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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES OF AZO DYES DERIVED FROM SULPHANILIC ACID

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Abstract

Azo dyes were synthesized using sulphanilic acid as the diazo component and betaoxynaphthoic acid(BON acid) and 1-naptholas the coupling components,the wavelength of maximum absorbtion is at 471.00 and 509.00 nm on the solvent ethanol for dye 1 and dye2respectively.thus the synthesized dyes were characterized using Gallan kamp melting point apparatus in which dye 1 and dye 2 have the melting point range of $298℃ - 200℃ respectively$. Fourier transform infrared spectroscopy studies shows that dye 1 has the following functional group and vibrational frequencies;3429.2 cm^{-1} NH-stretch,2981.9 cm^{-1} CH-stretch,1722.0 cm^{-1} ,1677.3 cm^{-1} N=N stretch.While dye 2 has the following;1722.0 cm^{-1} NH-stretch,2881.9 cm^{-1} CH-stretch,1722.0 cm^{-1} ,1677.0 cm^{-1} N=N stretch.The antimicrobial analysis performed shows that the dye were effective for all the concentration except 12.5,6.25 and 3.125 mg/ml for both dye 1 and 2.The highest zone of inhibition was 15mmat 3.125mg/mm for dye 1 against Escherichia coli and 13 mm against Staphylococcus aureus.The result for minimum bactericidal concentration shows that both dye 1 and dye 2 were active on all the tested organism.Hence the analysis carried out was observed that both dye 1 and dye 2 had no effect on the tested fungi which is Candida albicans respectively.

1.0 INTRODUCTION

Dyes are organic colour compounds which contains groups such as -N=N (azo group), -C=O (carbonyl group), (nitro) -NO, etc. and auxochromic group such as amino and substituted amino groups. In other words dyes are organic colored substance that chemically bonds to the substrate to which it is being applied, this distinguish dyes from pigments which do not chemically bind to the material they color. The dye is generally in an aqueous solution and may require a mordant to improve the fatness of the dye on the fiber. Both dyes and pigment are colored, because they absorb only some wavelength of visible light. Dyes are usually soluble in water whereas pigments are insoluble. Some dyes can be rendered insoluble with the addition of salt to produce a lake pigment. (Elmer, 2004).

The majority of natural dyes are derived from plant sources: roots, berries, barks, leaves, wood, fungi and lichens. Textile dyeing dates back to the Neolithic period. Throughout history, people have dyed their textile using common locally available materials. Scarce dye stuffs that produce brilliant and permanent colors such as the natural invertebrate dyes, tyrian purple and crimson kermes were highly prized luxury items in the ancient and medieval world. Plant based dyes such

as wood, indigo, saffron and madder ‘were important trade goods in the economies of Asia and Europe across Asia and Africa, patterned fabrics were produced using resistant dyeing techniques to control the absorption of color in piece-dyed clothes (Booth, 2000). Dyes from the new world such as coehineal and logwood were brought to Europe by the Spanish treasure fleets, and the dye stuffs of Europe were carried by colonialists to America. Natural dyes are colorants derived from plants, invertebrates and minerals. The majority of natural dyes are vegetable dyes from plant source. They are sustainable because they are renewable andbiodegradable but they cannot fulfill the huge demand from the textile sector in view of preferential use of land for food or feed purposes. Also, over exploitation of natural resources to obtain dyes may result in deforestation of natural resources. From the plant source e.g. indigo dye which is from *Endigoferattnctora,* madder root, we obtain red, pink and yellow color, pomegranate peel gives yellow color while onion peel gives red or maroon color. Insects were the main source of natural dye from animal origin. Most of these provide red color. We have octopus and cattle fish (Batler, 2009).

3.0 MATERIALSANDMETHODS

3.1Materials

Weighing balance, beaker, measuring cylinder (10 cm^3). Thermometer, funnel, melting point instrument, UV-visible spectrophotometer, round bottom flask, glass rod, spatula, boiling tube and Fourier transformed infrared (FT- IR) spectrophotometer, methanol, distilled water, sodium nitrite, hydrochloride acid, ice block, Betaoxynaphthoic acid (BON acid, and sulphanilic acid.

3.2 Procedure

3.2.1 Diazotization of the sulphanilic acid

Sulphanilic acid (4.0g, 1 mol) was dissolved in 50 ml of NaOH. The solution was then cooled in an ice bath. A solution of sodium nitrite (50 ml, 1 mol) and 10 ml concentrated HCl was added drop –wise with continous stirring for 30 minutes to form the diazonium salt. As shown in the reaction below.

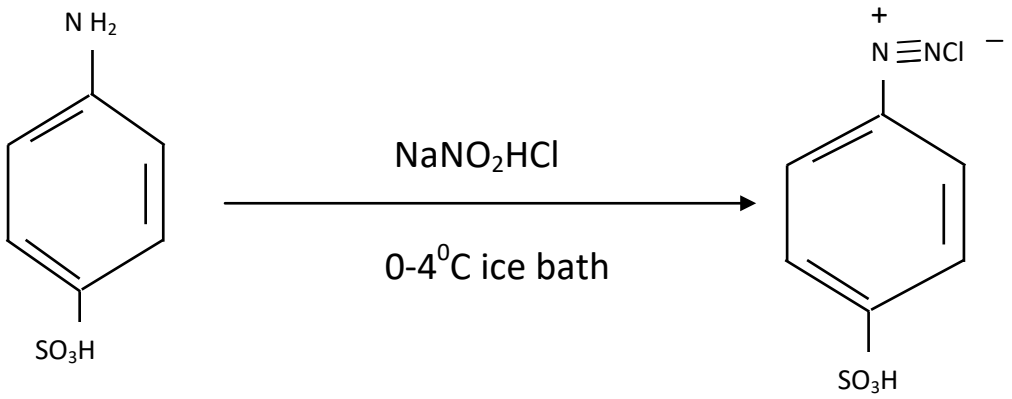


Fig: 3.1: Diazotization of sulphanilic acid

3.2.2 Coupling reaction with betaoxynaphtoic acid (BON acid)

Coupling reaction was carried out with betaoxynaphtoic acid and was added drop wise to the diazonium salt of sulphanilic acid in the ice bath. The mixture was stirred vigorously for a hour and left over night after which it was filtered, recrystallization and stored in refrigerator.

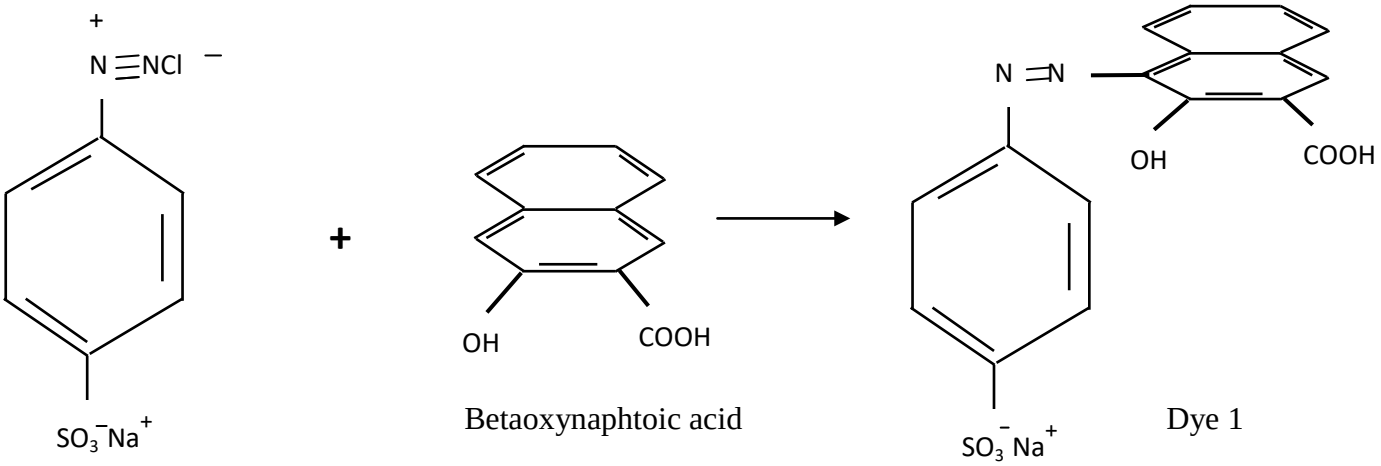


Fig: 3.2.2: Diazonium salt reacting with betaoxynaphtoic acid

3.2.2.1 Coupling with 2-naphtol

In a test tube 0.8 ml of diazomium salt was thoroughly mixed with 2-naphtol. The precipitate of the dye was filtered and allowed to dry at room temperature. As shown below;

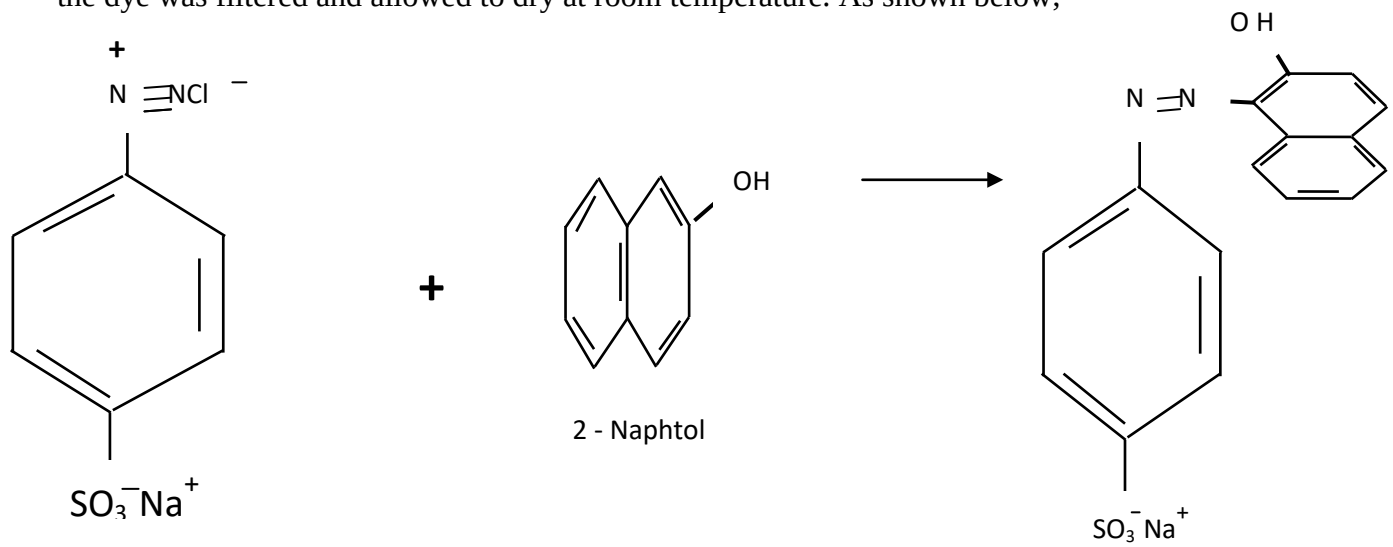


Fig: 3.2.2.1 Coupling with 2-naphtol

3.2.3 Melting point determination

The melting point was determined by the open capillary method and will be expressed in ℃. The dyes will be determined using gave ncanp. Electrothermal melting point apparatus.

3.2.4 Determination of visible absorption spectroscopy

Visible absorption spectra was recorded on ethanol and DMSO (Jen way UV/visible spectrophotometer, model: 6405) by (Dudley and Lan 1980)

3.2.5 Fourier transforms infrared spectroscopy Pelkin Elmer spectrum 100 (FT-IR)

The infrared spectra of the dyes were determined using the FT-IR spectrophotometer to determine the functional groups present.

3.2.6 The antimicrobial screening

The following pathogenic microorganisms such as, Methicillin resistant *Staphylococcus avrous, Staphylococcus aureus, Escherichia coli*, *Candida albicans* and *Candidatropicalis* was used to determine the antimicrobial activities of dye sample. The microbes was obtained from the department of medical microbiology, Ahmadu Bello University Teaching Hospital, Zaria all the isolates was checked for purity and cultured in slants of nutrient agar for the bacteria and sabourand dextrose agar for the fungi.0.5 of the dye sample was weighed and dissolved in 10 cm3 of distilled water so as to obtain a concentration of 50 mg/ml of the dye sample. This was the initial concentration of the dye sample used to check the antimicrobial activities of the dye sample. Muller-Hinton agar was the medium used as the growth medium for the microbes, the medium was prepared according to the manufacturer's instructure, sterilized at 121 ℃ for 15 minutes. The sterilized medium was then poured into sterilized petri-dishes, the plate was allowed to cool and solidified (Clinical and Laboratory Standard Institute, 2006).

Well diffusion method was used for screening the dye sample; the sterilized medium was seeded with 0.1 cm3 of the standard inoculums of the test microbes. The inoculums were spread evenly over the surface of the medium by the use of a sterilized swab. By the use sterilized cork borer of 6 mm in diameter a well was cut at the center of each inoculated medium 0.1 m of the solution of the dye sample of concentration of 50 mg/ml was then introduced into each well on the medium. The inoculated medium was inoculated at 37 ℃ for 24 hours, after which each plate was observed for zone of inhibition, the zone was measured with a transparent ruler and the result was recorded in millimeters (Clinical Laboratory Standard Institute, 2006).

3.2.7 Minimum inhibitory concentration (MIC)

MIC was determined using the broth dilution method. Muller-Hinton broth was prepared and 10 cm was dispersed into a test-tube, sterilized at 12 ℃ for 15 minutes and then allowed to cool. Mc-farland's turbid by standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared 100 cm^3 was also dispersed into sterile test-tube and the test microbe was carried out in the normal saline until the turbidity matches with that of the Mc-farland's scale by visual comparison, at this point the test microbe might have a concentration of about 1.5×10^3 cfu/ml, two fold serial dilution of the dye sample in the sterile both was made to obtain the concentration of 50 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml, the initial concentration resulted by dissolving 0.5g of the dye sample in 10 cm^3 of the sterile broth, having obtained the

different concentration of the dye sample in the broth, 0.1 cm^3 of the test microbes in the normal saline was then introduced into the different concentration of the dye sample in the broth. It was then incubated at 37 ℃ for 24 hours after which each test-tube was observed for turbidity, the lowest concentration of the dye sample in the broth which might show no turbidity was also recorded as the minimum inhibitory concentration (Wayne, 2006).

3.2.7.1 Minimum Bactericidal/Fungicidal (MBC/MFC)

This MBC was carried out to check whether the test microbes would be killed or the growth would be inhibited. Muller-Hinton agar was prepared and sterilized at 121 ℃ for 15 minutes, poured into the plates, the content of the MBC in the medium, incubated at 37 ℃ for 24 hours, after which each plate was observed for colony growth, the MBC/MFC was the plate of the lowest concentration of the dye sample without colony growth (Wayne, 2006).

4.0 RESULTS AND DISCUSSION

4.1: Physical Properties of the Synthesized Dyes

S/N	sample	Melting Points ℃	Colour
1	Dye 1	297-298	Purple
2	Dye 2	198-200	Orange

Table 4.2: Wavelenght of maximum absorption (λmax (nm))

S/N	Sample	Ethanol
1	Dye 1	471.00
2	Dye 2	509.00

Fig 4.1: Wavelength of Dye 1

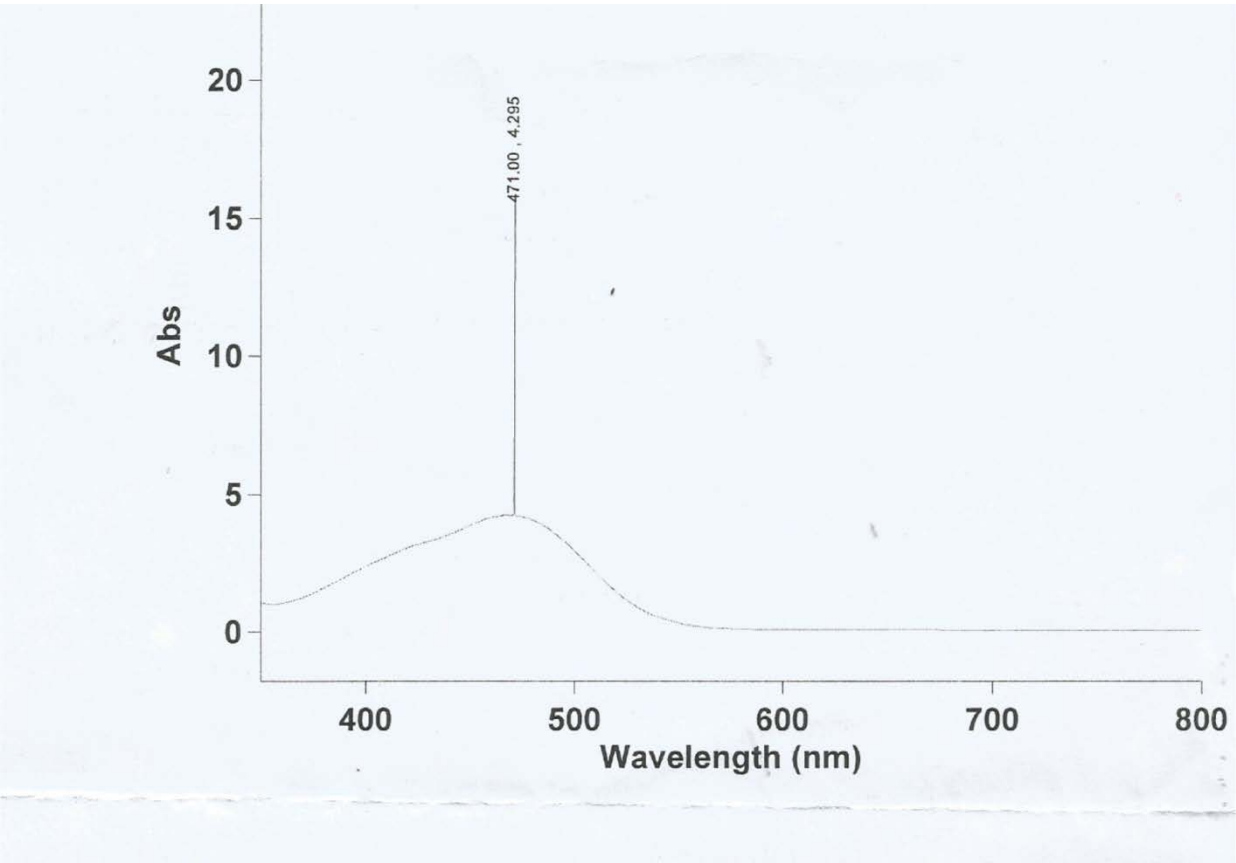
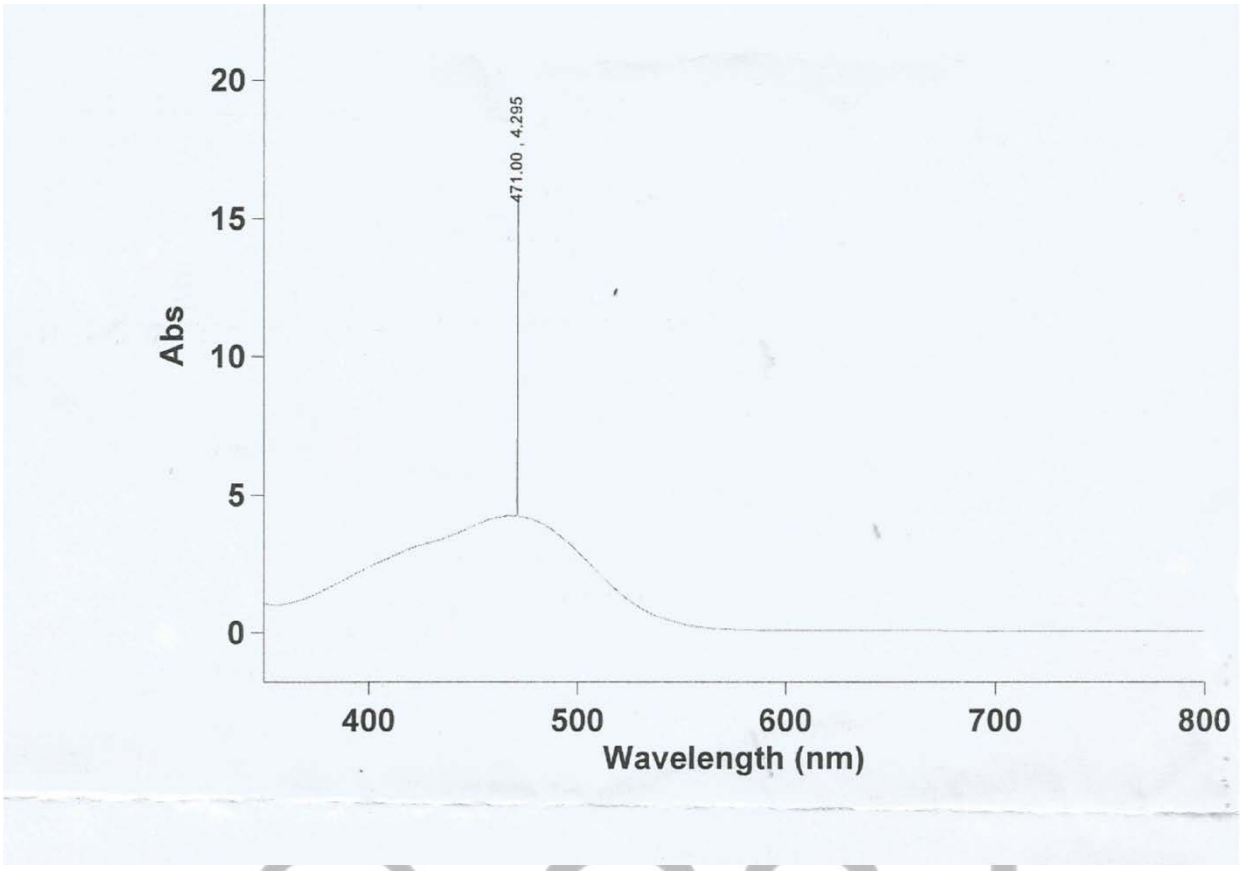


Fig 4.2 Wavelength of Dye 2



4.3 The Infra-red Absorption of the Synthesized Dyes

Table 4.3: IR-vibrational Frequencies of the Synthesized Dyes

Dyes	**IR Vmax (cm^{-1})**
1	3429.2 (NH-Stretch), 2981.9 (CH-Stretch), 1722.0, 1677.3 cm^{-1} (N=N)
2	3429.2 cm^{-1} (NH-Stretch), 2881.9 (CH-Stretch), 1722.0, 1677.3 (N=N)

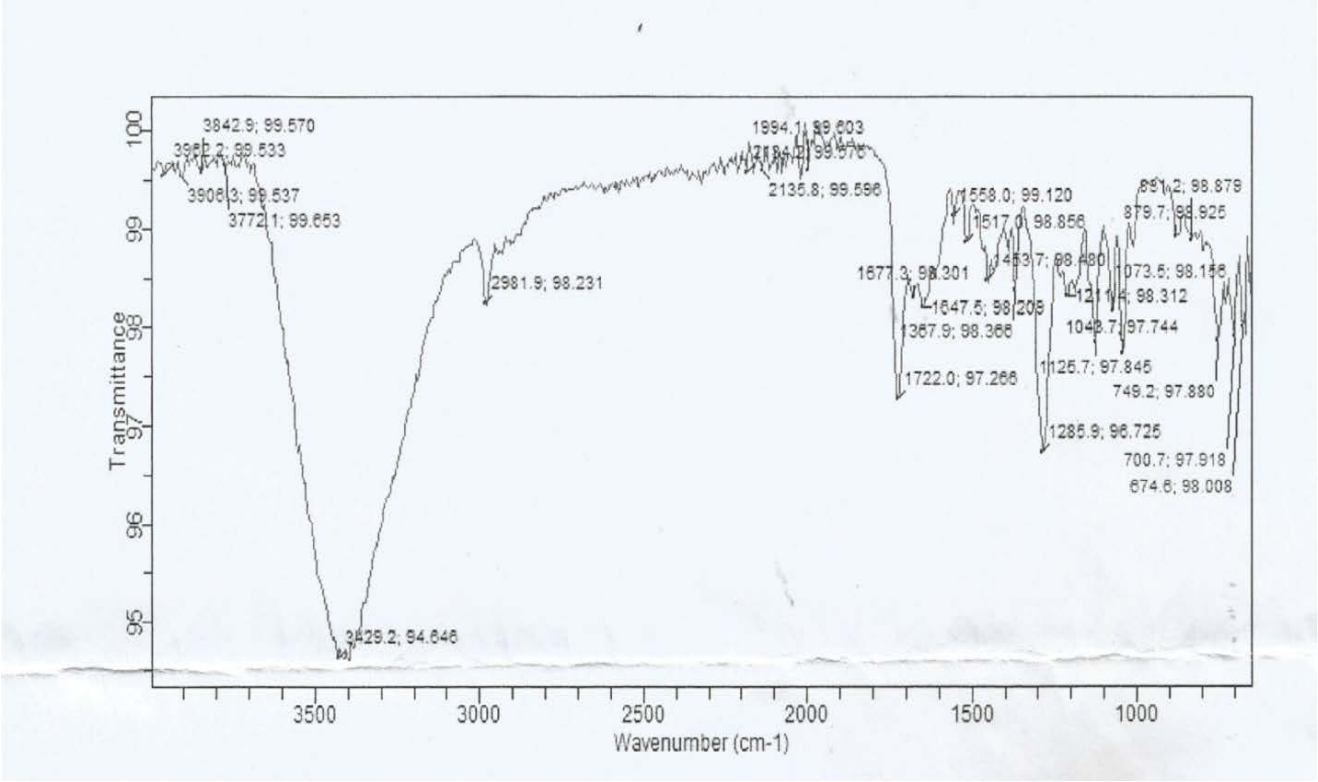


Fig: 3.2.4.1 FT-IR spectrum of Dye 1

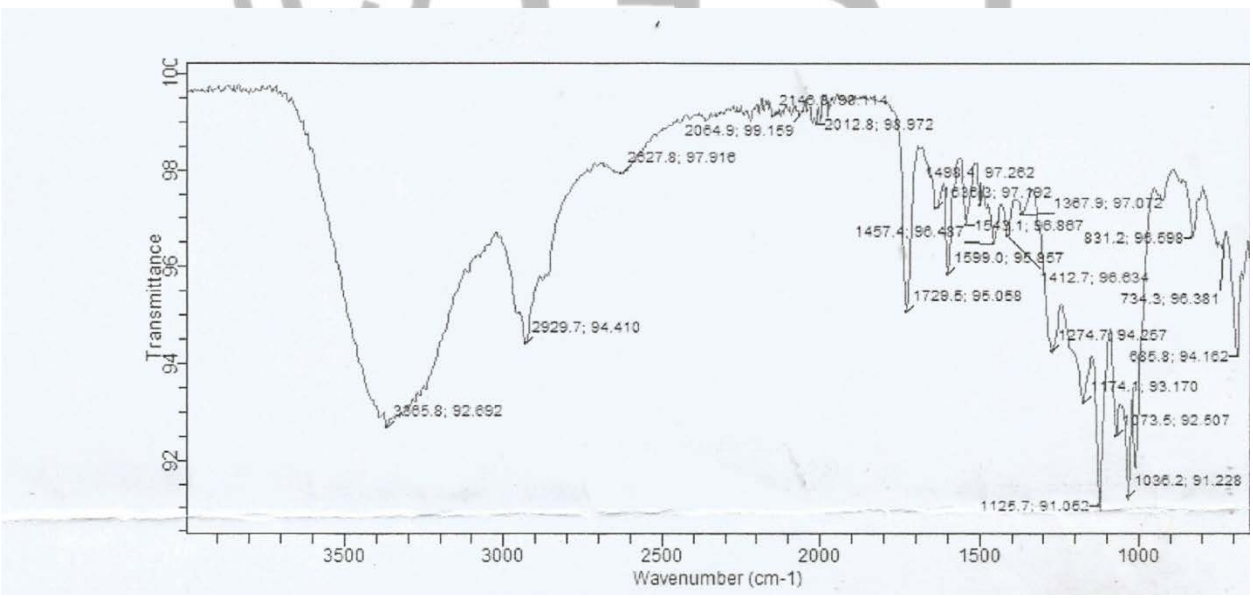


Fig: 3.2.4.2: FT-IR spectrum of Dye 2

Table 4.4: Determination of Inhibitory Activity (Sensitivity Test) of the Dye Sample Using Agar Well mg/ml)

Test Organism	Dye 1				Dye 2			
	50	12.5	6.25	3.125	50	12.5	6.25	3.125
Escherichia coli	15	-	-	-	-	-	-	-
Staphylococcus aureus	13	-	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-	-	-

Keys:

- No growth

\+ Growth

Table 4.5: Minimum Inhibitory Concentration (MIC) of the Synthesized Dyes Against Test Microorganisms (mg/ml)

Test Organism	Dye 1				Dye 2			
	50	12.5	6.25	3.125	50	12.5	6.25	3.125
Escherichia coli	C	C	ST	T	C	C	C	C
Staphylococcus aureus	C	C	C	ST				
Candida albicans	C	C	C	C	C	C	C	C

Keys:

C = Clear – No Growth

ST = Slightly Turbid

T = Turbid

Table 4.6: Bactericidal/Fungicidal Concentration (MBC/MFC)

Test Organism	Dye 1				Dye 2			
	50	12.5	6.25	3.125	50	12.5	6.25	3.125
Escherichia coli	+	+	-	+	-	-	-	-
Staphylococcus aureus	+	+	-	+	-	-	-	-
Candida albicans	-	-	-	-	-	-	-	-

Keys:

\+ Growth

- No Growth

Antimicrobial analysis of the synthesized dye samples.

Antimicrobial analyses of the sample were carried out and table 4.4 shows the inhibitory activity (Zone of inhibition) for all the test organisms. The result shows that the dye 1

was effective for all concentration except 12.5, 6.25 and 3.125 mg/ml concentration, dye 2 has no effect in all the concentrations. This is simply because of the dilution concentration of the dye sample in dye 2. The highest zone of inhibition was 50 mg/ml at 15 mm and 13 mm against *Staphylococcus aureus* and *Escherichia coli* respectively for dye 1, but for dye 2 there is no growth of inhibition which means dye 1 has high effect than dye 2 respectively.

- Table 4.5 shows the minimum inhibitory concentration of the synthesized dyes against the test microorganism;The result reveled that dye 1 completely inhibits the growth of microorganisms at 3.125 mg/ml, meaning that any concentration lesser than 3.125 mg/ml will allow the growth of these microorganism and above 3.125 mg/ml, there will be no growth for either bacteria or fungi.
- Table 4.6 shows the minimum bactericidal/fungicidal concentration of the synthesized dyes against the test organisms.For dye 1;at 6.25 mg/ml *Staphylococcus aureus* and *Escherichiacoli* had a complete bactericidal effect and *Candida albicans* is not antifungal which means it has no effect on the organism in all the concentrations.For dye 2;*Staphylococcus aureus, Escherichia coli* and *Candidaalbicans* had no effect against all the concentrations respectively. According to wolfgang,usually the more concentration on antimicrobial agent the more rapidly microorganisms are destroyed (Wolfgang, 1999).Dye 1 and dye 2 as an antimicrobial agent kills or inhibits the growth of microorganisms.However the larger the zone of inhibition the more susceptible (sensitive) the organism is to the antibiotic.

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