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Synthesis, Characterization and Antimicrobial Analysis of 1-(2-Nitrophenylazo)-2-Naphthol

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Abstract

Scientists are searching for efficient antibiotic medications to treat pathogenic microorganism-caused illnesses since these microbes have become a major medical threat because of the illnesses and infections they can induce in their hosts. Azo naphthol dyes are often vividly coloured substances with a variety of industrial applications. Lately, they have demonstrated efficacy against specific pathogenic microbe strains. In this study, 2-nitroaniline and 2-naphthol underwent an azo coupling reaction to create 1-(2-nitrophenylazo)-2-naphthol azo dye. The compound was identified using the information gleaned from the IR, NMR, and UV-vis spectra. The results of the antimicrobial investigation demonstrated that the azo compound has the ability to suppress certain types of bacteria, including *Escherichia coli* and *Staphylococcus aureus*, as well as fungus, such as *Aspergillus sp*. The compound concentration of 150 µg/mL was resisted by the fungi but inhibited the bacteria at the range of 0.5 – 0.8 mm while 50 µg/mL and 100 µg/mL concentrations were resisted across by the organisms. Then, 200 µg/mL, raw, and 30 µg/mL control drugs (Oflocitoxin and Augmentin) concentrations inhibited the organisms at the range of 0.5 – 1.2 mm, 1.1 – 1.7 mm, 1.6 – 24 mm (1.6 – 15 mm and 2.5 – 24 mm), respectively. The minimum inhibitory concentration of the compound was estimated at 150 µg/mL for the bacteria, and 200 µg/mL for the fungus used. The results showed that the compound can be used as an antimicrobial agent for a lot of industrial and medical applications.

Keywords: Azo dyes; 2-Nitroaniline; 2-Naphthol; 1-(2-Nitrophenylazo)-2-naphthol; Antimicrobial; Azonaphthol

1. Introduction

Nitrogen-nitrogen double bonds, or azo groups (N=N), are typically present in azo compounds and dyes. However, depending on how many azo groups are present in a given chemical, azo compounds can be categorized as monoazo, diazo, or triazo compounds. Because of potential structural differences in the chemical structures, azo dyes are used in a variety of ways compared to other dyes [1]. Azo-naphthol dyes can be utilized as antibiotic agents against some harmful bacteria and have good colour fastness properties [2–7]. In order to create diazonium salt, a primary amine must first be diazotized. This is achieved by treating the primary amine with nitrous acid, which is created when strong mineral acids (preferably hydrochloric acid) react with sodium nitrite. This process is known as azo coupling, which is the electrophile's (diazonium salt) attack on the nucleophile (electron-releasing group), typically an amino or hydroxyl group [8]. A few characteristics of azo compounds or dyes include their strong colour intensity, affordability of production, resistance to oxidizing agents, non-toxicity, mild acidity, and non-basicity [9]. Azo dyes made from benzidine have the potential to cause cancer because certain bacteria convert them to aryl amines through skin contact

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during perspiration. As a result, dyestuff rules must be followed properly [10]. Azo dyes have been found to have intriguing uses in the identification of metal flaws and cracks, recording layers in disks (DVD/CD), ink and paper technology (CI Direct Yellow 28 and also CI Direct Yellow 29), and they are also used as biological stains and pH indicators, pharmaceutical drugs, and food colorants [11–14]. Additionally, some infections caused by pathogenic microorganisms may benefit from the use of azo dyes if taken care of [4–7].

2. Materials and Methods

2.1. Materials

The chemical reagents were utilized without additional purification after being acquired from BDH Chemicals. The instruments used are pH meter (PHS-3C), melting point apparatus, precision weighing balance (Y-502N), incubator, Autoclave (Desco), magnetic stirrer (constant temp. HY-3D), thermocool refrigerator (HTF-259H), NMR spectrometer (Agilent-NMR-vnmrs400), UV-visible spectrometer (Metro UV-5800PC), and FT-IR spectrometer (Perkin-Elmer GX2000 FTIR). The microbes: *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus fumigatus* were obtained from Vetech Research Centre and Laboratory, Institute of Management and Technology, IMT, Enugu, Nigeria.

2.2. Synthesis of 1-(2-Nitrophenylazo)-2-naphthol

This was carried out following standard methods [4–7], with little modifications. A mixture of concentrated hydrochloric acid (16 cm^3) and distilled water (16 cm^3) was used to dissolve 5 g of 2-nitroaniline. After gently shaking the reaction mixture to remove any possible hydrochloride separation, the solution was cooled to 5 ^oC. At a temperature of 0–5 ^oC, 4 g of sodium nitrite that had been dissolved in 20 cm^3 of water and 1 spatula of urea, were added while the mixture was continuously stirred. By progressively adding a cold solution of sodium nitrite to a cold solution of 2-nitroaniline while stirring continuously and making sure the temperature never went beyond 5 ^oC, diazotization was accomplished. 2-Naphthol (5 g) was dissolved in 45 cm^3 of 10 % NaOH in a 250 cm^3 beaker while being constantly stirred to create a 2-naphthol solution. The reaction mixture was then allowed to cool further at 5 °C in an ice bath (containing 25 g of broken ice). This was followed by the slow addition of the cold diazonium salt solution. A silver-brown hue and crystal begin to form, and finally separating. After being continuously stirred in an ice bath for 30 minutes, the reaction mixture was filtered using a Buchner funnel and cleaned with deionized water. Three days were spent air-drying the residues. The yield as a percentage was 92.5 %. The Scheme 1 below represents the synthetic pathway of the compound: 1-(2-Nitrophenylazo)-2-naphthol.

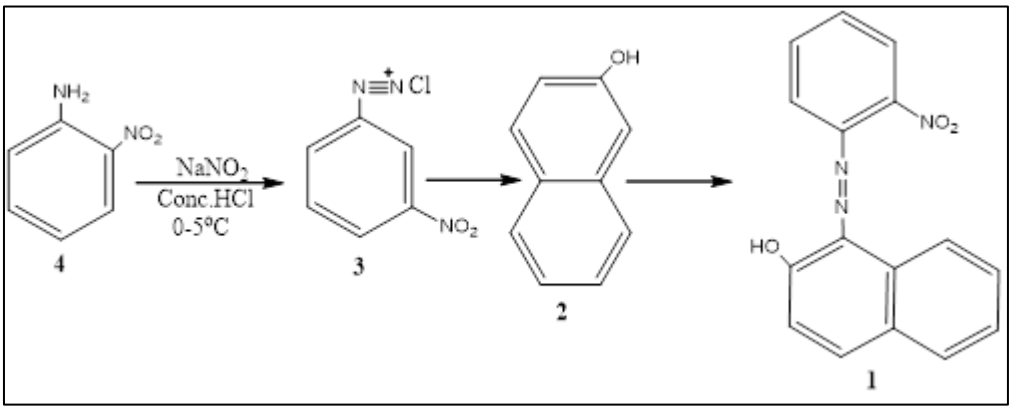

Figure 1 Synthesis of 1-(2-Nitrophenylazo)-2-naphthol

2.3. Determination of the Zone of Inhibition and Minimum Inhibitory Concentration Estimation (disks method)

Procedure of Onunkwo and Ejikeme [4] was followed in determination of the zone of inhibition. Discs (with a diameter of 5 mm) were created using a paper borer and Whatman filter paper (No. 1). Subsequently, the ready-made discs were placed in appropriate containers. Subsequently, the discs were autoclaved for 15 minutes at 121 °C to sanitize them, and they were then allowed to cool down. Subsequently, the discs were permitted to absorb, in turn, the sample filtrate at concentrations of 50 μg/mL, 100 μg/mL, 150 μg/mL, 200 μg/mL, unprocessed or raw, and 30 μg/mL of each of the control antibiotics, Augmentin (Au) and Oflocitoxin (OFX) drug discs, which were kept for a subsequent analysis. About 0.01 mL of the sample concentrations can be absorbed by each of the generated discs. After being produced, the plates inoculated with *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Escherichia coli*, were subjected (placing them on top)

with the discs containing the corresponding concentrations and incubated for a whole day. According to Onunkwo and Ejikeme [4], Onunkwo and Okerulu [5], Onunkwo *et al*. [6], and Onunkwo *et al*. [7], the minimum inhibitory concentration was estimated after the zone of inhibition was seen and measured in millimeters.

2.4. Instrumental Characterization of the 1-(2-Nitrophenylazo)-2-naphthol

The formation of the 1-(2-nitrophenylazo)-2-naphthol was determined using UV-visible spectrometer (Metro UV-5800PC) in the range of 190–1000 nm. FT-IR spectrometer (Perkin-Elmer GX2000 FTIR) was used for the analysis of the reactivity and functional groups present in the synthesized 1-(2-nitrophenylazo)-2-naphthol in the range of 4000 – 500 cm^{-1}. The NMR spectrometer (Agilent-NMR-vnmrs400) is a highly specialized instrument that captured the proton (^{1}H NMR) and carbon 13 (^{13}C or C13 NMR) spectra of the synthesized compound.

3. Results and Discussion

3.1. Characterization of the 1-(2-Nitrophenylazo)-2-naphthol

The synthesized compound was elucidated and the data obtained. The UV-visible data showed max. at 430 nm and 510 nm based on colour transitions and absorptions of the compound. The Infra-red data (Figure 3) showed the functional group O-H (3375 cm^{-1} broad), N=N (1472.29 cm^{-1} stretch), NO_2 (1315.11 cm^{-1} stretch) C=O (1624 cm^{-1} stretch), C=C (1562.0 cm^{-1} stretch), C-N (1445.32 cm^{-1} stretch), C-O (1125.65 cm^{-1} stretch), C-C (1125.65 cm^{-1} – 1096.60 stretch) and C-H (970.5 – 637.48 cm^{-1}). The HNMR spectrum (Figure 3) showed the δ 6.645 – 6.669 (more shielded ^{1}H group), δ 8.259 – 8.405 (more deshielded ^{1}H group) and δ 7.195 – 7.734, corresponding to the aromatic proton groups observed in the compound. C13NMR (Figure 4) solvent ($CDCl_3$) signal was observed at δ 108.133; then, C-OH (δ 181.195), C-NO_2 (δ 173.135), C-N (δ 145.666), and δ 117.757 – 143.427, were the observed ^{13}C signals in the aromatic compound. The data corresponds to the standard as shown in the works of Donald *et al*. [15], Onunkwo and Ejikeme [4], and Ayuk *et al*. [16].

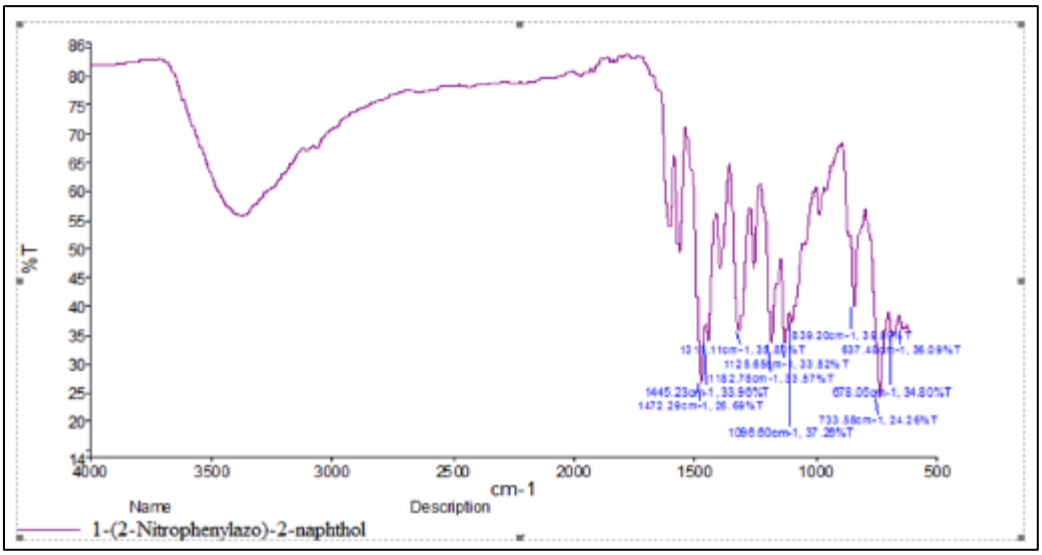

Figure 2 Infra-red (FT-IR) Spectrum of 1-(2-Nitrophenylazo)-2-naphthol

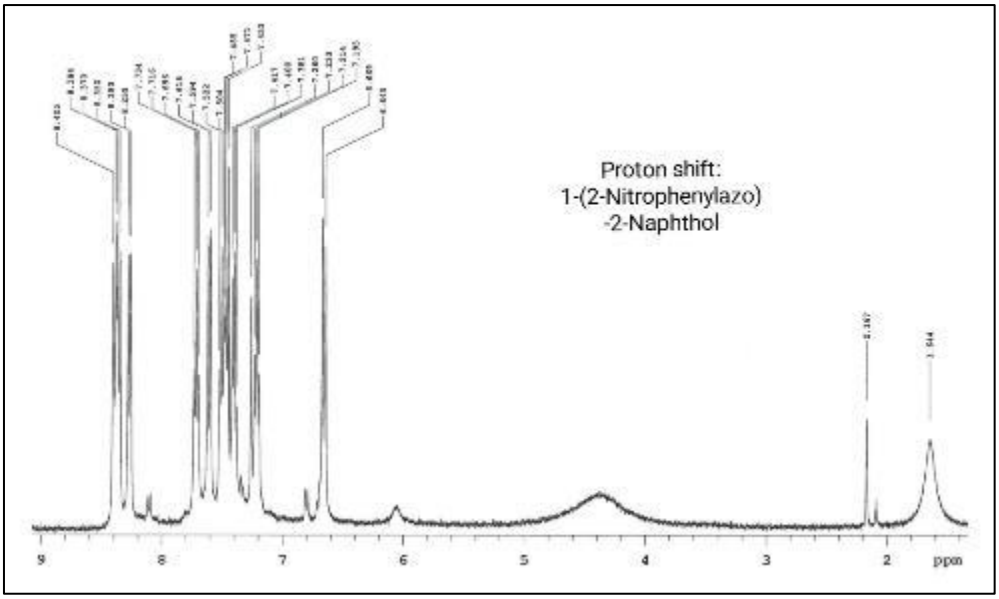

Figure 3 HNMR Spectrum of 1-(2-Nitrophenylazo)-2-naphthol

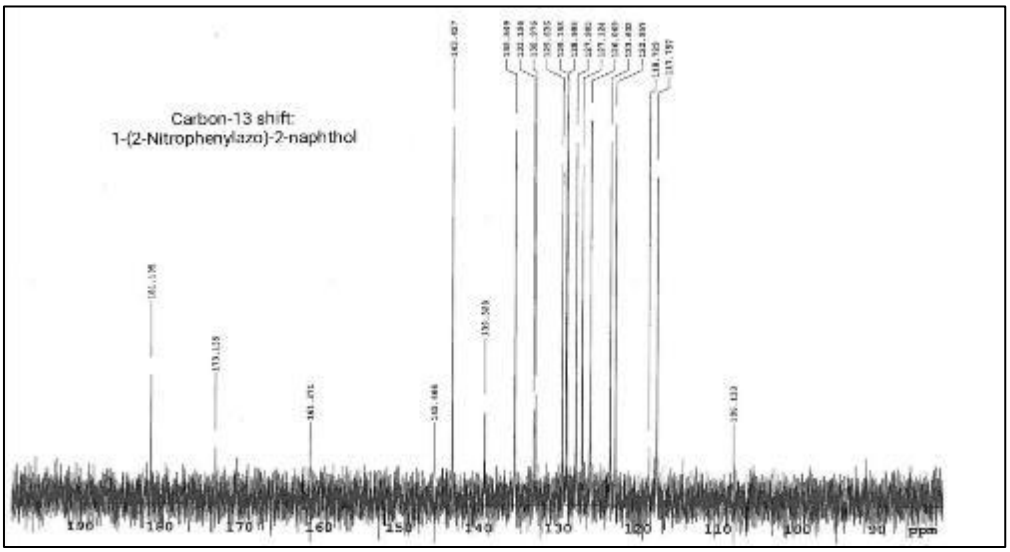

Figure 4 C13NMR Spectrum of 1-(2-Nitrophenylazo)-2-naphthol

3.2. Antimicrobial Studies of the 1-(2-Nitrophenylazo)-2-naphthol

The antimicrobial analysis of the 1-(2-nitrophenylazo)-2-naphthol was carried against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus fumigatus*. Augmentin (Au) and Oflocitoxin (OFX) were used as positive control drugs.

Table 1 Impact of varying sample dilution and control against the pathogenic strains

Organisms	Unit	1-(2-Nitrophenylazo)-2-naphthol (μg/mL)					Control (μg/mL)		MIC (μg/mL)
		50	100	150	200	Raw	Au	OFX	
Staph. sp.	(mm)	R	R	0.8	0.9	1.4	20	10	150
E. coli		R	R	0.5	1.2	1.7	24	15	150
Aspergillus sp.		R	R	R	0.5	1.1	2.5	1.6	200

R=Resistant

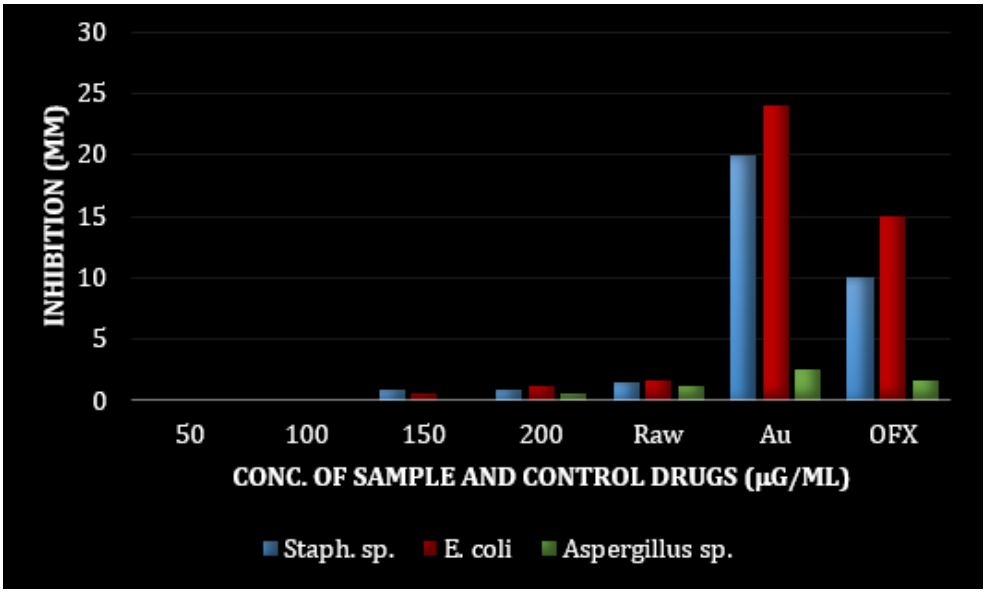

Figure 5 Antimicrobial analysis of 1-(2-Nitrophenylazo)-2-naphthol, and control [Augmentin (Au) and Oflocitoxin (OFX)], against *S. aureus*, *E. coli* and *A. fumigatus*

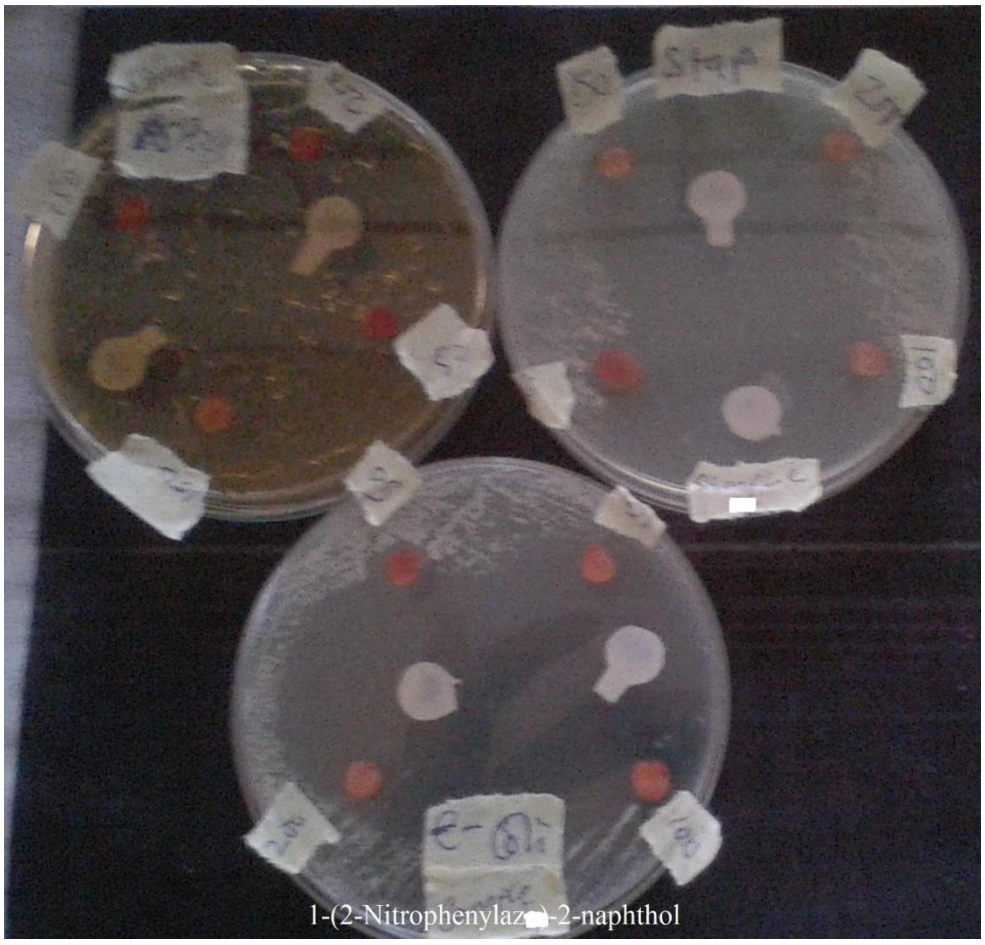

Figure 6 Antimicrobial study of the 1-(2-nitrophenylazo)-2-naphthol, and control [Augmentin (Au) and Oflocitoxin (OFX)], against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus fumigatus*

From Table 1, Figure 5 and Figure 6, the *Staphylococcus aureus* was found to be resistant to 50 µg/mL and 100 µg/mL concentrations of the compound but was inhibited by the raw, 150 µg/mL, and 200 µg/mL concentrations at 1.4 mm,

0.8 mm, and 0.9 mm respectively. The control drugs Au and OFX tested against the organism was able to inhibit the growth of the organism at 10 mm and 20 mm, respectively, and the minimum inhibitory concentration of the compound against *Staphylococcus aureus* was estimated at 150 μg/mL.

Escherichia coli was found to be resistant to 50 μg/mL and 100 μg/mL concentrations of the compound but was inhibited by the raw, 150 μg/mL, and 200 μg/mL concentrations at 1.7 mm, 0.5 mm, and 1.2 mm, respectively. The control drugs Au (30 μg/mL) and OFX (30 μg/mL) tested against the organism were able to inhibit the growth of the organism at 24 mm and 15 mm, respectively, and the minimum inhibitory concentration of the compound against *Escherichia coli* was estimated at 150 μg/mL.

Aspergillus fumigatus was resistant to 50 μg/mL, 100 μg/mL, and 150 μg/mL concentrations of the compound but was inhibited by the raw, and 200 μg/mL concentrations at 1.1 mm, and 0.5 mm, respectively. The control drugs Au (30 μg/mL) and OFX (30 μg/mL) tested against the organism were able to inhibit the growth of the organism at 2.5 mm and 1.6 mm, respectively, and the minimum inhibitory concentration of the compound against *Escherichia coli* was estimated at 200 μg/mL. The results differ slightly when compared to the work of Onunkwo and Ejikeme [4]. There was a slight difference with the results obtained in this research in comparison with that from the research carried out by Onunkwo and Ejikeme [4], and Onunkwo and Ezechi [17]. For example, Onunkwo and Ejikeme [4] reported that at 200 μg/mL, 1-(1-phenylazo)-2-naphthol had 0.1, and 0.2 mm inhibitions against *S. aureus*, and *E. coli*, respectively. Also, they also stated there was no effect of the compound and the control drugs on *A. fumigatus* at the concentrations used. The low turnout of the compound when compared to the control drugs in terms of the inhibitions of the organisms, showed that the compound may be less toxic compared to the control drugs at the concentrations tested and may be used as antimicrobial agent when toxicity effect is highly put into consideration [17]. However, more research at other concentrations can be carried out to assess further its inhibition effects. Therefore, increasing the concentrations and exposure time of the compound, as well as having appropriate understanding of the genomics and proteomics of the specie of the fungi, can help in their targeting and enhance the compound's efficacy. This is because several fungi species and strains have varying level of susceptibility to inhibitory compounds, and lack of knowledge about their chemical and biological compositions may pose a hindrance [4, 12–14, 17].

4. Conclusion

The compound, 1-(2-nitrophenylazo)-2-naphthol, synthesized by diazotization and azo coupling reactions possesses antimicrobial properties against *S. aureus*, *E. coli*, and *Aspergillus sp.* at all concentrations and was inhibited by the control drug. In that case, it will function effectively as antimicrobial agent in different formulations to fight infections.

Compliance with ethical standards

Acknowledgment

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Disclosure of conflict of interest

The authors declare no conflict of interest regarding this article.

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