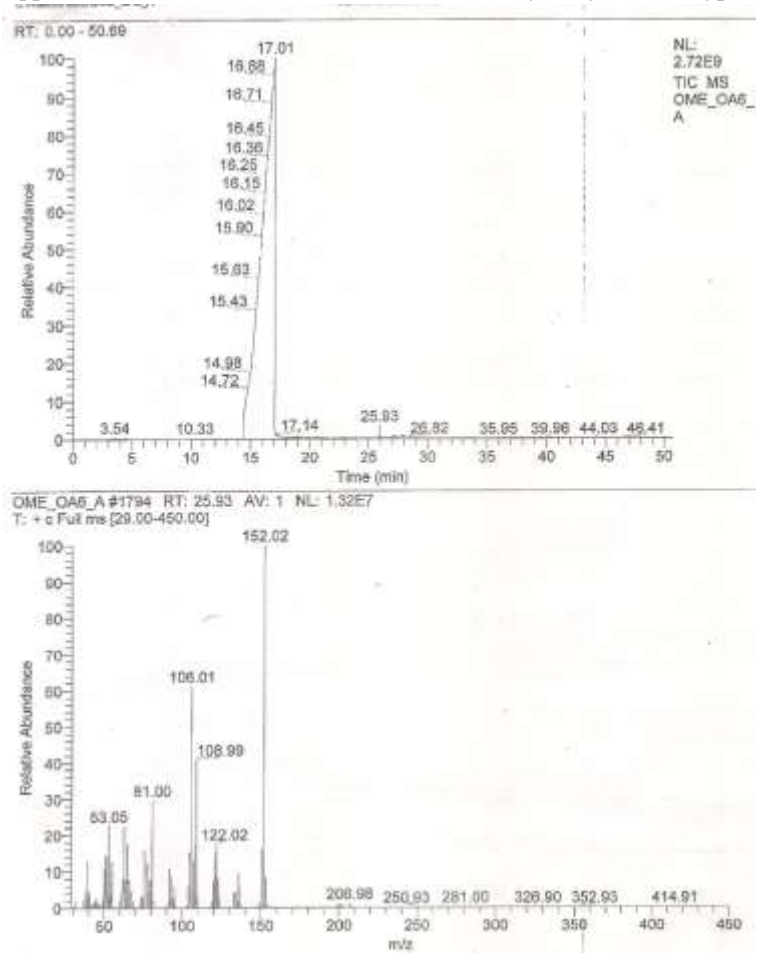
Appendix 6 (IR SPECTRA: Iron (II) 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid)



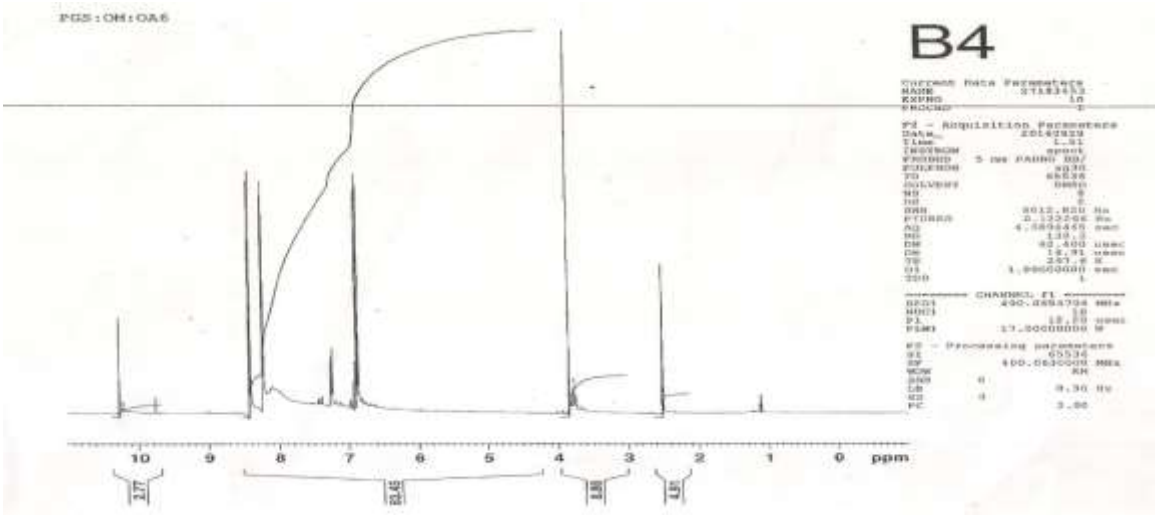
Appendix 7 (UV SPECTRA: Iron (II) 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid)



Appendix 8 (GC-MS SPECTRA: Iron (II) 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid)



Appendix 9 (^{1}H-NMR SPECTRA: Iron (II) 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid)



Appendix 10 (^{13}C-NMR SPECTRA: Iron (II) 2-{[(2-hydroxy-5-methoxyphenyl) methylidene]amino} nicotinic acid)