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Synthesis, Spectroscopic Characterization and Biological Studies Of 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid and Iron (II) complexes

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Abstract *Iron (II) complexes of 2-{[(2-hydroxy-5-nitrophenyl) 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 their potentials antimicrobial activity with reference to some known drugs. 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 better than that of synthesized Schiff base.*

Key Words: *Synthesis, characterization, Schiff base, iron complex, antimicrobial activity*.

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1.0 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 It is usually formed by the condensation of an aldehyde or ketone with a primary amine (Loudon, 2002). Several Schiff bases have been synthesised, characterized and most of them have been found to displayed strong antimicrobial activities. For example, Chohan *et al*., (2002) synthesised nicotinic acid derived Schiff bases and their transition metal complexes including Co^{2+}, Ni^{2+} and Zn^{2+}. They observed that the Schiff bases acted as a deprotonated tridentate ligand for the complexation of the metal ions. Screening results for antibacterial activity indicated that they exhibited strong activity against some pathogenic strain of *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*. However, the activities of the metal-Schiff base complexes were better than that of the Schiff base itself. Naglah *et al*., (2015) used microwave radiation to synthesized some isatin Schiff bases that were linked to nicotinic acid through some amino acid bridge. The Schiff bases were characterized using FTIR, ^{1}H NMR and mass spectra data. Their elemental constituents and antimicrobial activities were also analysed. The synthesised Schiff bases were confirmed to showed strong antimicrobial inhibitory activities with MIC values ranging from 50 to 500 µg/mL. Although More *et al*., (2017) did not work on nicotinic acid-based Schiff bases, they were able to show that spectroscopic methods (UV, IR, NMR and thermal analysis are good methods for characterizing Schiff bases. The prepared Schiff bases exhibited strong antimicrobial activities against 5 ESBL (extended spectrum beta-lactamase) and 5 MBL (metallo beta-lactamase). According to Tolulope *et al*. (2017), the resistance of microorganism to classical antimicrobial compound possess 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 expand their pharmaceutical applications. Therefore, the aim of this study is to synthesise, characterize, and coordinate Schiff bases synthesized from the coupling of 2-{[(2-hydroxy-5-nitrophenyl) methyl-idene]amino} nicotinic acid and its iron (II) complexes.

The synthesis of some target Schiff bases and possibility of altering their biological activity via coordination to metal ions has been extensively studied (Afanas *et al,* 1989). This present work is an extension of such studies and is considering 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 hetero aromatic amines. A detailed literature survey revealed that the synthesis of the Schiff base reported in this research has not been widely reported.

2.0 Materials and Method

Reagents used for the study were 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 analytical grade and were supplied by Sigma-Aldrich, Merck, Germany.

2.1 Organisms

Bacterial isolate used in the work were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aeruginosa,* and *Proteus mirabilis, 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.

2.2 Equipment/apparatus

Equipment used in the study were Gas chromatography, Mass spectrometry (GCMS) (manufactured by Thermal Scientific DSQ II Focus Instrument Model). Fourier Transform Nuclear Magnetic Resonance Spectrometer (FTNMR) (manufactured by Bruker 400MH_z machine), Ultra-violet visible spectrophotometer (manufactured by Hitachi U-2000 double beam spectrophotometer); Infra-red spectrophotometer (manufactured by Hitachi Model 200-50 IR spectrophotometre). Melting points were taken on a Gellenkamp melting pointapparatus. All instrumental determinations were carried out in Durham University, Chemistry Department, United Kingdom.

2.3 Syntheses of Schiff base, 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid

Equimolar portion of 2-aminonicotinic acid (0.01mol) and 5-nitrosalicylaldehyde (0.01mol) were mixed together in ethanol (30-40 ml) containing few drops of concentrated H_2SO_4 at a pH of 3.5 to 4.5. The resultant mixture was heated under reflux for 2 hours and filtered while still hot using suction filtration. The product of reaction was allowed to crystallize from the filtrate which was maintained at 25 °C for two days. The crystals formed were recrystallized hot in ethanol and dried in a desiccator over $CaCl_2$ vacuum and the yield was calculated. The scheme for the synthesis is shown below,

2.3.1 Synthesis of iron complex of 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid

The metal complexes of iron (II), was prepared by the reaction of equimolar (0.01 mol) of each metal salt with a corresponding (0.01 mol) of the Schiff base ligand. The mixture was refluxed in ethanol for 2 hours. They were all filtered and washed with ethanol after which they were allowed to stand for 24days.

2.4 Antimicrobial assay

The synthesised compounds (the Schiff base/complex) were assayed for their antimicrobial activities using the disc diffusion technique (Emma *et al.,* 2008). The Whatman filter paper (No. 1) was cut into sizes of 6 mm diameter with office perforator and sterilized at 105 ^{0}C for 1 hour. The

sterile discs were impregnated with 20 uL of 100mg/mL of the synthesized Schiff base or complex and dried in the oven at 60 ^{0}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 2mg of the compound were placed on the plates. They were then incubated at 37°C 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 results were recorded in millimeters (mm). The assay was done in duplicates. Sterilized discs were soaked in 100% DMSO as negative and 2 mg/mL of ampicillin-cloxacillin (ampiclox) for bacterial isolates and ketoconazole for fungi as positive control.

Scheme 2: Proposed synthetic route

2.4.1 Preparation of inoculum

A loopful of the test organisms was taken from their respective agar slants and subculture into test-tubes containing Mueller Hinton broth for bacteria and Saborauddextrose liquid for fungi. The test-tubes were incubated for 18hours at 37 °C for bacteria and for 48 hours at 30 °C for the fungi. The microorganisms in the broth were standardized using normal saline to obtain a population density of 100cfu/mL for the bacteria. However, for the fungi, fungal spores were harvested after visible notice of growth and suspension was standardized.

2.4.2 Preparation of the media

38 g of Mueller Hinton Agar, 52g of SDA were weighed independently into different conical flask. 100 mL of distilled water was added and capped with a cotton wool. The media were boiled to dissolution and then sterilized at 121 °C at 15 minutes. The media were allowed to cool to 45 °C and 20 mL of the sterilized medium was poured into sterile petri-dishes and allowed to solidify upon cooling. 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 °C for 30 minutes.

2.4.3 Minimum inhibitory concentration (MIC) – 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 °C for 15 minutes and 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 the different concentrations of the compound in the broth. The test tubes of the broth were incubated at 37 °C for 24 hours and at 30 °C for 1-7 days for bacteria and fungi respectively. They were observed for the occurrence of turbidity. The lowest concentration which showed turbidity in the test tube was recorded as the MIC.

2.4.4 Minimum bactericidal/fungicidal concentration – Macro Broth dilution method

Fresh Muller Hinton agar media were prepared, sterilized at 121 °C for 15 minutes and was 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 for 1-3 days at 37 °C for 24 hours and at 30 °C for bacteria and fungi respectively. It was then observed for colony growth. The MBC/MFC was the plate with the lowest concentration of extract without colony growth.

3.0 Results and Discussion

4.1 *The Schiff base, 2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino} nicotinic acid* obtained after synthesis was found to be 68% of the yield and had a yellow colour. The melting range was 140-141 °C. The IR spectrum revealed (KBr, Cm^{-1}): 1741.82 (OH, carboxylic acid) 3285.85 (OH,

phenol), 1382.04 (C=O, carboxylic acid), 1631.83(HC=N), 1529.5 (C=N, pyridine); the ^{1}H NMR (DMSO-d_6δ, ppm); 7.30 (d, IH, d=7.81H_3, phenyl C_3-H), 6.90 (dd, IH, d=7.82, 5.23H_3, pyridine C_5-H), 8.32 (d, IH, d = 7.82H_z, pyridine C_4-H), 8.41 (d, IH, d=7.81, 2.33H_z, phenyl C_6-H), 8.68 (d, IH, d=2.33H_3, phenyl C_6-H), 8.75 (d,1H,d=5.23$H_z$pyridine C_6-H), 8.93 (S,IH, CH=N), 10.45 (S,IH, OH), 11.42 (S,IH, COOH).

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

Its yield was 69% and appeared as reddish crystals. The melting point range (decomposed) was 189-191°C while the IR spectrum revealed the following frequencies and assignments: 1741.82 (OH, carboxylic acid) 3285.85 (OH, phenol), 1382.04 (C=O, carboxylic acid), 1631.83(HC=N), 1529.5 (C=N, pyridine); the ^{1}H NMR (DMSO-d_6δ, ppm); 7.30 (d, IH, d=7.81H_3, phenyl C_3-H), 6.90 (dd, IH, d=7.82, 5.23H_3, pyridine C_5-H), 8.32 (d, IH, d = 7.82H_z, pyridine C_4-H), 8.41 (d, IH, d=7.81, 2.33H_z, phenyl C_6-H), 8.68 (d, IH, d=2.33H_3, phenyl C_6-H), 8.75 (d,1H,d=5.23$H_z$pyridine C_6-H), 8.93 (S,IH, CH=N), 10.45 (S,IH, OH), 11.42 (S,IH, COOH), 530 (M-N), 455 (M-O).

4.3 IR spectrum of Schiff base due to 2-aminonicotinic acid and the salicylaldehyde

The IR spectra (Fig. 1) 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. 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 1610 cm^{-1} in all the synthesised structures. The spectrum of the iron(ii) complex is found in Appendix 1.

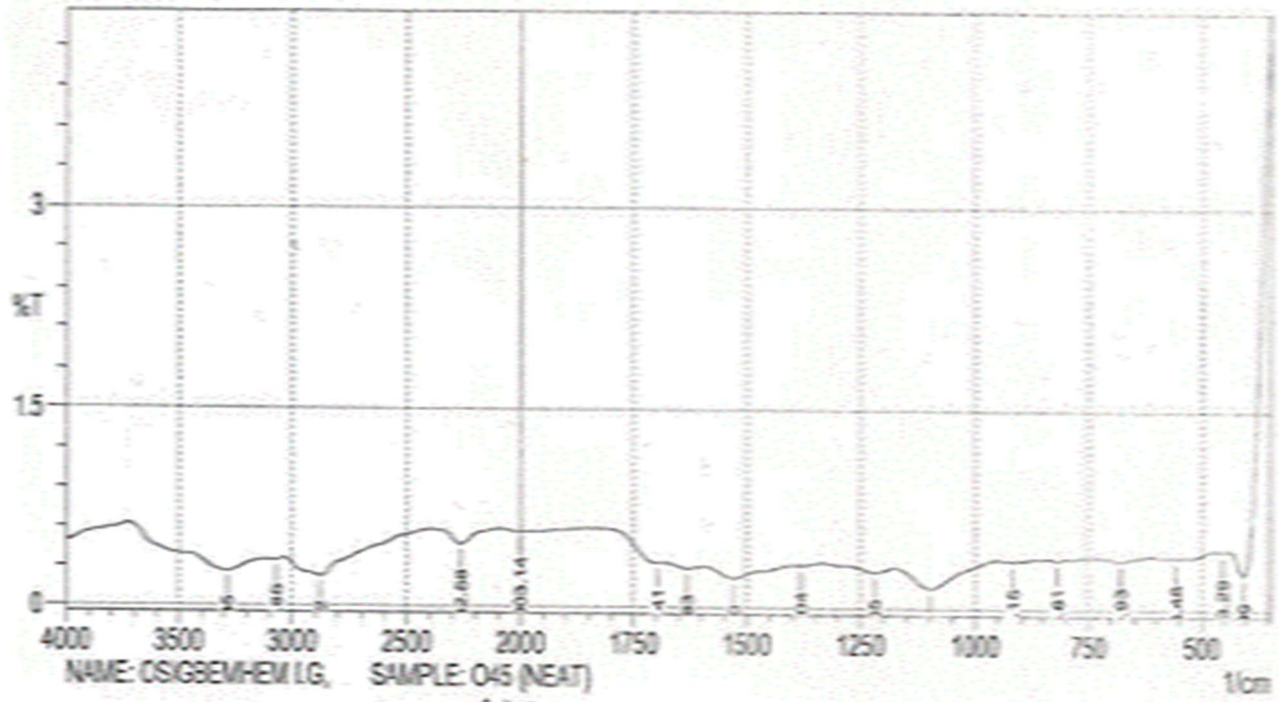

Fig. 1: FTIR spectrum of 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid

4.4 *^{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 is seen inductive effect of HC=N function and 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 (Fig; 2 and Appendix 2).

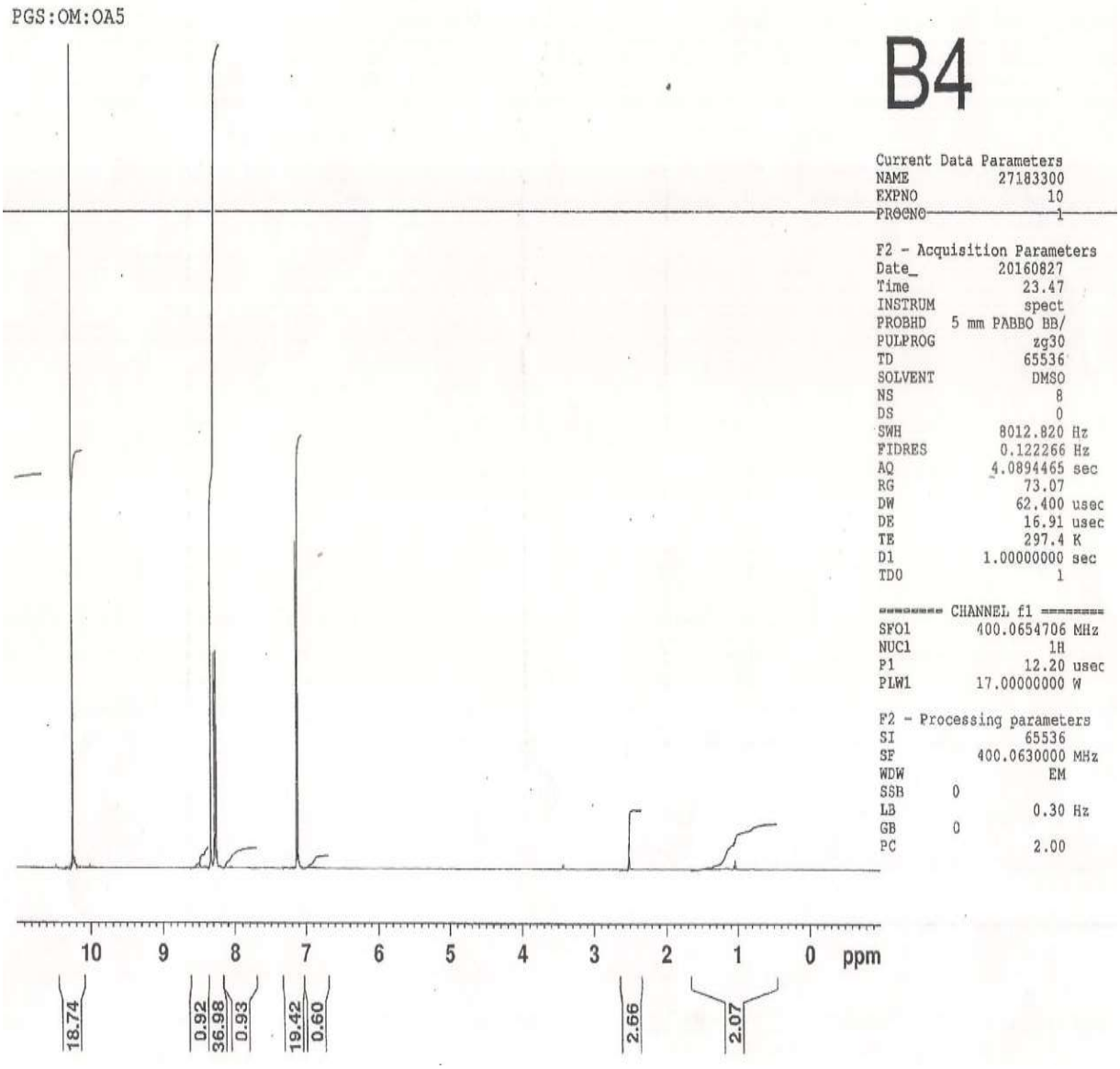

Fig.2: ^{1}H-NMR spectrum of 2-{[(2-hydroxy-5-nitrophenyl) methylidene] amino} nicotinic acid

4.5 ^{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 (Fig. 3). The carbonyl in carboxylic group experiencing a de-shielding effect occurred 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 spectra of the iron (ii) complex is found in Appendix 3.

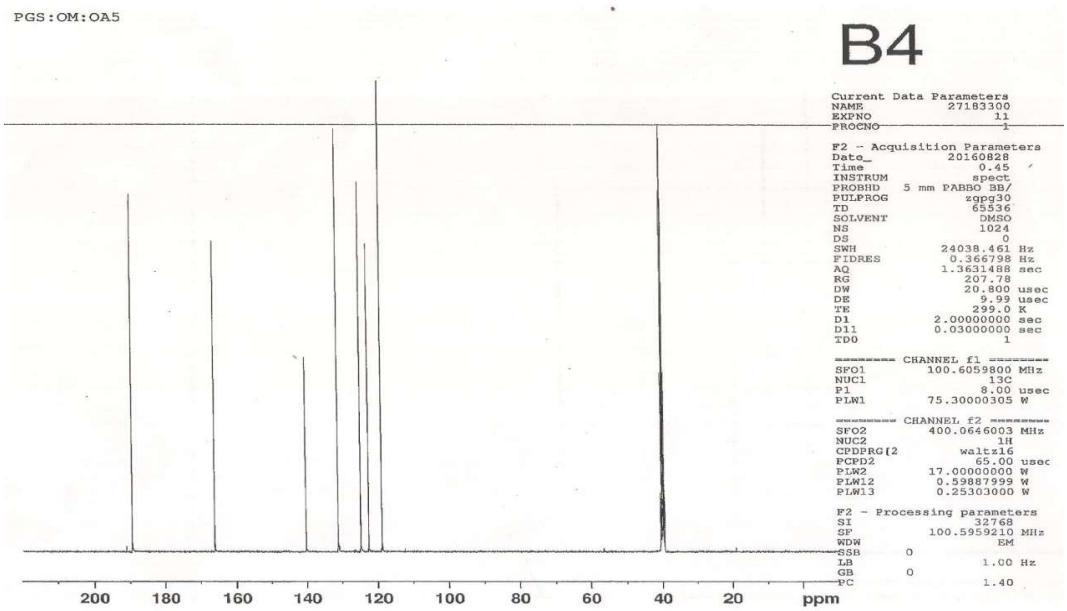

Fig.3: ^{13}C-NMR spectrum of 2-{[(2-hydroxy-5-nitrophenyl)methylidene] amino} nicotinic acid

4.6 GC-Ms fragmentation of2-{[(2-hydroxy-5-nitrophenyl)-methylidene]amino} nicotinic acid

The GC-MS showed the mass ion at 287.2 and major fragment at 167, 166, 139, 120, 105, 93, 79, 57(base peaks) 44, 43 (Fig. 4 and Appendix 4). The proposed scheme is given in Fig. 5

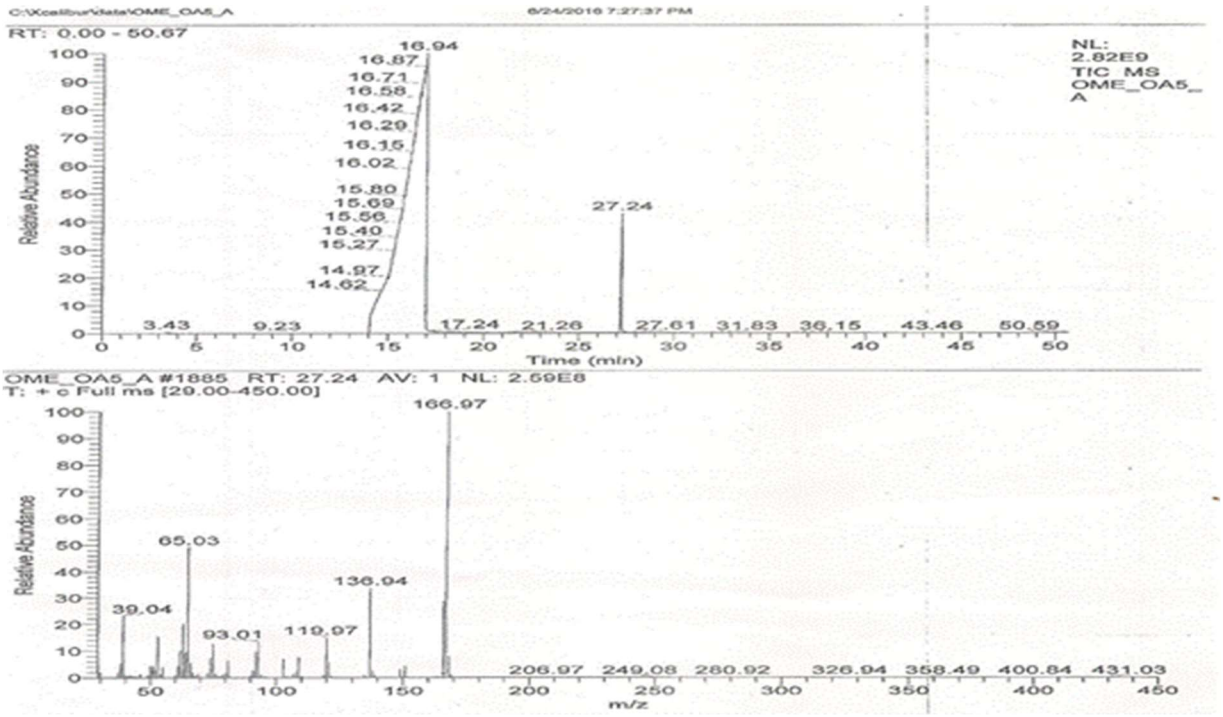

Fig. 4: GC-MS spectrum of 2-{[(2-hydroxy-5-nitrophenyl)methylidene] amino} nicotinic acid

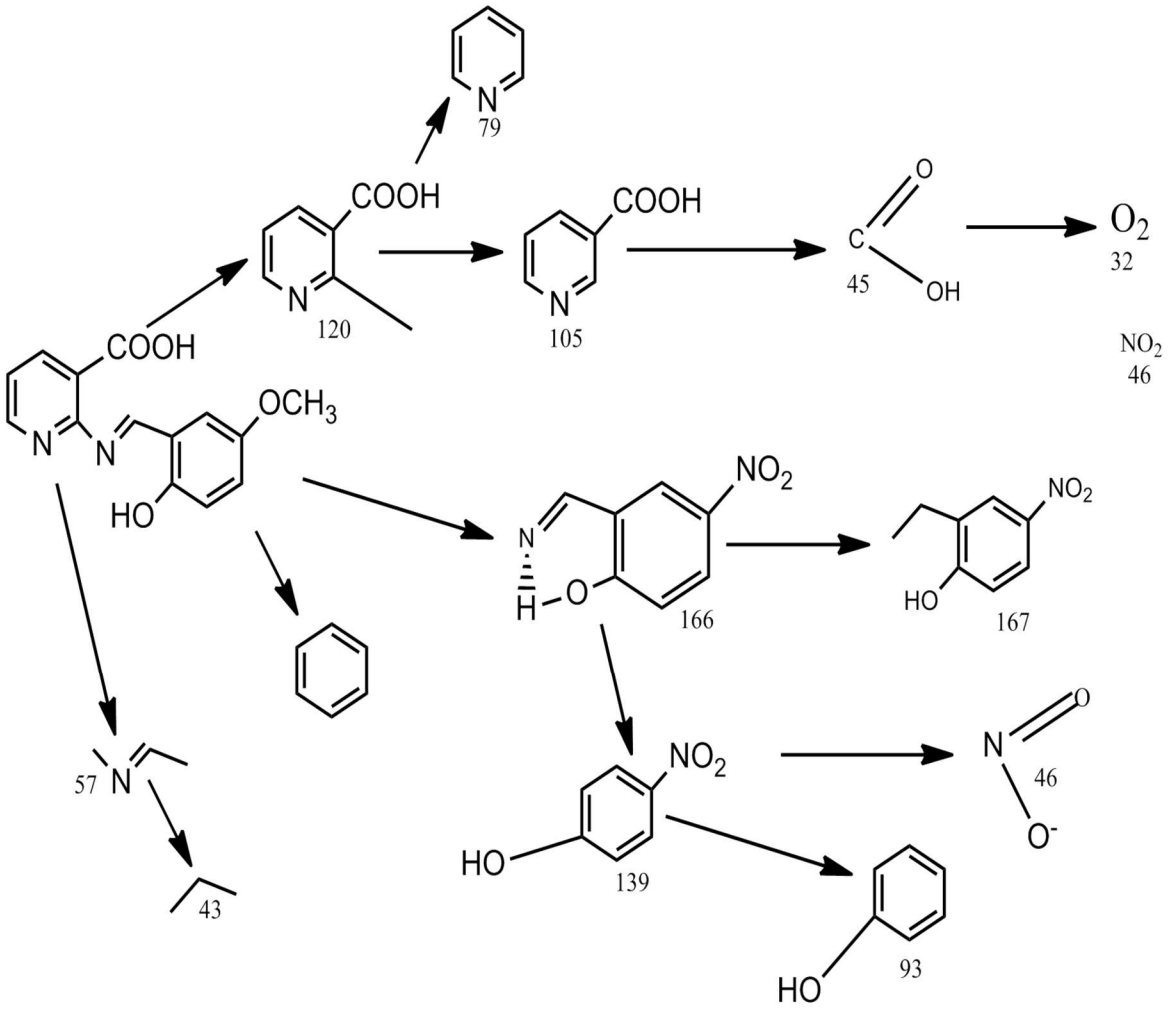

Fig. 5: The GC-Ms fragmentation of 2-{[(2-hydroxy-5-nitrophenyl)-methylidene]amino} nicotinic acid

4.7 Biological activities of the ligands and Its metal complexes

Results obtained from preliminary screening for antimicrobial action of 2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid, ampiclox and DMSO are recorded in Table 1.

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Table 1: *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-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid	24	24	25	14	12	30	12
Ampiclox	19	0	0	0	17	19	0
DMSO	0	0	0	0	0	0	0

Table 2: *In vitro*-anti-fungi activities of Schiff base

Compounds	Diameter zone of inhibition (mm)		
	Aspergillus niger	***Candida albicans***	***Penicillium notatum***
2-{[(2-hydroxy-5-nitrophenyl)methylidene]amino} nicotinic acid	29	0	35
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-nitrophenyl)methylidene] amino} nicotinic acid	1.2	1.5	0.9	1.2	1.2	1.5	1.5	1.8	1.5	1.8	1.5	1.8	1.5	1.8

*****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-nitrophenyl)methylidene] amino}nicotinic acid	**1.2**	**1.5**	**0**	**0**	**0.9**	**0.2**

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

The results of *in vitro* antimicrobial activities were presented in Tables 1-4. Diameter zone of inhibition were observed 24 hours after incubation at a constant temperature of 37 ºC for bacteria and 30 ºC at 2-5 days of incubation for fungi. The observed diameter zone of inhibition indicates that most of the Schiff bases were active against the bacteria and fungi isolates more than the standard (ampicillin-cloxacillin for bacteria and Ketoconazole for fungal infections). From the results of the antimicrobial activity screening, the Schiff base 2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino} nicotinic acid exhibited antimicrobial action against all bacterial and fungal isolates except for *C. albicans* which recorded zero diameter zone of inhibition. Bacterium isolate *S. aureus* and fungus *P. notatum* were most susceptible to it with record of 30- and 35-mm diameter zone of inhibition respectively.

In this work, antimicrobial activities of the metal complexes were also compared with those of the standard drugs ampicillin-cloxacillin (ampiclox)

and Ketoconazole. The overall results of diameter zone of inhibition obtained indicated that the metal complexes were more active against the bacteria and fungi isolates than the drugs used as standards. This result agrees with the findings of Fasina and Ogundele (2014), who 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 microbe's cell or differences in the ribosomes in the microbial cells (Gupta *et al.,* 2008; Gajendra *et al.,*2010).

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: Schiff base 2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino} nicotinic acid notably exhibited high and good MIC and MBC results, fairly MIC/MFC result on *P. notatum*.

4.0 Conclusion

The UV- Visible, IR, ^{1}HNMR, ^{13}CNMR and GCMS data of the synthesised Schiff bases and their metal complexes led to the proposed structure of the Schiff base and their complexes. The Schiff bases and their metal complexes have strong activity against tested microorganism 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 results, fairly MIC/MFC result on *P. notatum*.

5.0 Acknowledgement

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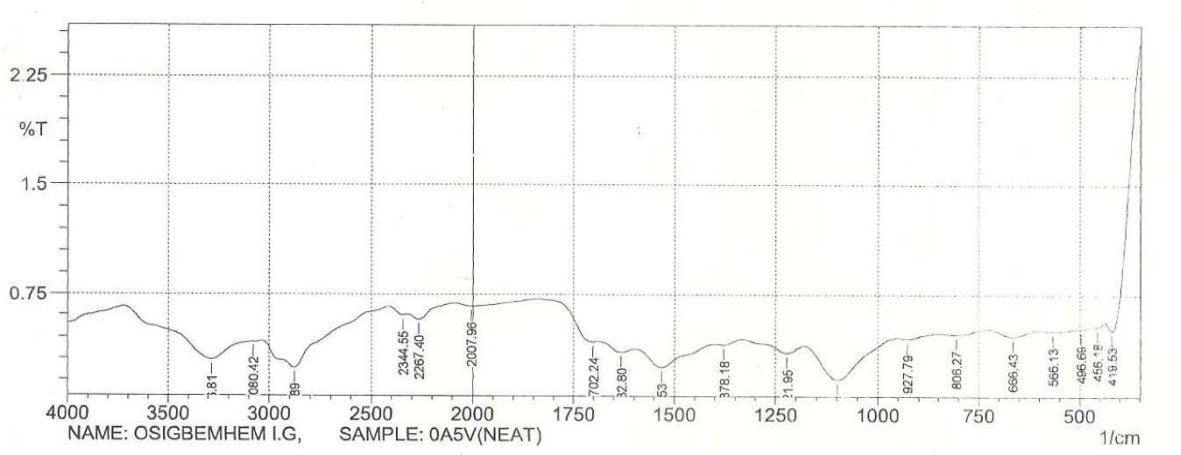
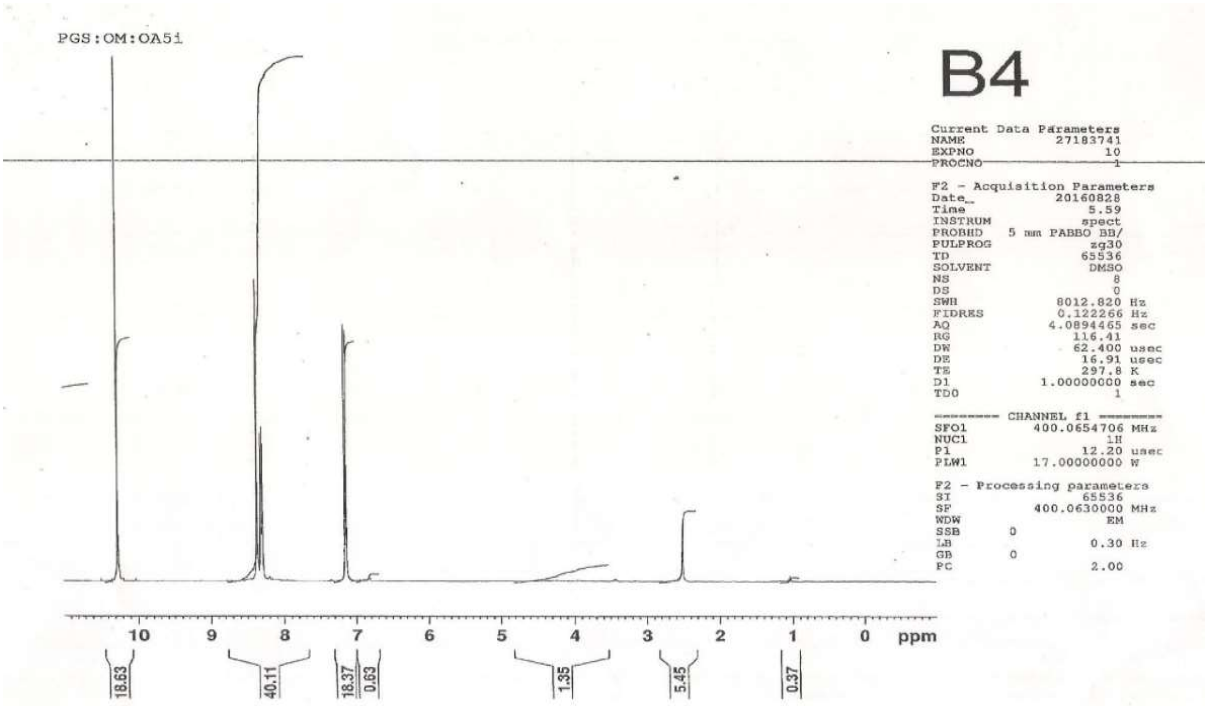
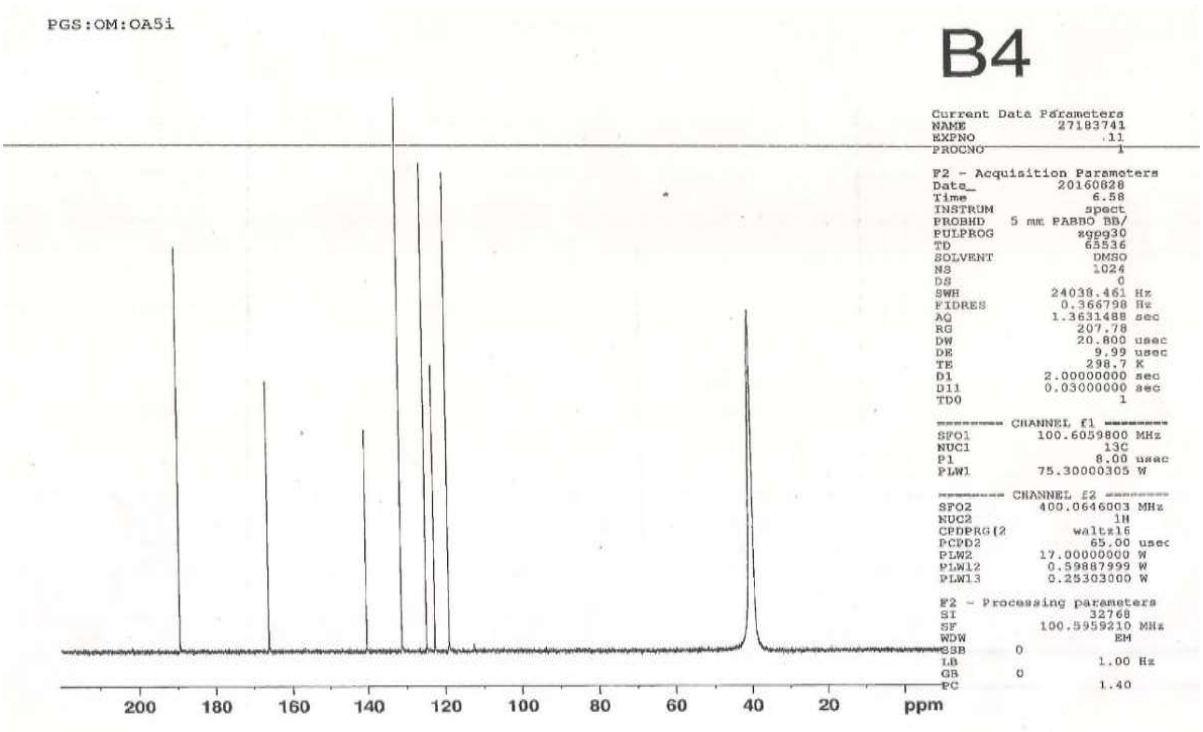
7.0 Appendices



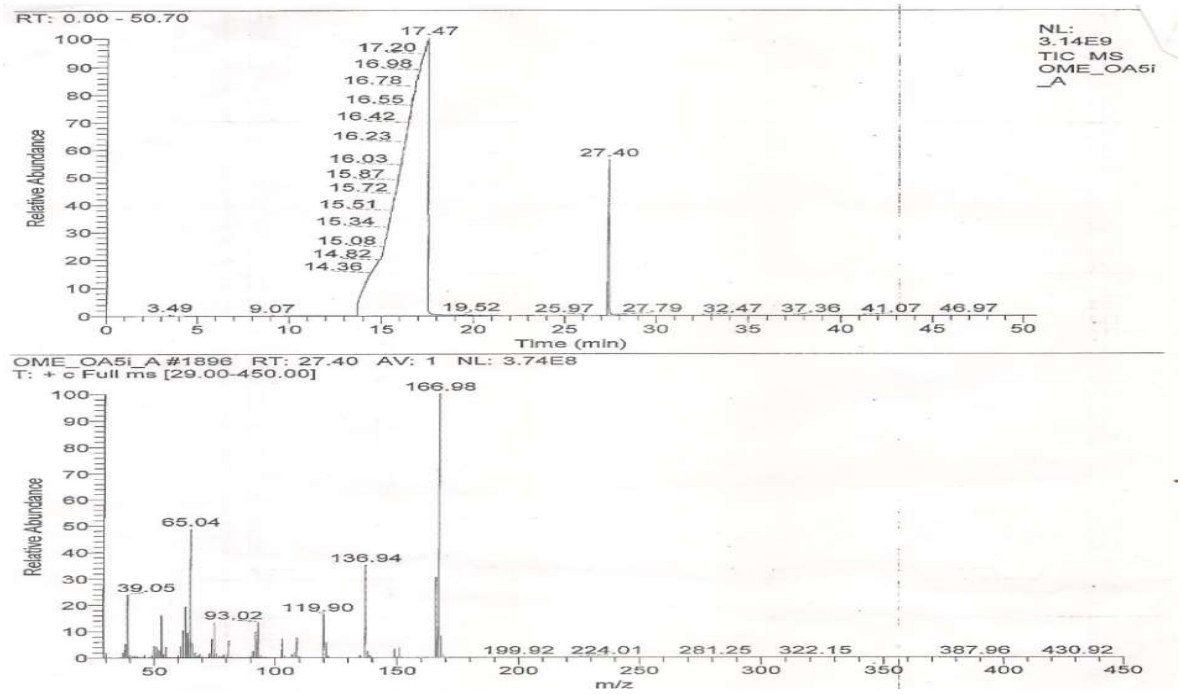
Appendix 1: FTIR spectrum of iron (II) 2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino} nicotinic acid



Appendix 2: ^{1}H-NMR spectrum of iron (II) 2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino} nicotinic acid



Appendix 3: ^{13}C-NMR spectrum of iron (II) 2-{[(2-hydroxy-5-nitrophenyl) methylidene]amino} nicotinic acid



Appendix 4: GC-MS spectrum of iron (II) 2-{[(2-hydroxy-5-nitrophenyl) methylidene] amino} nicotinic acid