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Research Article

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Synthesis, Characterization and Antimicrobial studies of (E)-N-(4-chlorobenzylidene) Aniline Schiff base.

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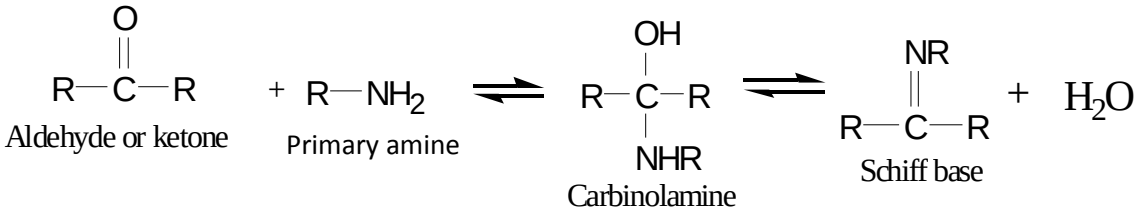
Abstract

Schiff bases are organic compounds containing the imine group (-C=N-) and are products obtained from the condensation of ketones or aldehydes with primary amines.In this study, a Schiff base was synthesized using the reflux method. Aniline was used as the primary amine while 4-chlorobenzaldehyde was the aldehyde. The synthesized Schiff base was characterized using infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR). The antibacterial and antifungal activities of the imino product was determined using the following inoculums: *Cadida albicans, Saccharomyces cerevisiae, Rhizopus oligastus*, *Aspergillus flavus, Aspergillus fumigatus, Fusa equisetti,* and *Aspergillus niger* for the antifungal activity while antibacterial activities were investigated using *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Shigella sonnei*, *Shigella dysenteriae* and *Escherichia coli.* The investigations showed that the compound had a broad spectrum antifungal and antibacterial activity.

Keywords: Schiff base, (E)-N-(4-chlorobenzylidene) aniline, *Saccharomyces cerevisae, Staphylococcus aureus*.

Introduction

Schiff bases are compounds containing the imine group (-C=N-) and are actually products obtained from ketones or aldehyde condensation with primary amines. Schiff bases could be secondary aldimines that is azomethine (R-C=NR[1]) where R[1] is not H.



In 1864, Schiff base was first reported by Hugo Schiff and it captured the interest of researchers as it found relevance in the making of other important compounds in medicine, catalysis, coordination chemistry, pharmaceutical chemistry and even in agriculture (Cimerman et al 2000). Their reported biological activities have provided endless research opportunities for scientist and industrialists alike (da Silva et al. 2011;Arun et al., 2014 and Sashidhara et al.,2008).On the other hand, different means of Schiff base synthesis have been documented since their discovery and they are reflux method, the magnetic stirring method, the ultrasound irradiation and microwave assisted method etc, (Singh, et al 1975; Qin et al. 2013). It was also reported that different solvent can be used with reaction conditions altered. These alterations are geared towards synthesizing Schiff bases of good yields under ecofriendly conditions. (Savalia et al 2013 and Umofia et al 2016).

Several moieties have been employed by Chemists to study the structure activity relationships in Schiff bases. While some have been dropped for inactivity others have been explored further given the susceptibility of microbes in their presence. The halogens are one of such chemical functional groups that has proven to be promising in the search for potent antimicrobials (Kartikayan et al.,2000; Umofia et al., 2017)

This current work therefore will study the activity of a chlorinated aniline derived Schiff base on some selected microorganisms.

Experimental

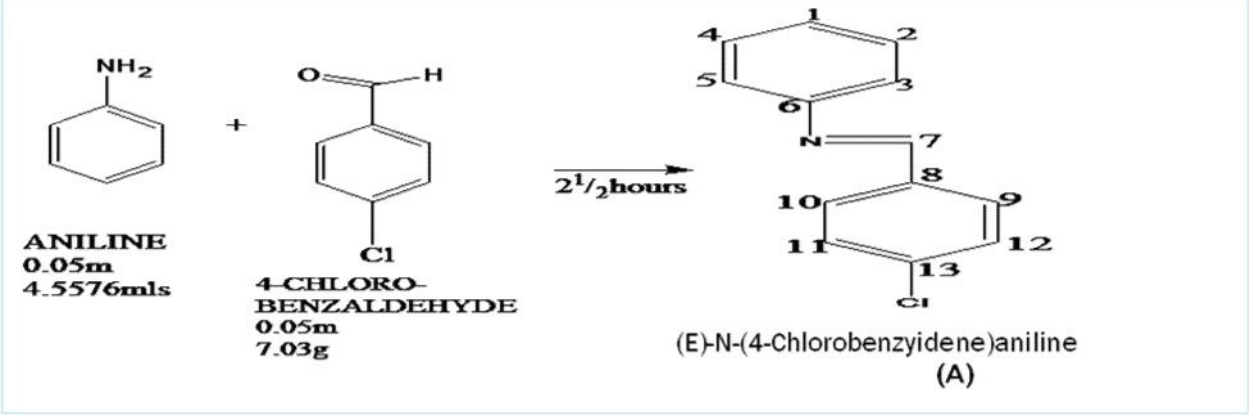
Material and Method

The chemicals were analytical grades gotten from Sigma Aldrich. The melting point was determined with the melting point apparatus. Tin layer chromatography (TLC) was carried out using pre coated silica gel plates and visualized using ultraviolet lamp at 256nm. The IR spectrum of the sample was recorded on an Agilent Technology carry 630 FTIR spectrometer in the range 4000 to 650 cm^{-1} using the transmittance method while the NMR spectra(^{1}H and ^{13}C) of the sample was recorded using Agilent Technologies 400mHz Premium + AR.

Synthesis

The reflux method was used in this synthesis. 4.56mls of aniline was homogenously mixed with 15mls of ethanol while 7.03g of 4-chlorobenzaldehyde was also homogenously mixed with 15mls of ethanol. The mixture of the amine was carefully added into a 250mls flat bottom flask containing the aldehyde.

The mixture was refluxed with a heating magnetic stirrer at 80˚C for two and a half hours. However few drops of hydrochloric acid were added to the mixture after one hour of the commencement of the reflux. The reaction was monitored using tin layer chromatography (TLC) and the plates viewed under ultraviolet lamp at 256nm.



Biological Activity

Preparation of the Schiff base.

The Schiff base was dissolved in dimethyl sulphoxide (DMSO) to obtain concentrations of 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml which were stored in the refrigerator overnight at 15° C.

Preparation of Fungal/Bacterial Isolates.

The fungi which were initially characterized were introduced into petri dishes which were incubated at 37°C for 48 hours after the purity and viability of the inoculums were confirmed. Uniform holes were made in the plates. The different concentrations of the Schiff base synthesized were introduced into the holes and the zones of inhibition were assessed in millimeter.

While in the preparation of the bacterial isolates, the agar was transferred into petri dishes after being sterilized. The plates are then inoculated with the cultures of the bacteria. Uniform holes were made in the dishes and the different concentrations of the Schiff base introduced into them. The zones of inhibition were also accessed in millimeters.

Results and Discussion

Spectral data of compound

IR: 1625.1(C=N) ,1587.8(Aromatic C=C) 3063.8 (Aromatic C-H), 1098.4(C-N), 760.4(C-C).

^{1}HNMR: 8.389 (N=CH(S), 6.503-7.082(Ar-H)

^{13}CNMR: 159.022 (C=N), 113.610-126.343(Aromatic C_1-C_5), 129.013-129.354 (Aromatic C_8-C_{13})

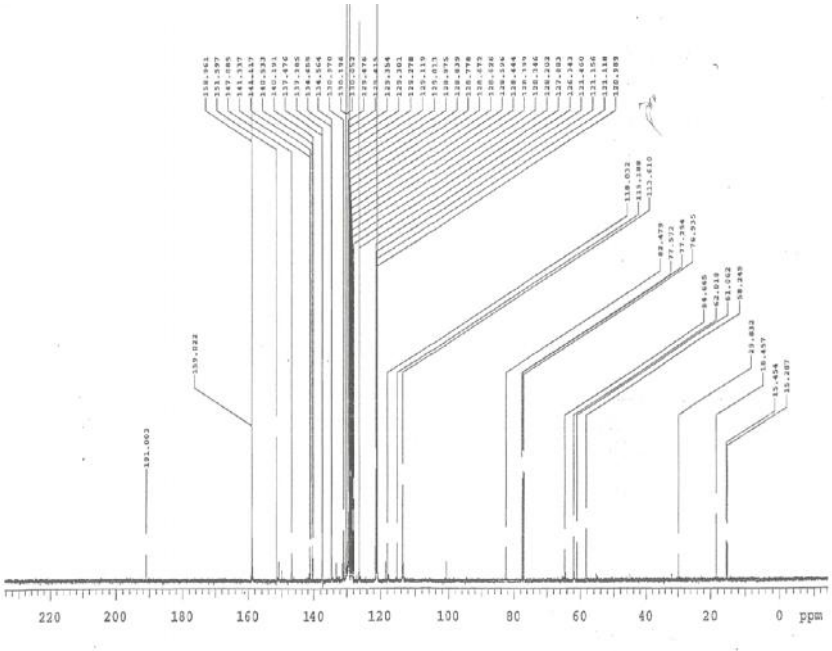


Figure 1: ^{13}CNMR Spectrum of compound A

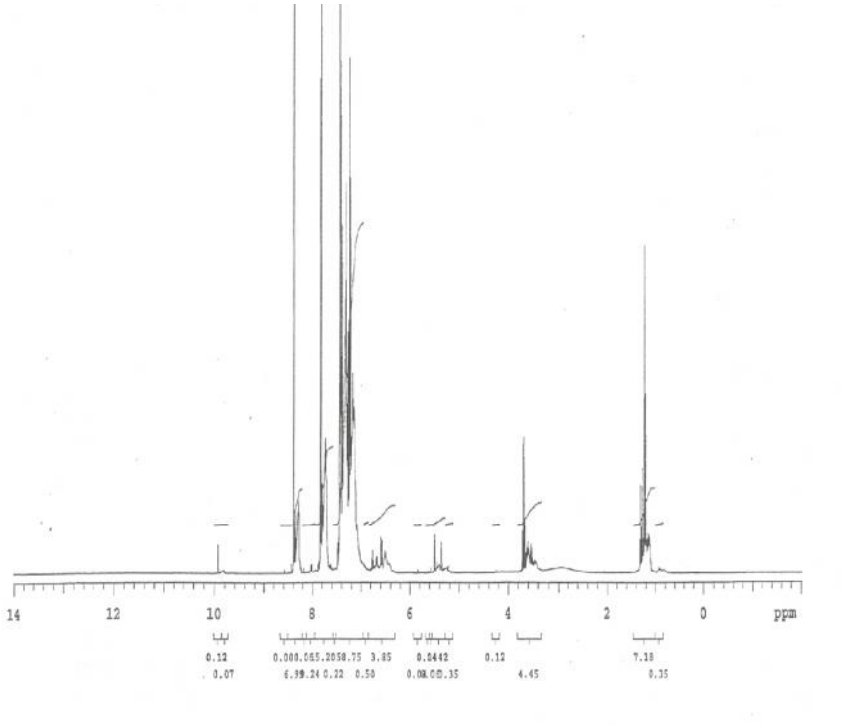


Figure 2: ^{1}HNMR spectrum of compound A

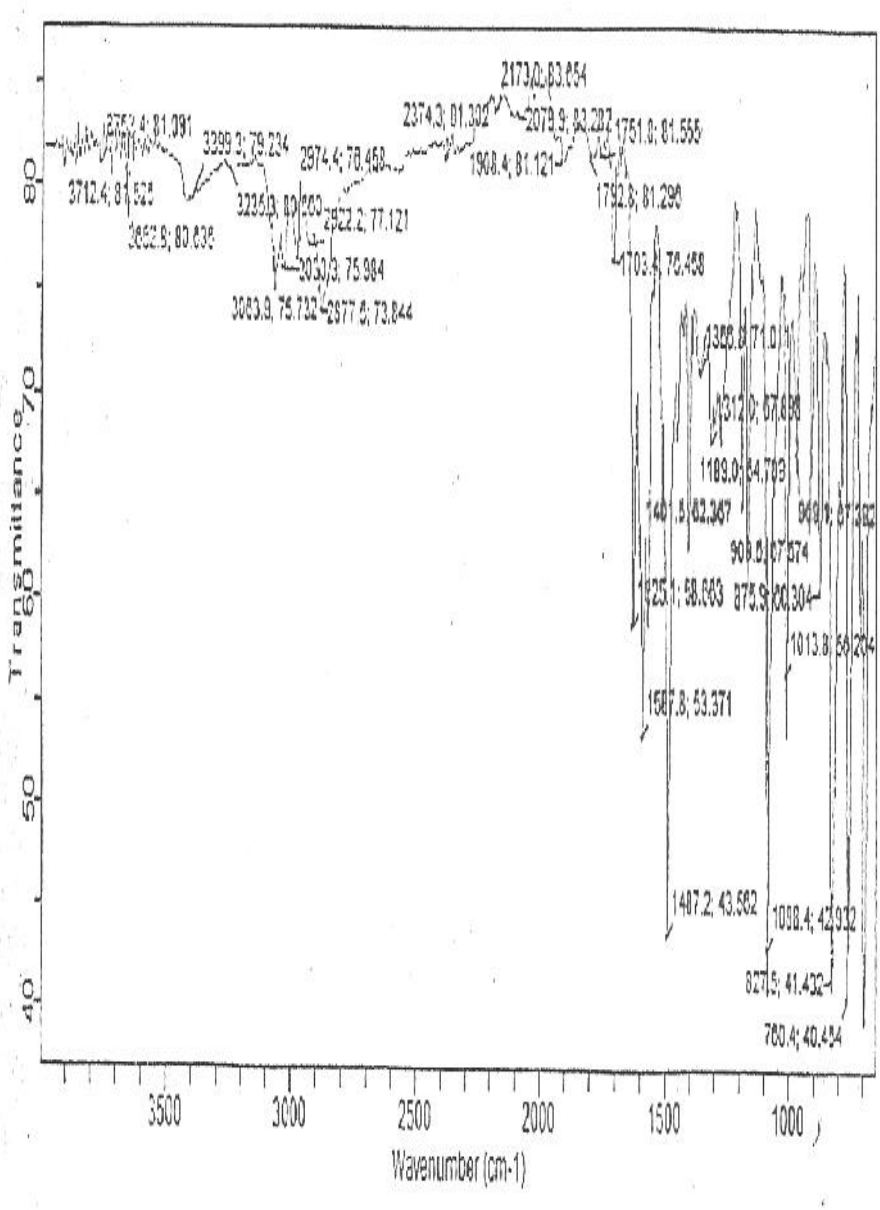


Figure 3: IR spectrum of compound A

Table 1: physicochemical properties of the schiff base

Physical state	Color	Rf Value	Melting point (^{0}C)	% Yield
Crystalline solid	Brown	0.76	64-68	63.7

Table 2: solubility test of the Schiff bases

Water	Ethanol	Methanol	DMSO	DCM	Ethyl acetate
Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble

Table 3: Susceptibility test of the Schiff bases on the bacterial

Bacteria – Gram Positive

	Unit	250mg	200mg	150mg	100mg	50mg	25mg	12.5mg
Staphylococcus	mm	22	17	7	0	0	0	0

Bacteria – Gram Negative

	Unit	250mg	200mg	150mg	100mg	50mg	25mg	12.5mg
Salmonella typhi	mm	16	6	0	0	0	0	0
Salmonella paratyphi	mm	16	4	0	0	0	0	0
Pseudomonas aeruginosa	mm	20	14	2	0	0	0	0
Shigella sonnei	mm	15	9	1	0	0	0	0
Shigella dysenteriae	mm	14	6	0	0	0	0	0
Escherichia coli	mm	20	9	0	0	0	0	0

Table 4: susceptibility test of the schiff base on fungi

Fungi

	Unit	250mg	200mg	150mg	100mg	50mg	25mg	12.5mg
Candida albicans	mm	18	10	2	0	0	0	0
Saccharomyces cerevisiae	mm	12	3	0	0	0	0	0
Rhizopus oligastus	mm	10	2	0	0	0	0	0
Aspergillus flavus	mm	15	7	0	0	0	0	0
Aspergillus fumigatus	mm	15	6	0	0	0	0	0
Fusa equisetti	mm	10	2	0	0	0	0	0
Aspergillus niger	mm	13	2	0	0	0	0	0

The broad single peak at the range of 4-6ppm which is associated with the proton of the amines ($–NH_2$) was absent in the spectra of the product. This indicated that the Schiff base was formed.

Also the signal exhibited at 8.389ppm is associated with the proton of the imine functional group (CH=N), indicating the formation of the Schiff base. The ^{13}CNMR data of the compound corresponded with those of Schiff base structures proposed by other researchers. (Roman and Andree, 2001) and Pavia *et al.*, 1996). The Schiff base showed a broad spectrum antifungal and antibacterial activity remarkably for concentrations of 200mg and 250mg.It is worthy of note that the activity of the gram positive bacterium (*Staphylococcus aureus*), showed activity at 150mg. Furthermore this broad spectrum antimicrobial activity of chloro substituted compounds is known ‘(Mc Donell and Russel, 1999). Hence the usage of these compounds as antiseptics, antibiotics, disinfectants etc., has gained wide acceptance.

Conclusion

This research has further established the potency of chloro-substituted Schiff bases as antimicrobial agents. Further study of the structure activity relationship of the compound, (E)-N-(4-chlorobenzydene) aniline could produce even more potent antimicrobial agents.

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