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Green Synthesis and Antibacterial Activity of a 3-Nitroaniline-3-Nitrobenzaldehyde Schiff Base

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Abstract:
The reactants, 3-nitroaniline and 3-nitrobenzaldehyde were refluxed in ethanol for 2 hours in an acidic medium to afford pale-yellow crystals of a Schiff base at 58% yield. The melting point and retention factor (Rf) of the product were checked, giving a value of 151 – 152°C and 0.54 respectively. The IR spectrum showed the following bands (cm-1); 1637 (imino, C=N), 1577 (Aromatic C=C stretch), 3074 and 3096 (Aromatic C – H stretch), 1346 and 1525 (Aromatic NO2 stretch) and 896 (Aromatic NO2 scissors). In addition, the 1HNMR spectrum showed a singlet at 8.44 ppm which represented the 1 H of the imino group, and a 8H multiplet between 7.18 – 8.41 ppm representing the aromatic protons. Furthermore, an antibacterial screening was done using the Schiff base on four (4) bacteria strains; Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, and Escherichia coli. The result from the screening portrayed that the highest zone of inhibition (ZOI) recorded for all bacteria was at a concentration of 200 mg/ml of the test compound, while the lowest was at a concentration of 50 mg/ml. This gave a ZOI of 12 mm, 10 mm, 13 mm, and 11mm for P. aureginosa, S. aureus, S. marcescens, and E. coli respectively. The results generally showed an increase in ZOI with increased concentration of the product. The best result observed was that obtained for S. marcescens which showed ZOIs of 7 mm, 10 mm, 12 mm, and 13 mm for concentrations of 50, 100, 150, and 200 mg/ml respectively.

Keywords: Ethanol, Escherichia coli, Geen solvent, 3-nitroaniline, 3-nitrobenzaldehyde, Pseudomonas aeruginosa, Schiff base, Serratia marcescens, and Staphylococcus aureus.

I. INTRODUCTION

Schiff bases are a subclass of a group of organic compounds called azomethines or imines. They are known to possess the imino functional group which is responsible for their various properties. The imino group is characterized by a carbon-nitrogen double bond (-HC=N-) (Ibrahim et al., 2006; Nic et al., 2005). Schiff bases are commonly made by the reaction between a carbonyl compound (aldehyde or ketone) and an amine in an acidic medium. Popularly, aromatic aldehydes and amines have been employed in synthesizing this compound (Mahmood, 2022). Due to the availability of various possible starting materials, thousands of Schiff bases have been formulated and investigated for their different properties. In addition, the synthesis of these compounds has been achieved using both toxic and green solvents, though majorly with the toxic ones (Abirami and Nadaraj, 2014). Such toxic organic solvents that have been used include but are not limited to, dichloromethane (Mishra et al., 2012), methanol (Essa et al., 2012), tetrahydrofuran, 1,2-dichloroethane (Bhagat et al., 2012), toluene, benzene and N,N-dimethyl formamide (Tomma et al., 2014).

Furthermore, green solvents like water (Bhagat et al., 2012; Sachdeva et al., 2014), ethanol (Endale and Desalegn, 2018; Ogbonda-Chukwu et al., 2023), and an ethanol-water mixture (Bhagat et al., 2012; Ogbonda-Chukwu et al., 2021) have also been used. Moreover, numerous techniques have been cited for the synthesis of Schiff bases including stirring at reflux temperature (Uddin et al., 2014; Ogbonda-Chukwu et al., 2023) and at room temperature (Khashi et al., 2018; Shipra et al., 2019), microwave irradiation (Bhagat et al., 2012, Shntaif and Rashid, 2016), ultrasonication (Karad et al., 2023), and solvent-free grinding (Liu et al., 2019; Sela et al., 2023). The conventional technique used for Schiff base synthesis is by stirring the reactants under reflux for a certain duration in the presence of an acid catalyst (Qin et al., 2013; Hussain et al., 2014).

Schiff bases have been found to possess a wide range of antimicrobial properties against some strains of bacteria and fungi (da Silva et al., 2011). Although, not all display a broad spectrum of bioactivity, some Schiff bases are very bioactive against various microbes. (Ceramella et al., 2022)

This paper uses a green route to synthesize a Schiff base from 3-nitrobenzaldehyde and 3-nitroaniline. Additionally, the antibacterial activities of this compound will also be explored. The objective of this research was to confirm if this compound could be obtained using an eco-friendly solvent and method, and also, to check if the product had any pharmacological viability.

II. MATERIALS

The chemicals used for this research were synthesis-grade chemicals purchased from Sigma-Aldrich. The melting point was determined with a melting point apparatus and was

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uncorrected. Thin Layer Chromatography (TLC) was carried out using a Merck pre-coated silica gel plate (10 x 10 cm), the Rf value was obtained by using a 2:1 mixture of dichloromethane and methanol as the mobile phase and the spot located and visualized using an ultraviolet lamp at 256 nm. The IR spectrum of the sample was recorded on a Fourier Transform Infrared spectrometer, Carry 630 Agilent Technologies in the range of 650-4000 cm-1. 1H NMR spectrum of the sample was recorded on a BRUKER AVIII 400 NMR spectrophotometer using deuterated methanol (MeOD).

III. Methods

SYNTHESIS OF 3-NITROANILINE-3 NITROBENZALDEHYDE SCHIFF BASE

An equimolar amount of 3-nitroaniline (1.39 g) and 3-nitrobenzaldehyde (1.51 g) were dissolved separately in 20 ml ethanol.

The reaction mixture was stirred continuously under reflux at 80 0C for 1 hr followed by the addition of a few drops of concentrated hydrochloric acid (HCl). The reaction mixture was then stirred for one more hour. The reaction was monitored to completion using thin-layer chromatography (TLC) with dichloromethane and methanol (2:1) as the mobile phase. At the end of the reaction, the hot mixture was allowed to cool to room temperature. On cooling, pale yellow crystals of the Schiff base were observed. The product was filtered, recrystallized using hot ethanol, filtered again, and air-dried. The general equation for this reaction is shown in Figure 1 below.

The title compound was characterized using FTIR and 1HNMR analysis and the spectral data are illustrated in Table 1. Figures 5 and 6 show the spectrum of the FTIR and 1HNMR analysis respectively. Rf: 0.54, m.p: 151-152oC, yield: 58%.

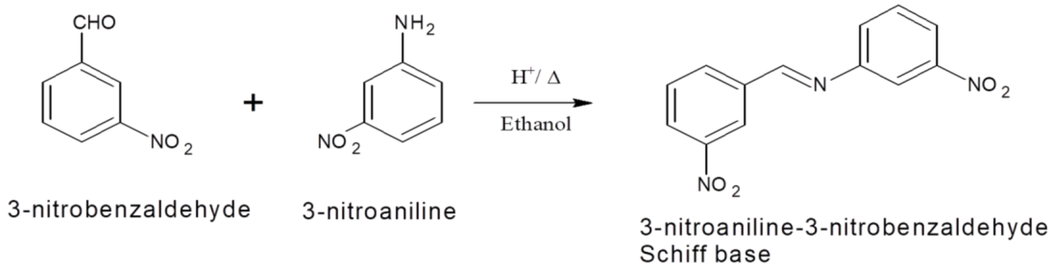

Figure 1: Reaction equation for the synthesis of 3-nitroaniline-3-nitrobenzaldehyde Schiff Base

ANTIBACTERIAL ANALYSIS

The Agar diffusion method was employed for this analysis, according to Eruteya and Odunfa, (2016). From overnight cultures of the various bacteria (Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, and Escherichia coli) on nutrient agar plates, a 1× 108 cell/mL McFarland standard was prepared, and 0.1mL aseptically transferred to sterile Petri dishes before adding 20 mL molten Mueller Hinton agar cooled to 45-50oC. The contents were thoroughly mixed and then allowed to solidify. Four holes (5.0mm) were made in each plate using a cork borer and 0.2ml of the various Schiff base concentrations dissolved in dimethylsulfoxide (DMSO) was transferred into each hole aseptically using a pipette. The plates were allowed to stand for pre-diffusion for 1 hr before incubation at 29±2oC for 24 hrs. Zones of inhibition were measured in millimeters (mm).

The plates are illustrated in Figures 2-4.

Figure 2: Plate showing ZOI for growth of P. *aureginosa*

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Figure 3: Plate showing ZOI for growth of S. marcescens

Figure 4: Plate showing ZOI for growth of S. aureus

IV RESULTS AND DISCUSSION

TABLE 1: IR AND ^{1}H NMR DATA OF 3-NITROANILINE-3-NITROBENZALDEHYDE SCHIFF BASE

Functional groups	**imino C=N**	**Aromatic C=C stretch**	**Aromatic C–H stretch**	**Aromatic NO_2 stretch**	**Aromatic NO_2 scissors**
IR bands (cm^{-1})	1637	1577	3074 3096	1346 1525	896

^{1}HNMR(ppm)	**(8H, aromatic protons)**	**1 H, imino proton**
	7.18 – 8.41, m	8.44, s

TABLE 2: ANTIBACTERIAL ACTIVITY OF 3-NITROANILINE-3-NITROBENZALDEHYDE SCHIFF BASE

Concentration (mg/ml)	***Zone of Inhibition (mm)***			
	Pseudomonas aeruginosa	***Staphylococcus aureus***	***Serratia marcescens***	***Escherichia coli***
50	8	5	7	6
100	9	7	10	8
150	10	8	12	9
200	12	10	13	11

The synthesis of this compound gave a moderate yield of 58% which is relatively high when compared to that obtained by Elemike et al., (2018), in their synthesis of N1, N2-bis(3-nitrobenzylidene)phenylene diamine where they achieved 16.28% yield using a similar method used in this research. On the other hand, the synthesis of 1-nitro-4(1-imino-4-nitrophenyl) benzene, was achieved using the same solvent but on reflux for 4 hrs and with a base catalyst to give 97.60% yield (Chetana et al., 2019). The difference in yield could be compound and method-specific.

The data obtained from the spectral analyses (table 1) established that the title compound formed as expected. The structure was confirmed by the presence of an IR band at 1637 cm-1 for the Schiff base portraying the presence of the imino group (C=N), and the absence of the amino and carbonyl group peaks at 3400-3250 cm-1 and 1740-1720 cm-1 respectively (figure 5). Additionally, the 1H NMR result showed the presence of the imino proton, with a single peak at 8.44 ppm (figure 6). The values showing the presence of the peaks of interest, the imino functional group, for the IR and HNMR data revealed that they were within range and close to that observed in Lee Hee and Lee Soon (2005) and Elemike et al., (2018).

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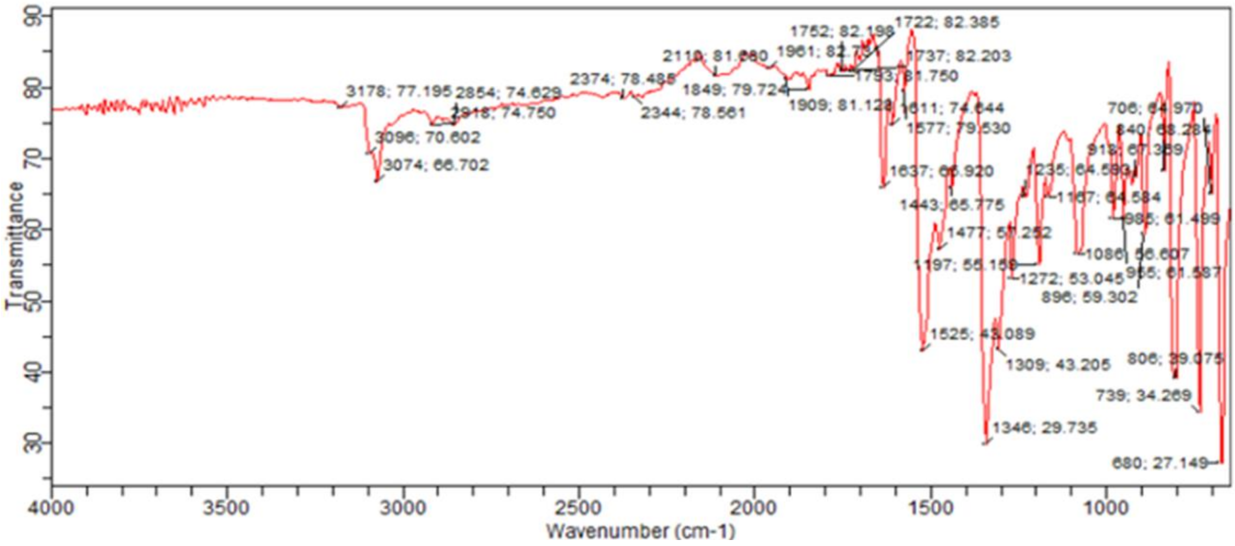

Figure 5: IR spectrum of 3-nitroaniline-3-nitrobenzaldehyde Schiff base

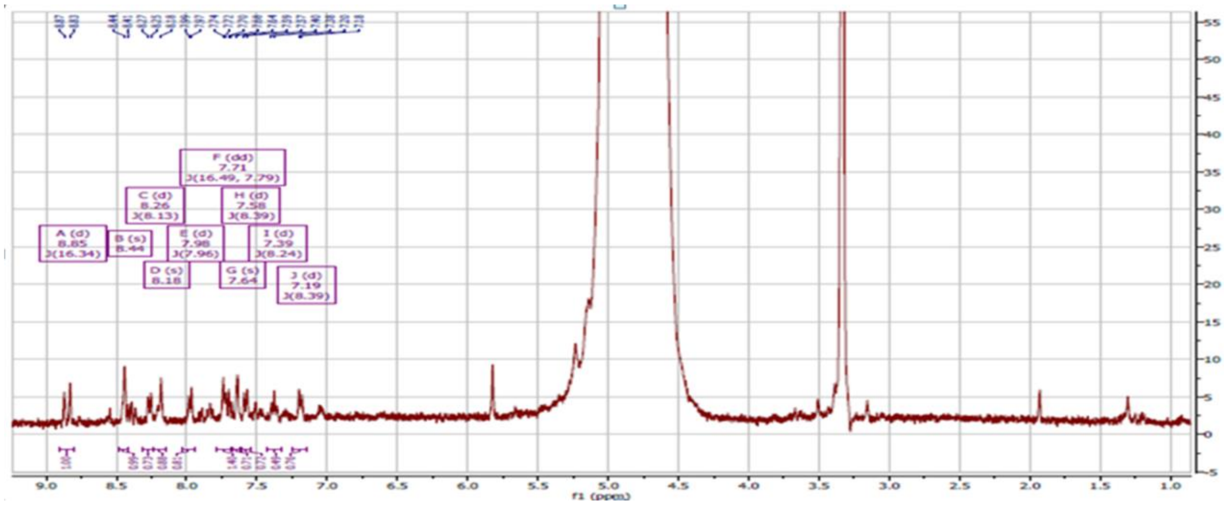

Figure 6: [1]H NMR spectrum of 3-nitroaniline-3-nitrobenzaldehyde Schiff base

The antibacterial screening results (table 2) showed that the compound was active against the four bacteria tested, *Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, and Escherichia coli* at all concentrations. It was noted that the ZOIs recorded for all the strains increased with an increase in the concentration of the product. At the highest concentration of 200 mg/ml, *S. marcescens* showed the highest ZOI value while *S. aureus* showed the lowest, with values of 13 mm and 10 mm respectively.

Furthermore, at the lowest test concentration of 50 mg/ml, *P. aeruginosa* gave the highest ZOI measurement while *S. aureus* showed the lowest, with values of 8 mm and 5 mm respectively. In addition, the results for *S. marcescens* stood out, displaying the highest values of 10, 12, and 13 mm at concentrations from 100 to 200 mg/ml. This may be because it has the least resistance mechanism against the Schiff base being examined.

IV. Conclusion

The results from this investigation demonstrate that the 3-nitroaniline-3-nitrobenzaldehyde Schiff base can be synthesized using a green route to give a relatively good yield. Furthermore, this research has found that the title compound possesses a good range of antibacterial properties making it a promising pharmacological compound for the drug or agrochemical industry.

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