World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Synthesis, Characterization and Antimicrobial Analysis of 1-(3-Nitrophenylazo)-2-naphthol

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World Journal of Advanced Research and Reviews, 2025, 28(03), 1199-1206

Publication history: Received on 10 November 2025; revised on 15 December 2025; accepted on 18 December 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.28.3.4179

Abstract

Because of the illnesses and infections that pathogenic microorganisms can cause in their hosts, these microbes have become a major medical risk. As a result, researchers are looking for efficient antibiotics to cure these illnesses. Azo-naphthol dyes are often vividly coloured substances with a variety of industrial applications. Lately, they have demonstrated efficacy against specific strains of pathogenic microbes. The azo dye, 1-(3-nitrophenylazo)-2-naphthol, was synthesized in the current investigation through diazotization and azo coupling reactions between 3-nitroaniline and 2-naphthol. The NMR, IR, and UV-vis spectra data verified the presence of the molecular frameworks of the azo compound. The raw and 200 µg/mL concentrations of the sample inhibited the bacteria strains (*Staphylococcus aureus* and *Escherichia coli*) at the range of 0.2 mm to 0.8 mm while all other concentrations of the compound employed were resisted by the *Aspergillus sp*. However, the control antibiotics (Augmentin and Oflocitoxin) used, had little inhibitory effects on the fungus but were effective on the bacterial strains. The minimum inhibitory concentration was estimated at around 200 µg/mL. As no research has been carried out on the compound and also in this area, this compound can act as an antibacterial agent for various uses.

Keywords: Azo dyes; 3-Nitroaniline; 2-Naphthol; Azonaphthol; 1-(3-Nitrophenylazo)-2-naphthol; Antimicrobial analysis

1. Introduction

Azo groups are nitrogen-nitrogen double bonds (N=N) that are typically present in azo compounds or dyes [1]. Azo dyes find extensive application compared to other dyestuffs due to potential structural differences in their chemical structures [2].

The azo dye compounds have shown some interesting phenomena such as isomerism (phenylazonitrobenzene, for example), tautomerism (para red forming ketohydrozone, for example) [3], photochromism (disperse red, for example), irreversible hydrogen bonding (typically appeared at hydroxyl group of azo compounds), as well as quaternization of cylic azo colorants for increased color intensity [4]. These phenomena are caused by light from ultraviolet, infrared, and nuclear magnetic resonance spectrometers [5].

A major benefit for those who produce azo dyes is their high and brilliant colour intensity as well as their economical manufacturing technique. Additional characteristics of azo dyes are their non-basicity, moderate acidity, and resilience

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to oxidizing agents. They are also non-toxic. This is unrestricted because some have some degree of antibacterial activity [6–7].

Azo dyes are used as pH indicators and biological stains (example methyl orange etc.), recording layers in DVD/CD disks, testing for flaws or cracks in metals (example CI Red 164), paper and ink (example CI Direct Yellow 28 and CI Direct Yellow 29), food colourant (Butter Yellow) and pharmaceutical [7–12].

Nevertheless, azo dyes made from benzidine have the tendency to be carcinogenic despite their usefulness. This is because certain bacteria in the human body convert azo dyes—through skin contact through perspiration—to aryl amines, which have been shown to be carcinogenic. As a result, azo dyes are properly regulated [13–15].

All the same, according to Onunkwo et al. [14], Arijit et al. [16], Onunkwo and Okerulu [17], azo-naphthol dyes have good color fastness properties and can be employed as antibiotic agents against some harmful bacteria [18–19]. For instance, Onunkwo et al. [14] from their study stated that 1-(1-phenylazo)-2-naphthol possesses some levels of antibacterial activity against some bacterial strains, and could be used for certain applications due to its less toxicity effects. This calls the need for more research on other azo-naphthol compounds to determine their antimicrobial properties, and the potentiality their utilization as antimicrobial agents in various applications.

2. Materials and Methods

2.1. Materials

Without additional purification, the chemical reagents that were acquired from BDH Chemicals were used. Precision weighing balance (Y-502N), pH meter (PHS-3C), Melting point apparatus, Incubator, UV-visible spectrometer (Metro UV-5800PC), Magnetic Stirrer (constant temp. HY-3D), Thermocool refrigerator (HTF-259H), Autoclave (Desco), Fourier Transform-IR spectrometer (Perkin-Elmer GX2000 FTIR) and Nuclear Magnectic Resonance (NMR) spectrometer (Agilent-NMR-vnmrs400) are among the apparatuses utilized. 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-(3-Nitrophenylazo)-2-naphthol.

The method of Onunkwo and Ejikeme [7] was employed with little modifications. Distilled water (16 cm^3) and strong hydrochloric acid (16 cm^3) were used to dissolve 5 g of 3-nitroaniline. After gently shaking the reaction mixture to dissolve any hydrochloride that may have separated, the solution was cooled to five degrees Celsius. At 0 to 5 °C, add 1 spatula of urea and 4 g of sodium nitrite that had been dissolved in 20 cm^3 of water, stirring the mixture continuously. Diazotization was accomplished by progressively incorporating a cold solution of sodium nitrite into a cold solution of 3-nitroaniline while stirring continuously and keeping the temperature below 5 °C. In a 250 cm^3 beaker, 5-grams of 2-naphthol was dissolved in 45 cm^3 of 10 % NaOH while being continuously stirred to create a 2-naphthol solution. After adding the cold diazonium salt solution gradually, the reaction mixture was placed in an ice bath to cool it down to an additional 5 degrees Celsius. Crushed ice (25 g) was also added directly to the bath to stimulate temperature. The crystal ultimately separates and takes on a cherry-red colour. After remaining in an ice bath for 30 minutes while being constantly stirred, the reaction mixture was filtered using a Buchner funnel and cleaned with deionized water. The residue was air-dried for three days. Eighty-six (86.0) percent was the yield percentage. Figure 1 below illustrates the compound's synthesis pathway: 1-(3-nitrophenyl)-2-naphthol.

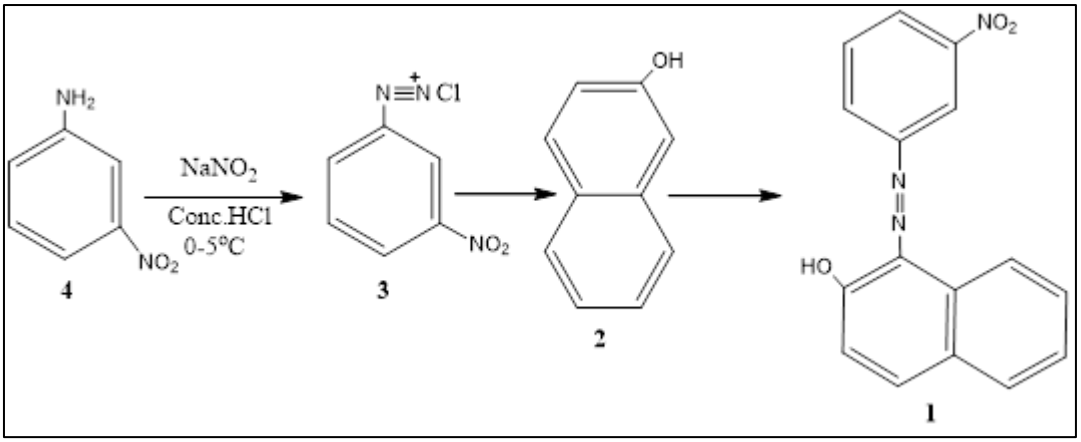

Figure 1 Synthesis of 1-(3-Nitrophenylazo)-2-naphthol

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

Using Whatman filter paper No. 1, paper borer was used to create discs with a diameter of 5 mm. The created discs were then placed in the appropriate containers. Once the autoclave was set to 121 °C for 15 minutes, the discs were subsequently placed under autoclaving to achieve sterilization and allowed to cool. Later, the discs were left to absorb, in turn, 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 [drug discs for Augmentin (Au) and Oflocitoxin (OFX)], which were kept for a subsequent analysis. Since the more concentrated a substance is, the more the efficacy; therefore, the rational for selecting the various concentrations was aimed to determine an optimal analytic effect. The capacity of each generated disc to absorb the sample concentrations is approximately 0.01 mL. The prepared plates inoculated with *Staphylococcus aureus, Aspergillus fumigatus,* and *Escherichia coli* were incubated for 24 hours after the discs with their corresponding concentrations were placed on them. The zone of inhibition was measured in millimeters, and the minimal inhibitory concentration computed as described by Onunkwo and Ezechi [12].

2.4. Instrumental Characterization of the 1-(3-Nitrophenylazo)-2-naphthol

The formation of the 1-(3-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-(3-nitrophenylazo)-2-naphthol in the range of 4000 – 500 cm^{-1}. The NMR spectrometer (Agilent-NMR-vnmrs400) is a highly specialized instrument that was used to capture the proton (^{1}H NMR) and carbon 13 (^{13}C or C13 NMR) spectra of the synthesized compound using $CDCl_3$ solvent.

3. Results and Discussion

3.1. Characterization of the 1-(3-Nitrophenylazo)-2-naphthol

The UV-visible data showed max. at 434 nm and 508 nm due the colour absorption and transition of the compound. The Infra-red data (Figure 2) showed the functional group O-H (2998 cm^{-1} broad), N=N (1497.44 cm^{-1} stretch), NO_2 (1344.29 cm^{-1} stretch) C=O (1625 cm^{-1} stretch), C=C (1527.40 cm^{-1} stretch), C-N (1437.5 cm^{-1} stretch), C-O (1248.65 cm^{-1} stretch), C-C (1125.65 – 1096.60 cm^{-1} stretch), C-H (999 – 503.42 cm^{-1}). The HNMR data (Figure 3) showed the δ 6.760 and δ 6.785 for the most shielded protons and the deshielded protons occurred at δ 8.451, δ 8.471 and δ 8.487; then, δ 7.260 – 7.430, δ 7.541 – 8.066 and δ 7.195 – 7.734 were other corresponding aromatic protons observed. C13NMR data (Figure 4) solvent ($CDCl_3$) signal was observed at δ 112.285, C-OH (δ 174.926); then, C-NO_2 (δ 149.378), δ 145.431 (C-N), δ 141.985, δ 133.082, and δ 120.611 – 130.964, were also observed, corresponding to the aromatic 13C groups in the compound. The data was found to fall within the reference data of Donald et al. [20] and Onunkwo and Ejikeme [7], Shahab et al. [21], and Onunkwo and Ezechi [12].

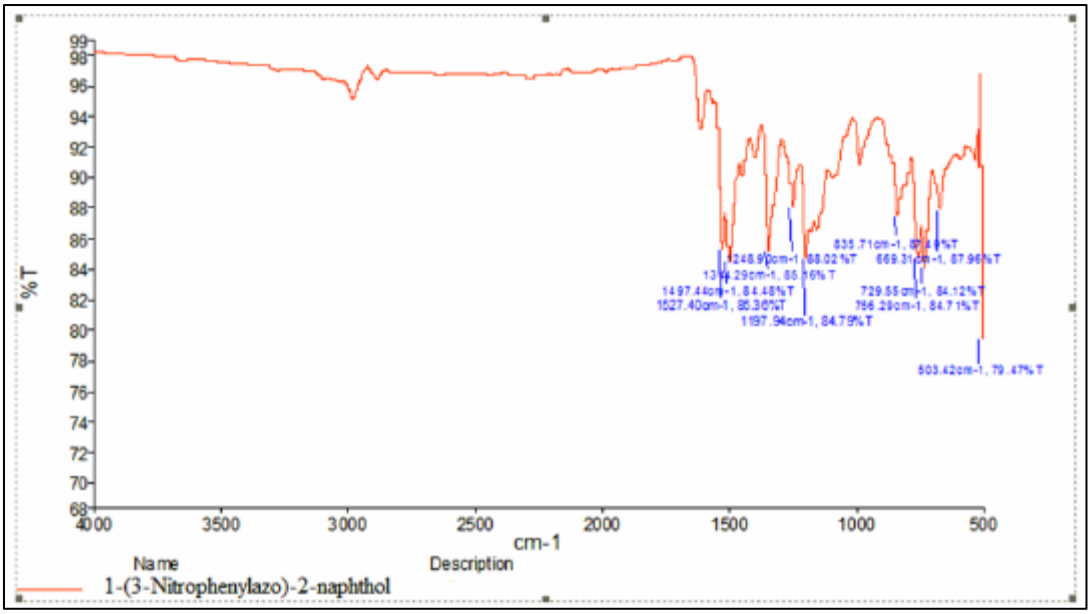

Figure 2 Infra-red (FT-IR) Spectrum of 1-(3-Nitrophenylazo)-2-naphthol

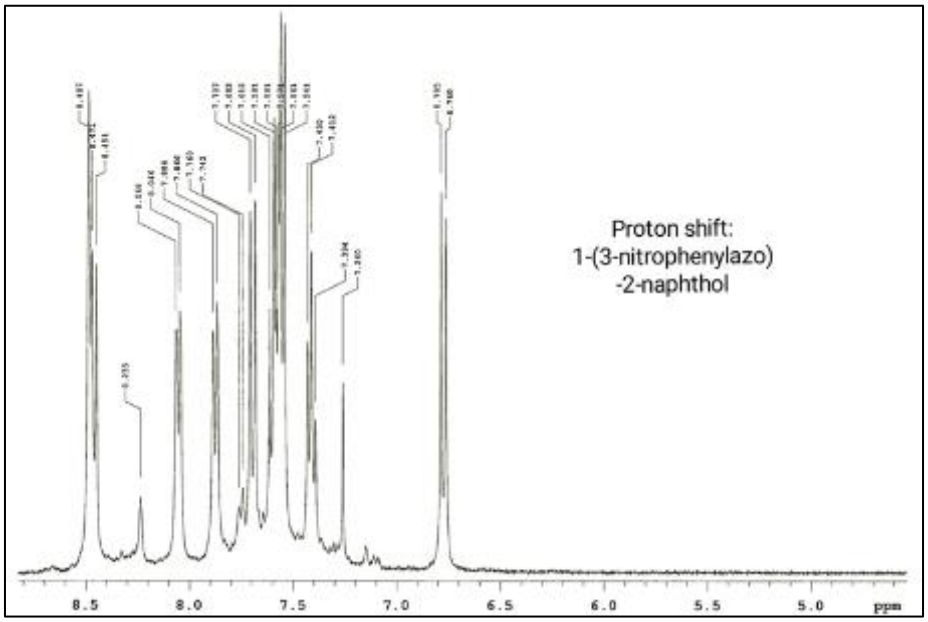

Figure 3 HNMR Spectrum of 1-(3-Nitrophenylazo)-2-naphthol

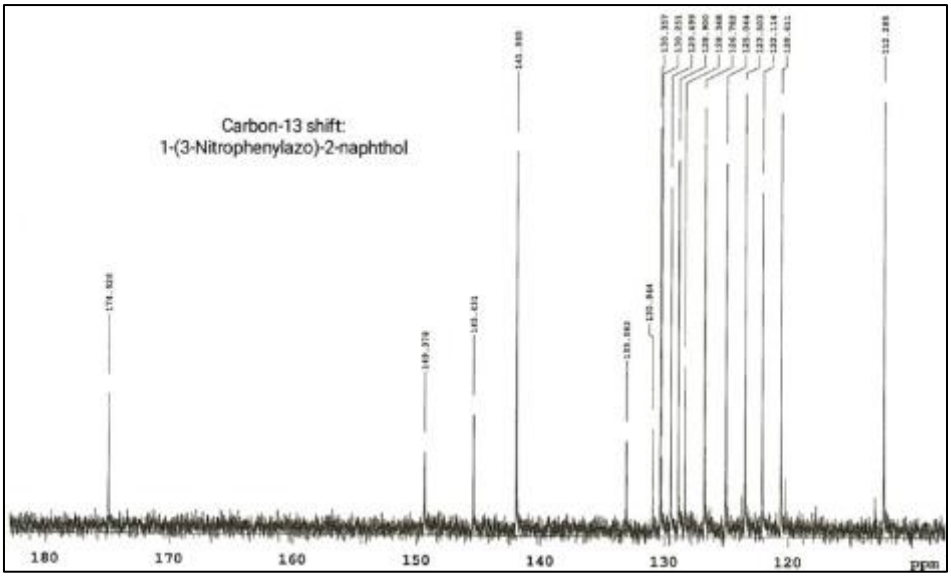

Figure 4 C13NMR Spectrum of 1-(3-Nitrophenylazo)-2-naphthol

3.2. Antimicrobial Studies of the 1-(3-Nitrophenylazo)-2-naphthol

The antimicrobial analysis of the 1-(3-nitrophenylazo)-2-naphthol was carried against *Staphylococcus aureus, Escherichia coli, Aspergillus fumigatus.* Augmentin (Au) and Oflocitoxin (OFX) were used as positive control drugs.

Table 1 How various sample dilutions and control affect the pathogenic strains

Organism	Unit	1-(3-Nitrophenylazo)-2-naphthol (µg/mL)					Control (30 µg/mL)		MIC (µg/mL)
		50	100	150	200	Raw	Au	OFX	
Staph. sp.	(mm)	R	R	R	0.2	0.5	20	20	200
E. coli		R	R	R	0.8	0.5	15	32	200
Aspergillus sp.		R	R	R	R	R	3.4	2.3	-

R=Resistant

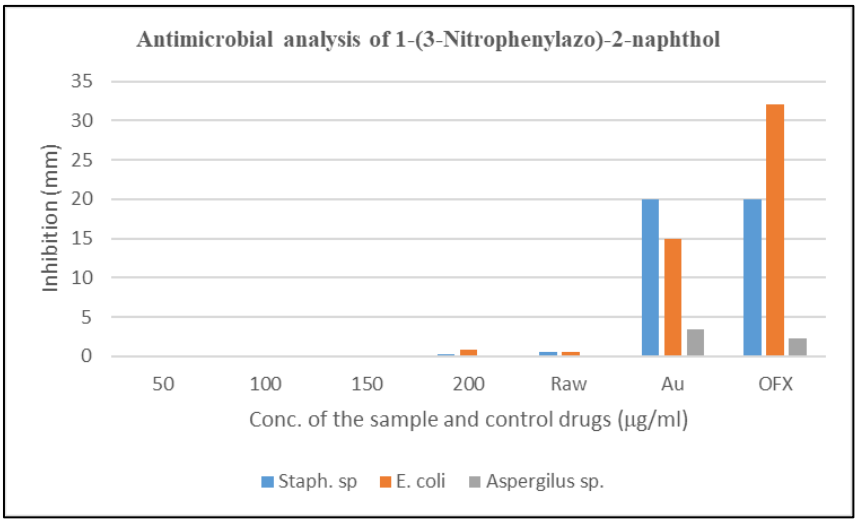

Figure 5 Antimicrobial analysis of the 1-(3-nitrophenylazo)-2-naphthol, and control [Augmentin (Au) and Oflocitoxin (OFX)], against *Staphylococcus aureus, Escherichia coli* and *Aspergillus fumigatus*

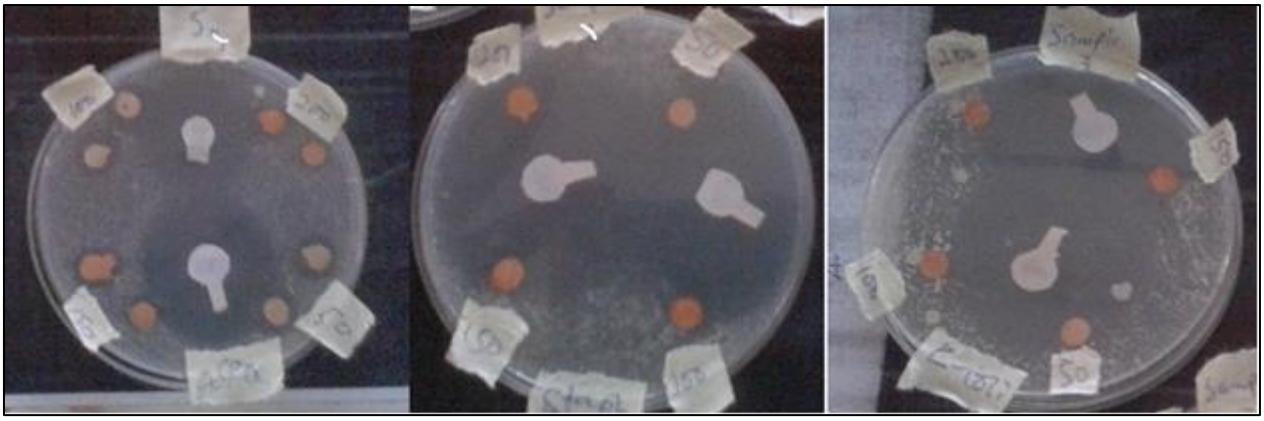

Figure 6 Antimicrobial study of the 1-(3-Nitrophenylazo)-2-naphthol against *Staphylococcus aureus, Escherichia coli, Aspergillus fumigatus*, and positive control [Augmentin (Au) and Oflocitoxin (OFX)]

From Table 1, Figure 5 and Figure 6, the organism (*Staphylococcus aureus*) was found to be resistant to the compound concentrations of 50 μg/mL, 100 μg/mL and 150 μg/mL but was inhibited by raw and 200 μg/mL concentrations of the compound at 0.5 mm and 0.2 mm, respectively. The 30 μg/mL concentrations of the control drugs [Augmentin (Au) and Oflocitoxin (OFX)] gave an inhibition of 20 mm each against the organism. The compound minimum inhibitory concentration (MIC) was estimated at 200 μg/mL. *Escherichia coli* resisted the compound's 50 μg/mL, 100 μg/mL, and 150 μg/mL concentrations but was inhibited by raw and 200 μg/mL concentrations of the at 0.5 mm and 0.8 mm respectively. The 30 μg/mL concentrations of the Augmentin (Au) and Oflocitoxin (OFX)], the control drugs, gave 15 mm and 32 mm, respectively. The compound's MIC was estimated at 200 μg/mL. The *Aspergillus sp.* resisted all the compound concentrations, which include 50 μg/mL, 100 μg/mL, 150 μg/mL, 200 μg/mL, and raw sample concentrations. As it is observed from the result, the compound performed very poorly against the fungus (*Aspergillus sp.*) when also compared to the bacteria. This may be as a result of insufficient concentration and exposure time of the compound, as fungi may have compensatory mechanisms that allow them to survive and grow despite the presence of an inhibitory compound. The 30 μg/mL concentrations of the Augmentin (Au) and Oflocitoxin (OFX), the control drugs gave 3.4 mm and 2.3 mm inhibitions respectively. The compound minimum inhibitory concentration was indeterminable for the fungus but was estimated at 200 μg/mL for the bacteria from the results. There was slight difference with the results obtained in this research in comparison with that from some the previous research [7, 12, 15, 17, 19]. For example, Onunkwo and Ejikeme [7] 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 turn of the compound 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 agents when toxicity effect is highly put into consideration. 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 [18]. 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 [22].

4. Conclusion

The compound 1-(3-nitrophenylazo)-2-naphthol synthesized by diazotization and azo coupling reactions possesses antibacterial properties against *Staphylococcus sp.* and *E. coli* but was resisted by *Aspergillus sp.* at all concentrations. The control drugs utilized were effective against the microbes, particularly against the bacteria. In that case, the synthesized compound can function effectively as an antibacterial agent in different formulations to fight infections.

Compliance with ethical standards

Acknowledgments

For the cooperation obtained in regards with the experimental and analytical elements of this research work, the authors would like to thank Prof. Okonkwo, O. J. of Tshwane University, Acadia, South Africa, Vetech Research Centre and Laboratory, IMT, Enugu, and Godfrey Okoye University, Enugu, Nigeria.

Disclosure of conflict of interest

The authors declare no competing interest.

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