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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF 1-(1-PHENYLAZO)-2-NAPHTHOL

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ABSTRACT

Pathogenic microorganisms are capable of causing various illnesses and diseases to its hosts (humans, animals and plants) and has prompted researchers in search of antibiotics to be used in treatment of such ill-health conditions. Azo dyes possess some interesting colours and usages in many industries. In the present study, 1-(1-Phenylazo)-2-naphthol azo dye was synthesized by diazotization of aniline and coupling reaction with 2-naphthol. The results obtained from the UV-vis, IR and NMR spectra were able to elucidate the important peaks in the compound. The concentrations of 200 µg/mL, 30 µg/mL control drugs [Augmentin (Au) and Oflocitoxin (OFX)] and raw sample of the compound were able to inhibit Staphylococcus aureus at 0.1 mm, 15 mm, 13 mm and 0.4 mm marks respectively while the concentrations of 200 µg/mL, 30 µg/mL Augmentin (Au, control drug), 30 µg/mL Oflocitoxin (OFX, control drug) and raw sample of the compound had inhibitions of 0.2 mm, 21 mm, 19 mm and 0.4 mm marks respectively against Escherichia coli; Aspergillus fumigatus was resistant across all prepared concentrations and raw sample of the compounds, including the 30 µg/mL control drugs [Augmentin (Au) and Oflocitoxin (OFX)] from the antimicrobial analysis carried out. The minimum inhibitory concentrations of the compound was estimated at 200 µg/mL concentration.

Contribution/Originality: The study contributes in the existing literature of 1-(1-phenylazo)-2-naphthol, and recommends its use as antibiotics for treatment of certain bacterial related diseases and infections in comparison with some commercial drugs which could be toxic and highly concentrated to the infected hosts.

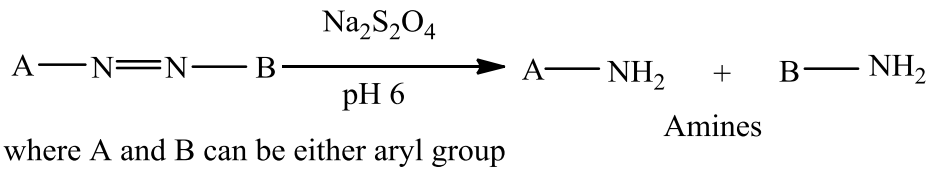
1. INTRODUCTION

Azo dyes possess at least one nitrogen-nitrogen double bond usually called azo group (N=N). However compounds with one azo group are monoazo compounds, while those with two, three are called diazo and triazo compounds. These side groups are necessary for imparting colours, causing high intensities and shades in material being applied [1]. Variations in the chemical structures of azo dyes are readily achievable and this has made the azo dyes attain a wide range of usage among other dyestuffs [2]. Azo-naphthol dyes showed a better hue with good colour fastness property to washing, rubbing and light [3]. The synthesis of most azo dyes involves diazotization of a primary amine, followed by the coupling with one or more nucleophiles usually amino and hydroxyl groups. Diazotization process involves the treatment of the primary amine with nitrous acid formed by the reaction of strong mineral acid (preferably hydrochloric acid) and sodium nitrite to produce diazonium salt while Azo coupling

is the attack of the electrophile (diazonium salt) on the nucleophile (electron-releasing groups), usually amino or hydroxyl group [4].

Effect of light from Ultraviolet, infrared, nuclear magnetic resonance spectrometer on azo dye compound have made them exhibit some interesting phenomenon such as isomerism (example phenylazonitrobenzene), tautomerism (example para red forming ketohydrozone), photochromism (example disperse red), irreversible hydrogen bonding (usually occurred at hydroxyl group of azo compounds) and quaternization of cylic azo dyes for increased colour intensity [5].

The work of Emerson [6] reported that azo dyes can be reduced to amines. Use of azo reductase/catalysts or sodium dithionite can cleave the azo groups to form amines.



The high and bright colours intensity of azo dyes coupled with their cost-effectiveness of the manufacturing process is a great advantage while people go into production of azo dyes. Other properties of azo dyes are their resistance to oxidizing agent and non-toxicity, as well as their non-basicity and slight acidity of the compounds [7].

Azo dyes have been found to be the most widely used dyes in today's industries (60-70 % of the market). They have been applied in textile. The discovery of German chemist William Böettinger in 1884 that ingrain azoic dyeing allows the dye to bond strongly to fabrics creating an excellent fastness properties have eased the process of dyeing of the material fabrics. Azo dyes are also used as oxidation hair dyes for hair colouring. Other uses of azo dyes are 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 colorant (Butter Yellow) and pharmaceutical [8-11]. However, despite of the functionality and usage of azo dyes, azo dyes derived from benzidine tends to be carcinogenic as some bacteria in human body converts the azo dyes (through skin interaction by sweating) to aryl amine, proven to be carcinogenic and this resulted to proper regulations on azo dyes [12]. 1-(1-Phenylazo)-2-naphthol also called Sudan 1 is an azo dye, possesses a strong colour shades and could possess some antimicrobial properties against some strains of pathogenic microorganisms.

2. MATERIAL AND METHODS

The chemical reagents purchased from BDH chemicals and were used without further purification. The equipment used are pH meter (PHS-3C), precision weighing balance (Y-502N), melting point apparatus, UV-visible spectrometer (Metro UV-5800PC), incubator, magnetic stirrer (constant temp. HY-3D), autoclave (Desco), thermocool refrigerator (HTF-259H), FT-IR spectrometer (Perkin-Elmer GX2000 FTIR) and NMR spectrometer (Agilent-NMR-vnmrs400).

2.1. Synthesis of 1-Phenylazo-2-Naphthol 1

Aniline **4** (4.5 cm^3) was dissolved in concentrated hydrochloric acid (16 cm^3) and distilled water (16 cm^3). The reaction mixture was shake gently to dissolve any hydrochloride which might have separated and the solution was cooled to a temperature of 5 °C. Sodium nitrite (4 g) dissolved in 20 cm^3 of water and 1 spatula of urea was added with constant stirring at a temperature of 0 - 5 °C. Diazotization was achieved by gradually adding the cold solution of sodium nitrite to a cold solution of aniline with constant stirring, making sure the temperature never exceed 5°C. A solution of 2-naphthol was prepared dissolving 2-naphthol **2** (5 g) in 45 cm^3 of 10 % NaOH in a 250 cm^3 beaker with constant stirring. This was followed by slow addition of the cold phenylazonium chloride **3** salt solution and

the reaction mixture was further cooled below 5 °C by placing it in an ice bath and by direct addition of crushed ice (25 g). A red-orange colour and red-orange crystal develops and eventually separate. The reaction mixture was further allowed to stand in an ice bath for 30 minutes with constant stirring, after which it was filtered through a Buchner funnel and washed with water. The residue was air dried for 3days and has 88.06 % yield. The compound possess melting point of 120 °C. The synthetic reaction Figure is shown in Figure 1 below.

The UV-visible data against ethanol showed max. at 440 nm and 504 nm of orange-red colour, indicating a conjugated and delocalized n – π^* (non-bonding to pie star) electron transition of the compound [13, 14]. The Infra-red data against KBr showed the functional group O-H (3100 cm^{-1} broad), N=N (1493.44 cm^{-1} stretch), C=O (1625 cm^{-1} stretch), C=C (1562.5 cm^{-1} stretch), C-N (1445.77 cm^{-1} stretch), C-O (1138.64 cm^{-1} stretch), C-C (1255.22 cm^{-1} stretch), =C-H (2938 cm^{-1} aromatic). The HNMR data using $CDCl_3$ of δ8.524 – 8.547, δ7.669 – 7.727, δ7.516 – 7.578, δ7.449-7.489, δ7.293 – 7.313, δ7.354 – 7.394, δ7.260 – 7.274, δ6.840 and δ6.863 are proton signals exhibited in phenyl and 2-naphthol aromatic rings.

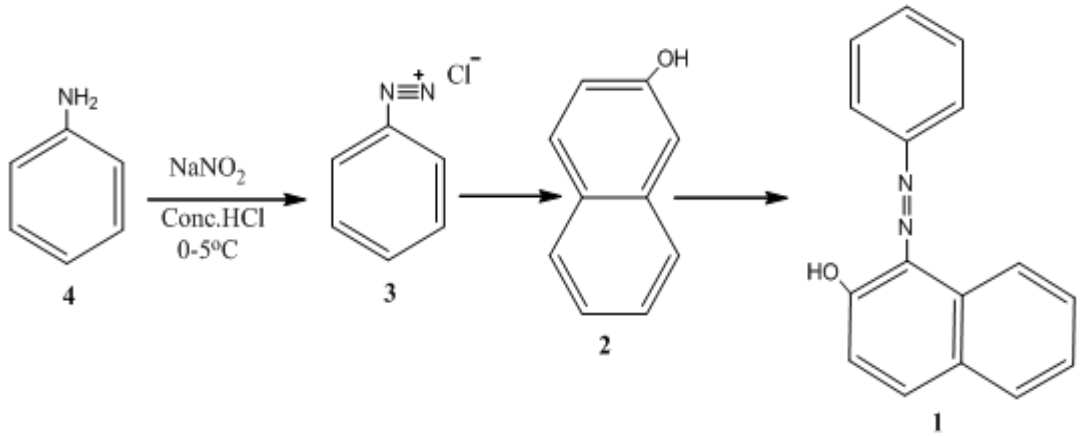


Figure-1. Synthesis of 1-(1-phenylazo)-2-naphthol.

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

By using Whatman filter paper No. 1, Discs of 5 mm in diameter were produced by using a paper borer. After that, the prepared disks were put in suitable containers. Then, the discs were subjected to autoclaving in order to sterilize the disks (adjusting the conditions of autoclave to 121°C for 15 mins) and left to cool. Later on, the discs were allowed to suck up the sample filtrate at 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, raw and 30 µg/mL each of control antibiotics [Augmentin (Au) and Oflocitoxin (OFX) drug discs] concentrations, maintained for later assay. The produced discs (each one) have the ability to absorb about 0.01 mL of the sample concentrations. The discs with concentrations were place on the prepared plates inoculated with *Staphylococcus aureus*, *Aspergillus fumigatus* and *Escherichia coli* and incubated for 24 hrs. The zone of inhibition was observed, measured in millimeter and the minimum inhibitory concentration estimated.

3. RESULTS AND DISCUSSIONS

Table-1. The effect of different dilutions of the samples against pathogenic isolates.

Organisms	1-(1-phenylazo)-2-naphthol (µg/mL)					Control		MIC
	50	100	150	200	Raw	Au	OFX	
Staph. Sp	R	R	R	0.1	0.4	15	13	200
E. coli	R	R	R	0.2	0.4	21	19	200
Aspergillus. sp	R	R	R	R	R	R	R	-

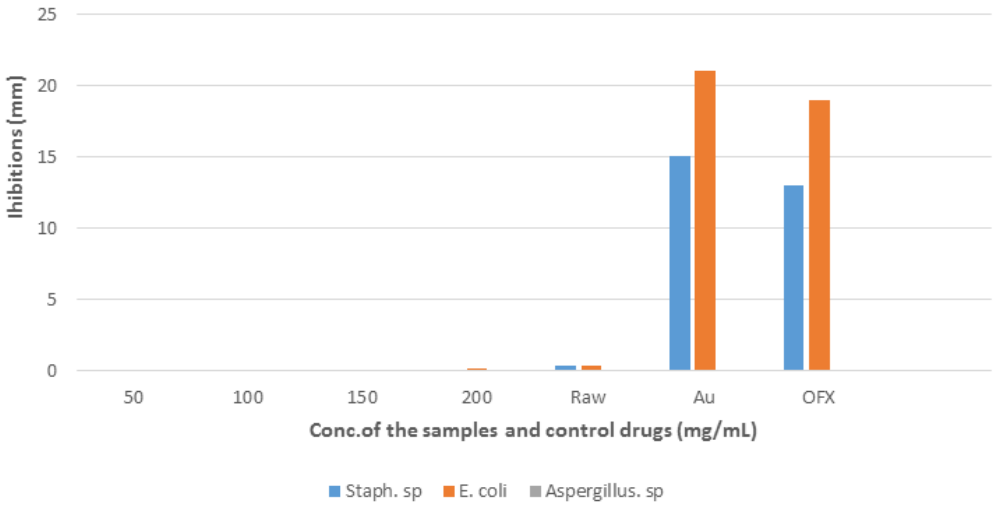


Figure-2. Antimicrobial analysis of 1-(1-phenylazo)-2-naphthol.

From the Table 1 and Figure 2, the organism (*Aspergillus sp.*) was resistant on all the concentrations (50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL and raw) of compound and the minimum inhibitory concentration is zero. The organism was also resistant against the control drug Augmentin and Oflocitoxin at 30µg/mL concentrations.

The organism (*E.coli*) was resistant against 50 µg/mL, 100 µg/mL and 150 µg/mL concentrations of the compound but 200 µg/mL and raw concentrations of the compound were able to inhibit the organism at 0.2 mm and 0.4 mm respectively. The minimum inhibitory concentration is 200 µg/mL while the control drugs Augmentin (Au) and Oflocitoxin (OFX) at 30 µg/mL concentrations have 21 mm and 19 mm inhibitions respectively.

The organism (*Staphylococcus. sp*) was resistant (R) against 50 µg/mL, 100 µg/mL and 150 µg/mL concentrations of the compound but 200 µg/mL and raw concentrations of the compound were able to inhibit the organism at 0.1 mm and 0.4 mm respectively. The minimum inhibitory concentration is 200 µg/mL while the control drugs Augmentin and Oflocitoxin at 30 µg/mL concentrations have 15 mm and 13 mm inhibitions respectively.

4. CONCLUSION

The red-orange crystal azo compound: 1-(1-phenylazo)-2-naphthol synthesized through diazotization and azo coupling reactions possess antimicrobial property against *Staphylococcus aereus* and *E. coli* but was resisted by *Aspergillus sp.* at all concentrations. This means that it can be used as antibiotics in drug formulations for treatment of certain bacterial related illnesses with regards to some commercial control drugs.

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