SYNTHESIS, EXTRACTION AND TOXICITY STUDIES OF INDIGO DYES AND THEIR APPLICATION ON COTTON FABRIC.

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Abstract
This paper aims to conduct a comprehensive study of the synthesis, extraction and toxicity of indigo dye and its application on cotton fabrics.it will employ an experimental approach to quantify the synthesis rate, extraction efficiency and toxicological properties of indigo dye, in order to determine its potential applicability for cotton fabric dyeing. The synthesis of the indigo dye was accomplished by reaction o-nitrobenzaldehyde and acetone under alkaline condition. The characterization was determine using UV-Visible spectroscopy, FTIR, and melting point decomposition. The extraction of indigo dye from the reaction mixture was studied in terms of the different solvent used and the concentration of toxicity of the indigo dye was accessed using Acute /Oral toxicity test. The results obtained showed that indigo dye can be successfully synthesis and extracted at high yield, and the toxicity level was found to be low level with record of no mortality rate. The results indicate that indigo dye is an appropriate candidate for used in dyeing cotton fabrics due to its low toxicity and excellent extraction efficiency. Further studies suggest to examine the parameter such as K.S value and NMR studies.

Keywords: Synthesis, Characterization, Indigo dye, Toxicity.

INTRODUCTION

A Dye is usually a coloured organic compound or a mixture that may be used for imparting colour to a substrate such as Cloth, Paper Plastic, and Leather in a reasonable permanent fashion. dyes were obtained from animal and vegetable source, today, most of the available dyes are synthetic prepared from aromatic Compound which are obtained from Coal tar or Petroleum.
The used of synthetic dyes has an adverse effect on all forms of life. Presence of Sulphur, naphthol, vat dyes, nitrates, acetic acid, Soaps, enzymes, Chromium compounds, and heavy metals like Copper, arsenic, Lead, Cadmium, Mercury, nickel and Cobalt and certain auxiliary chemical all collectively make the textile effluent highly toxic (Gurses et al,. 2016).
Dyes have various effect on human health depending on the application areas. Skin irritation and contact dermatitis have been reported for some synthetic dyes and the use of azo dyes made from carcinogenic amines have been banned by legislation in many countries (Gurses et al 2016).

The main aim of extracting a dye from plant (natural) source is to avoid the environmental pollution. Present days with global concern over the use of eco-friendly and biodegradable materials (Greetha 2013) the effluent problems of synthetic dyes occur not only during their application in the textile industries, but also during their manufacturing and possible during the synthesis of their intermediates and other raw materials. The use of natural dyes for textile dyeing purposes decreases to a large extend after discovery of synthetic dye in 1856. Dyes derived from natural source have emerge as an important alternative to synthetic dyes.

Plant are one of the sources of the important sources of dyes. Recently, interest in the use of natural dyes have been growing rapidly due to the result of stringent environmental standards imposed by many countries in response to the toxic and allergic reaction associated with synthetic dyes (Greetha, 2013).

Indigo dyes are part of the numerous marketed of organic colourant used for coloration of textile, paper, leather, plastic and for specialized application such as food, drugs, cosmetics, and phochemical production (Novotry et al,. 2016). Textile effluent containing indigo dye and other types of dyes such as azo and reactive dye make water toxic (Robinson et al,. 2001) thereby causing imbalance within different aquatic ecosystem food chain. The final toxic effect of the indigo dye effluent may be synthetic, additive or antagonistic as a function of the different effluent component that make up the effluent and not the dye in isolation (Mathias et al 2013).

The main idea of extracting dyes from a plant (natural) sources is to avoid environmental pollution. This is because of the stringent environmental standard imposed by many countries in response to the toxic and allergic reaction associated with synthetic dyes (Kamel et al 2005). Among the natural dyes, indigo has been obtained from a variety of plant source such as Indigofera tinctoria, Polygonum tinctoria, Nereum tinctoria, and Isatistintorium (Gubert and Cook 2001). Natural indigo is produced by Fermenting the leaves of indigo bearing plant. During fermentation, indicant in the leaves is hydrolyzed by to form Indoxyl and the glucose by the action of endogenous β-glucosidase and subsequently oxidized to form indigo in contact air through Oxidation (Song et al 2010).

(1)

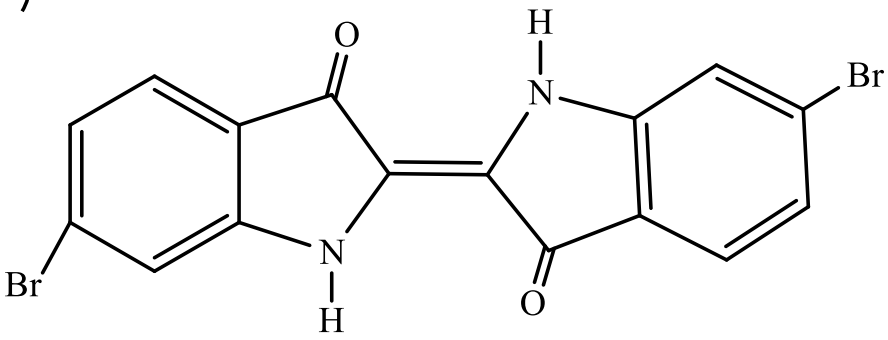
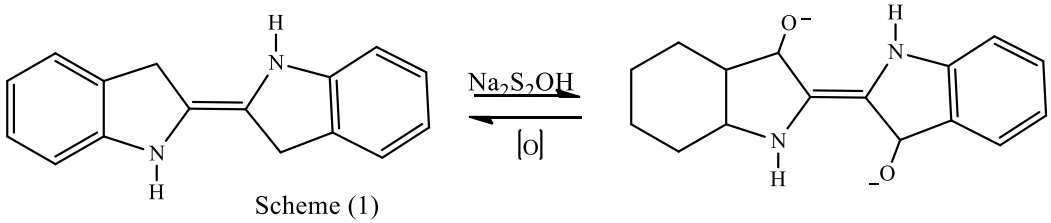


Fig. (1) showing the structure of 6'6-dibromoIndigo
also known as Tyran purple

Indigo dye been soluble in water due to the presence of dicarbonyl group usually undergoes a redox reaction, so to dye cloth, the indigo need to be made in to a water-soluble form, the insoluble indigo dye is then reduced with sodium hydrosulfide (sodium dithionite) as shown in figure (2).to the water soluble leucoindigo.

(2)



Scheme (1)

Fig. showing the conversion of the insoluble blue Indigo
due to the clear yellow water soluble Leuco or Indigo white

Natural Sources of Indigo

A variety of plants have produced indigo dye throughout the history, but most natural indigo was obtained from those in the genus Indigofera, which are native to the tropics, notably the Indian subcontinent. The primary commercial indigo species in Asia was true indigo (indigoferatinctoria) aiso known as (surnatrana). A common alternative used in the relative colder subtropical location such as Japans Ryukyu island and Taiwan is Strobillan thesusia.

Until the introduction of indigofera species from the south, polygonium tinctorum (Dyers knotweed) was the most important blue dyestuff in East Asia, however, the crop produced less dyestuff than the average crop of indigo and was quickly suppressed in terms of favour for the more economical indigofera suffruticosa, also known as anil. In Europe Isatitinctoria, commonly known as woad, was used for dyeing fabrics blue, containing the same dyeing compounds as indigo, also referred to as indigo.

Several plant contain indigo, when exposed to an Oxidizing source such as atmospheric oxygen, react to produce indigo dye, however, the relative low concentration of indigo in these plants make them difficult to work with the colour more easily tainted by other dye substances also present in these plants, typically leading to a greenish tinge.

The precursor to indigo is indicant, a colorless, water soluble derivatives of the amino acid tryptophan. Indicant readily hydrolyzed to release B-Dglucose and Indoxyl. Oxidation by exposure to air converts indoxyl to Indigotin. The insoluble blue chemical that is the endpoint of indigo. Indicant was obtained from the processing of the plants leaves, which contain as much as 0.2-0.8% of this compound. The leaves were soaked in water and fermented to convert the glucoside indicant present in the plant to the blue dye indigotin which precipated from the fermented leaf solution when mixed with a strong base which later dried and turn in to powdered. The powdered was then mixed with various other substance to produce different shades of blue and purple.

Properties of Indigo Dye

Indigo dye is a dark blue crystalline powder that sublimes at 390^0-392^0(734-738^0F). it is insoluble in water, alcohol, or ether but soluble in nitrobenzene and concentration sulphuric acid. It has molecular formula of $C_{16}H_{10}N_2O_2$, Density of $1.199 g/cm^3$, molecular mass = 262.27 g/mol.

Indigo dye been soluble in water due to the presence of dicarbonyl group usually undergoes a redox reaction, so to dye cloth, the indigo need to be made in to a water-soluble form, the insoluble indigo dye is then reduced with sodium hydrosulfide (sodium dithionite) as shown in figure (2).to the water soluble leucoindigo.

Toxicity Evaluation of Dyes

Toxicity of dyes

The toxic and allergic reactions of synthetic dyes are compelling the people to think about natural dyes. Natural dyes are renewable source of colouring materials. Besides textiles it has application in colouration of foods, medicine and in handicraft items. Though natural dyes are ecofriendly, protective to skin and pleasing colour to eyes, they are having very poor bonding with textile fibre materials, which necessitate mordanting with metallic mordants, some of which are not eco-friendly, for fixation of natural dyes on textile fibres. So the supremacy of natural dyes is somewhat subdued. This necessitates newer research on application of natural dyes on different natural fibres for completely eco-friendly textiles.

Classification of Indigo (Vat) Dyes

There are two main classes of Indigo vat dyes which includes:

I. Indigoid Vat dyes: derivatives of indigo or thio-indigo and the structure is shown in figure (3)

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(3)

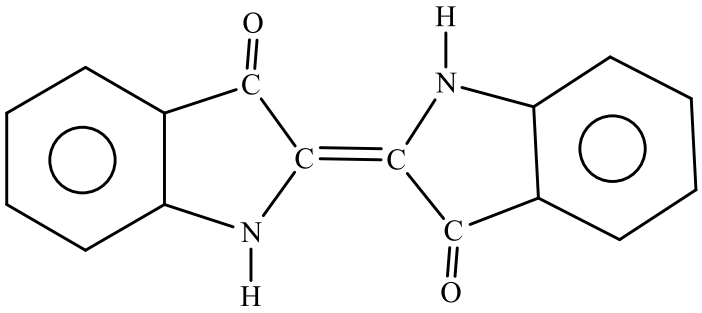


fig. showing the structure of Indigotine

Anthraquenone Vat dyes: the structure is shown in figure (3.1)

(4)

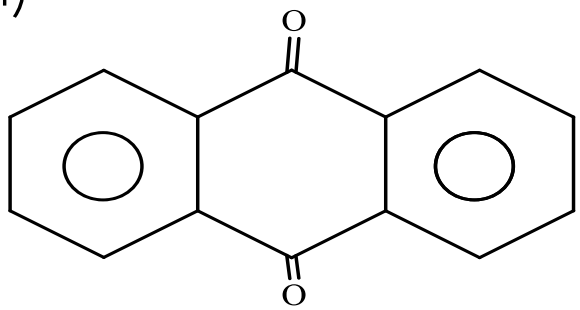
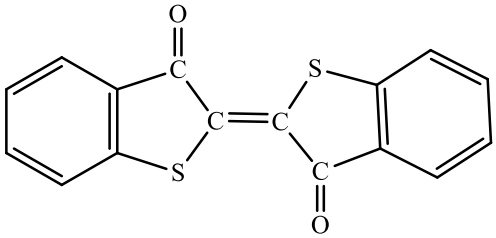


Fig. showing the structure of Anthraqunine

Derivatives of Indigo Dyes

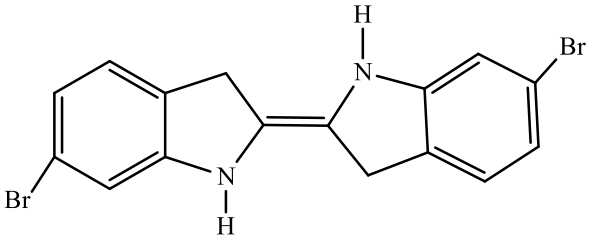
The benzene rings in indigo can be modified to give a variety of related dyestuff. Thioindigo where the two NH groups are replaced by S atoms, is deep red, Tyrian purple is a dull purple dye that is secreted by a common mediterenean snail. It was highly prized in antiquity in 1909, its structure was shown in in figure (4) to be 6'6 dibromoindigo (red) 6- broindigo (purple) is a component bases. The relative Ciba blue (5,7,5,7 tetra bromo indigo) is however of commercial values.

(5)



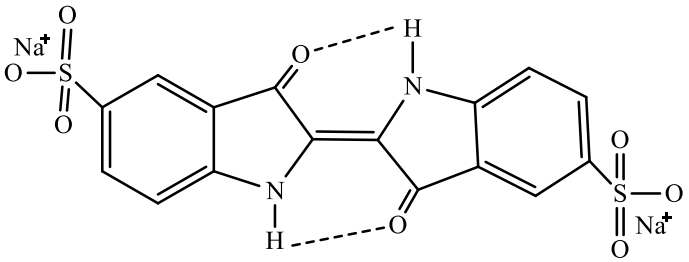
Showing the structure of thiondigo

(6)



Showing the structure of Tyrian purple thindigo and Tyrian purple

(7)



Structure of Indigo Carmine

Fig. Showing the structure of Indigo Carmine

Treatment with sulfuric acid convert indigo in to a blue green derivative called indigo Carmine (sulfonated indigo) as shown in figure (4) and it became available in the mid-18th century. It is used as colorant for food, pharmaceutical and cosmetic.

Structural Studies of Indigo

The interatomic distance …. N…….H……. O… indicate that the formation of two intramolecular hydrogen bonds between ketone and amine groups.

In the monomer, amino and carboxyl groups occupy the position that would otherwise be the most reactive ones for nucleophilic and electrophilic attacks. In the dimmer, amino and carbonyl group on different monomers form intermolecular multicenter non-linear hydrogen bounding in six-member rings protecting again the same reactive centers. Then inter and intramolecular H- bound formation help in protecting the reactive centers and centers stability to the compound in both gas and solid phases.

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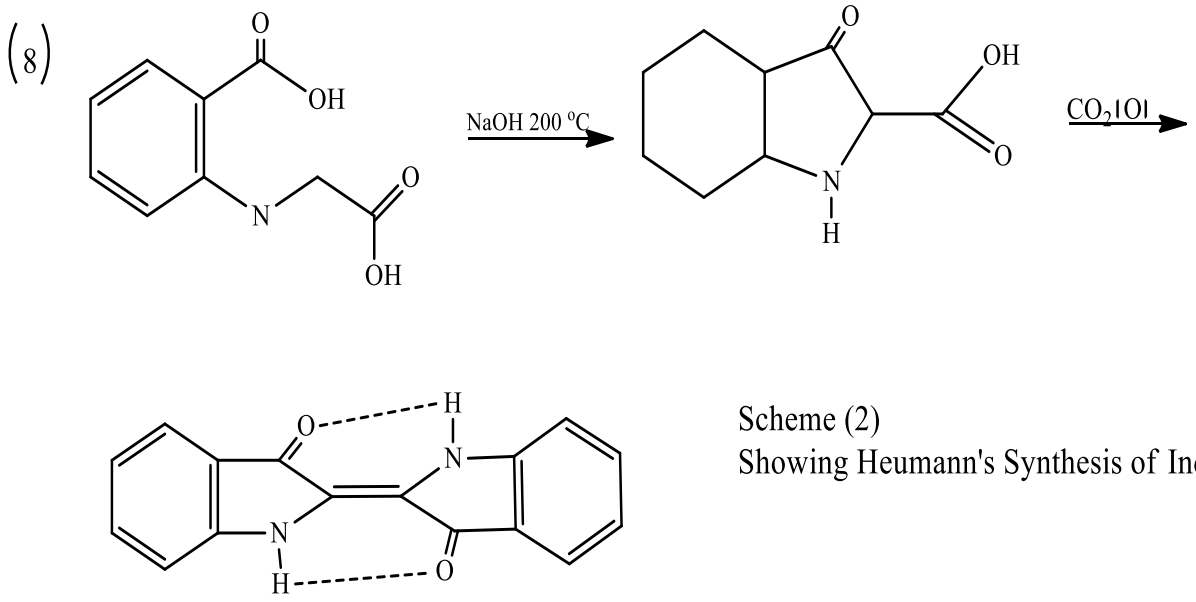
Chemical reactivity of indigo with OH free radical

The interaction energy between the indigo molecule and the free radical OH were calculated under the assumption that OH can approach any of the atoms of carbon (from one to eight) but only give consistence result when interaction was on C1, C3, and C6.

Indigo is very stable due to large π conjugation inside the molecule along 10 carbon atoms. The intra and intermolecular, nonlinear and multicenter hydrogen bridges are confirmed by the presence of two internal orbits in the dimer that overlaps between low-energy orbitals from the monomer.

Chemical Synthesis of Indigo

Given its economic important, indigo has been prepared by many methods. The Baeye-Drawson indigo synthesis date back to 1882. It involves an aldol condensation of O-nitrobenzendehyde with acetone, follow by clydization and oxidative dimerization to indigo. This route is highly useful for obtaining indigo and many of its derivative on the laboratory scale, but however, Pfleger and Kari Heuman eventually come up with the industrial mass production synthesis od in Indigo as shown in figure (5).



Scheme (2)
Showing Heumann's Synthesis of Indigo

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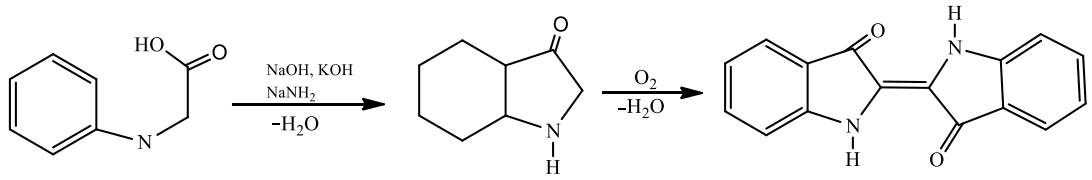
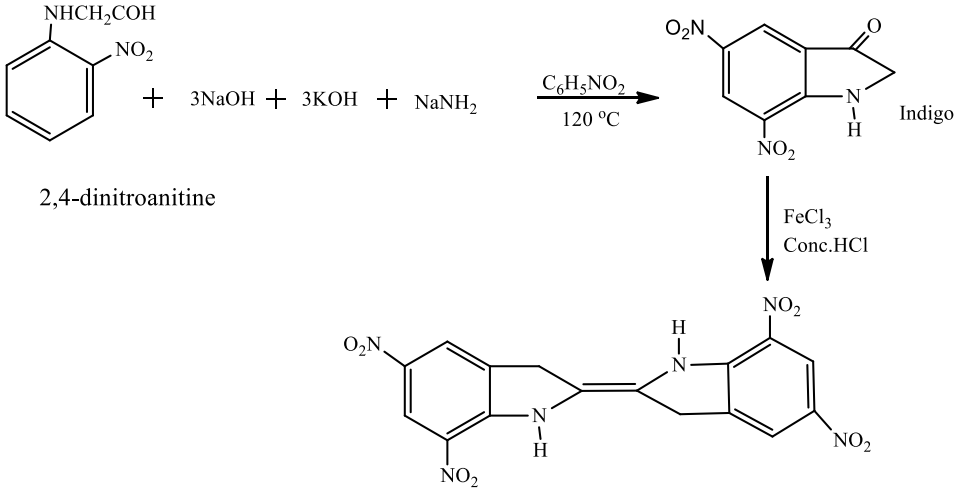
(10)



Flegere's Synthesis of Indigo

Scheme ( )



2,4-dinitroanitine

Scheme showing the synthesis of Vat dye using 2,4-dinitro anitine with chloroacetic acid

A new blue indigo vat dye was prepared from 2,4- dinitro aniline which used as nucleophile and treated with chloroacetic acid (Nwokkonkwo ,2016). This paper aimed at synthesis, extraction, characterization and toxicity evaluation of indigo dyes and applications on cotton fabric.

Materials and Method

The following are the materials and reagents that was used for the synthesis of Indigo Vats dye: Beakers of different size, Test tube, Filter funnel, Filter paper, Pipettes, Plastics container, Washing bottles, Ice bath, 2-Ntrobenzaldehyde, Acetone, Deionized water, Ethanol, Sodium dithionite, Cotton fabric etc.

All the chemical and reagent that would be use for this research both the extraction and the synthesis would be of analytical grade and not Industrial grade as they are pure and free from impurities and would not require further purification.

Extraction of Indigo dye

Fresh leaves of Indigo (Indigofera Tinctorium) were obtained from the National Research Institute of Chemical Technology(NARICT) Basawa Zaria. 400g of leaves were weighed and cut in to small pieces of 1cm3, the leaves were allowed to undergo fermentation for three to four days, after the fermentation, the leaves were separated from the liquid extract and the dyestuff was then extracted by using four thousand mill of distill water at normal temperature. Aeration of the extract was allowed for two hours using aeration machine that supplied oxygen to the extracted liquor, the PH of the extract was maintain at 11.0 throughout the extraction and finally decantation was carried out were by the Indigo dye was obtained.

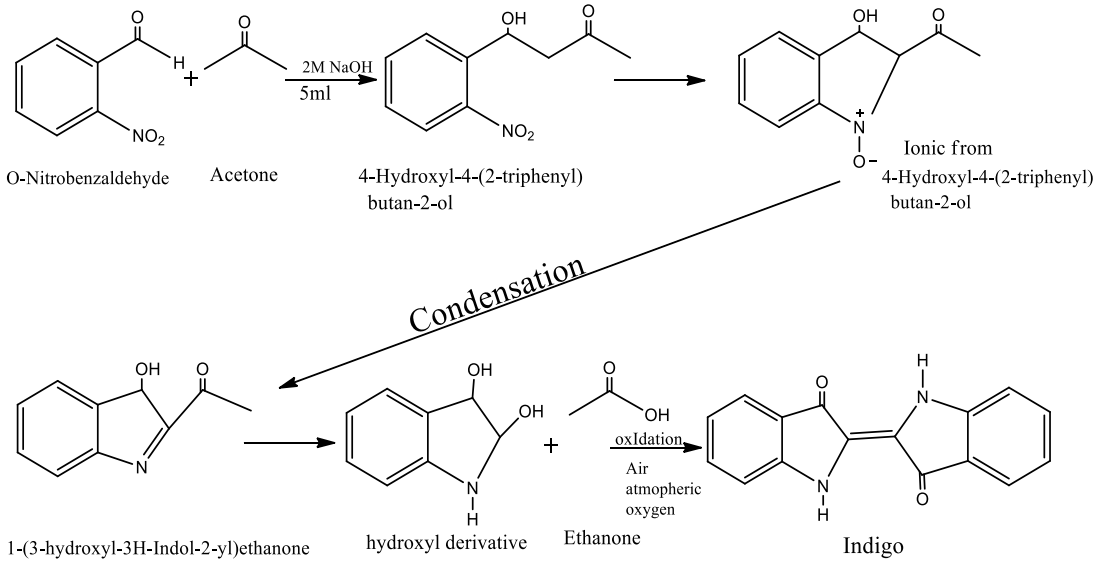
Synthesis of Indigo dye

Procedure

5ml acetone, 5ml of distilled water, and 0.50g of O-nitrobenzaldehyde was stirred using a 1cm stirbar. 2.5ml of 1M of NaOH was added dropwise over a period of 5 minutes. The mixture was stirred for about 5 minutes to allow it return to room temperature. The contents of the vial were filtered using a Hiesch funnel as quickly as possible (indigo form very fine crystal that may clog the filter to become dyed while the filtrate or the system will clog). The Vial was rinsed with 10ml of waer and add to the filtrate before the products become dry. The filtrate was washed with 5ml cold

95% **ethanol** before the liquid passes through the filtrate completely. The product was removed from the filter and allowed to air dried. The weight of the dried product was taken (James, 1991).

Synthesis of Indigo dye



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Application of Indigo on Cotton Fabric

Materials

White cotton fabric, Indigo vat dyes, Sodium hydrosulphite,Sodium hydroxide (Caustic soda) Stirer, Beakers, Hand gloves, Weighing balance, Conical flask, Measuring cylinder, Thermometer, PH meter, and Volumetric flask.

Method of application

3g NaOH pellets was dissolved in 10ml of H_2O and was added to the solution to a 50ml filtrate flask with an operating stir bar. The indigo produced was then added to the above. The mixture was stired and heated to boil in a shallow san bath. While waiting for the mixture to boil, 3g of sodium hydrosulphite (sodium dithionite $Na_2S_2O_4$) was stired in to 27ml H_2O to mark an approximately 10% solution. When the indigo solution reaches boiling solution drop wise, this continue until the blue colour has completely left the solution yielding leucoindigo, a clear yello liquid.

Acute-Oral Toxicity test (OECD-425)

Acute Oral toxicity studies will be carried out by the both extracted and synthesis indigo dye on anima mainly rats which is require to kill 50% of the animal population within 14 days and of which will determine the level of toxicity of the indigo dyes, lethal dose (LD 50) of 2000mg/kg was measured and dosed orally to one of the mice and was observed for 24 hours, then followed by the remaining four which was monitored for forty eight hours and it was observed for fourteen days on the basis of each dye(Umbuzeiro *et al.,* 2019).

Characterization

The techniques employed for the characterization of both extracted and synthesis indigo were Infra-red (FTIR) using KBr pellets, UV visible spectrophotometer which will be carried out at NARICT. Research Institute Zaria, ^{1}HNMR (Agilent -NMR-Vnmrs 400nm) which will be carried at multipurpose laboratory ABU Zaria, Samaru main campus.

Fastness properties

Light fastness test (255-ISO 105-2013)

An artificial light source e.g as build in to a light fastness tester was used for this study. The blue wool light fastness standard was cut in to 5cm x 1cm strips and staple across a black cardboard of 10 x 5 cm. The effect of the light was followed by inspecting the specimen frequently. The results will be expressed by comparing the faded specimen with those of the blue standards.

Washing fastness (8032- ISO105-C06-2010)

A solution of 5g per litre of soap will be made using a liquid soap and potassium hydroxide solution. The specimen will then be treated in the solution of a liquor ratio of 50:1 for 30minutes under the following condition. ISO TEST NO 3 at 60 ± 2 °C

The results were established by using the grey scales to assess the stained specimen for change of shade.

Rubbing fastness (180- ISO 105-B02-2013

The sample specimen will be cut in to 21cm*6cm and form the white fabric of specified substance cut circular sample of 65mm diameter. The top plast of the staining tester will be lifted up and placed the colored specimen on the flat bed. The while specimen on the rubbing head was fastened using the rubber rings provided. The electric motor of the machine was activated and press the rubbing button to allow the machine make several working cycles. The experiment was repeated using a moistened white specimen on the rubbing head. The grey scales were used to assess the degree of staining on white sample. Both dry and when moistened.

Results and Discussion

Table 1: Shows the physicochemical parameters of synthesis indigo dye

Name	Colour	Melting point	Molecular weight	Molecular formula
Synthesis indigo dye	Dark blue	389-391 ^{0}C	262	$C_{16}H_{10}N_2O_2$
Extracted indigo dye	Blue	390-392 ^{0}C	262	$C_{16}H_{10}N_2O_2$

The results in table one shows the physicochemical parameters of the extracted and synthesized indigo dye which revealed the color to be dark blue in the synthesized while blue color in the extracted indigo dye, other parameters such as melting point, molecular weight, and molecular formula were found to be identical.

Table 2: shows the Solubility test of indigo dye

S/N	SOLVENTS	INDIGO DYE SYNTHESIS	INDIGO DYE EXCTRACT	POLARITY
1	ETHANOL	IS	IS	P
2	METHANOL	IS	IS	P
3	ACETONE	IS	S	P
4	DISTILLED WATER	IS	IS	P
5	D.M.F	IS	S	P
6	CHLOROFORM	IS	IS	P
7	BENZENE	S	SS	NP
8	DIETHYLETHER	S	SS	NP
9	N-HEXANE	S	SS	NP
10	TWEEN-80	S	S	NP

The solubility test was carry out on the two samples using different solvents of different polarity to determine the rate of solubility of the indigo dyes as shown in table 2, the results found that most of polar solvents such as methanol, ethanol, acetone, distilled water and DMF were not soluble in synthesized indigo dye while the non-polar solvent were found to be soluble, this may be due the organic nature of the precursors used in the synthesis were from organic substance, while the acetone and acetone and DMF were found to be soluble with extracted indigo dye, this could also attributed due to solvent used for the extraction as distilled water which is also a polar solvent in nature. However, non-polar dissolve extracted while the polar dissolved the synthesized indigo dye.

Table 3 shows the % EXHAUSTION OF INDIGO DYE SAMPLES

SAMPLE	OD1	OD2	%EXHAUSTION	WAVELENGHT
DS	0.901	0.621	31	600mn
DE	0.906	0.492	46	555mn

DS= Indigo dye synthesis
DE= Indigo dye extract
The table 3 shows the results of percentage exhaustion of the dyed samples using the extracted and the synthesized indigo dye which the value of 31 and 46 % respectively, this shows that extracted have better percentage exhaustion than the synthesized indigo dye.

Table 4 shows the UV-VISIBLE SPECTROSCOPY RESULTS

SAMPLES	WAVELENGTH(nm)	ABSORBANCE
DS	600.00	0.534
DE	555.00	0.305

The UV-Visible spectroscopic results as shown in table 4 shows DS the wavelength value of 600nm with absorbance of 0.534 and DE with 555nm with absorbance of 0.305 respectively, the values show that they are within the visible region of UV and that indicates the appearance of blue color of indigo dye.

Table 5: shows the FTIR Results of Indigo Dye Synthesis

Functional group	N-H cm^{-1}	C=O cm^{-1}	C-H cm^{-1}	C-O cm^{-1}	C=C cm^{-1}	O-H cm^{-1}	N=C=N cm^{-1}
Types of vibration	Stretching	Stretching	Stretching	Stretching	bending	Stretchg	Stretching
Wave number	3041.5	1707.1	2878.0	1380.3	1606.5	3753.4	2117.1
			1461.1	1297.1		3662.8	
			1945.7				

Table 6: shows the FTIR Results of Indigo Dye extract

Functional group	N-H	C=O	C-H	C-O	C=C	CO-O
Types of vibrations	Stretching	Stretching	Bending, Stretching	Stretching	Stretching	Stretching
Wave number	3250.2	1796.6	2922.2	1170.4	1610.2	1069.5
			1405.2	1125.7		

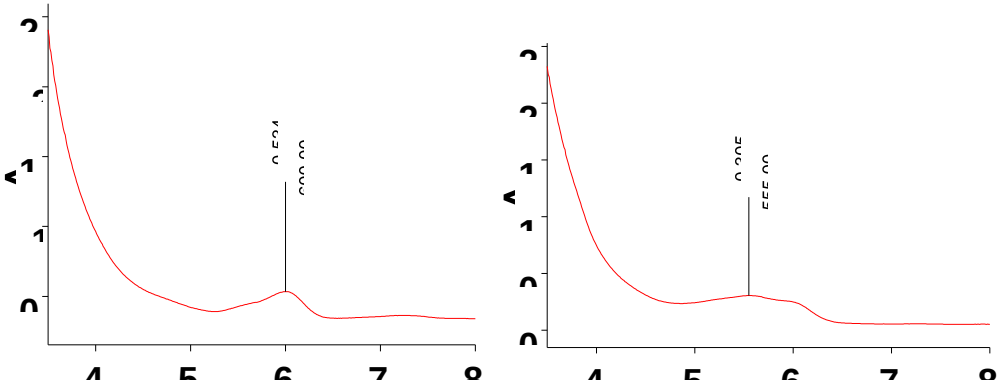
Table 5 and 6 shows the FTIR results of the two samples (synthesized and extracted indigo). The difference values were shown at the spectral among which the prominent are: N-H, C=O, C-C, and C-H with their wave number at different peaks, these functional groups correspond with the structure of indigo dye as shown in figure 1, as having two conjugates bond of carbonyl C=O and two alternate N-H group which are linked with carbon carbon stretching.

Table 7: shows the results of fastness properties of Indigo dye

Light fastness	Indigo synthesis		Indigo extract	
	Dry	wet	Dry	Wet
Rubbing	½	2	¾	4
Washing	¾		½	
Light	4		3	
perspiration	3		2/3	

The results of fastness properties as shown in table 7 shows a good to moderate light and perspiration with the rating of 4-3 while rubbing and washing shows fair, the results also shows good in the rubbing as wet with rating of 4 as compared to dry with ¾.

Table 1: shows the UV-Visible results of Indigo dyes



Wavelength (nm) **Wavelength (nm)**

Table 2: shows the FTIR results of Indigo dyes

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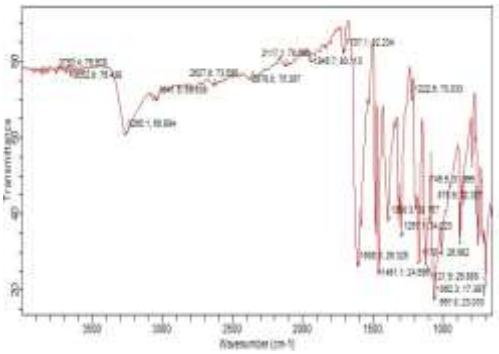
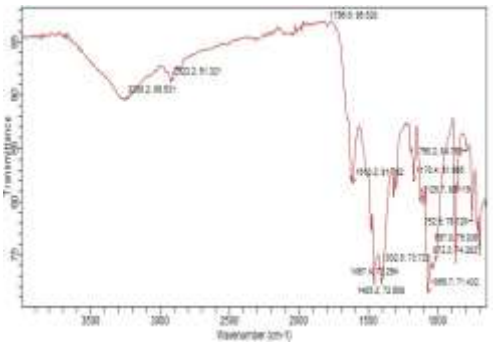
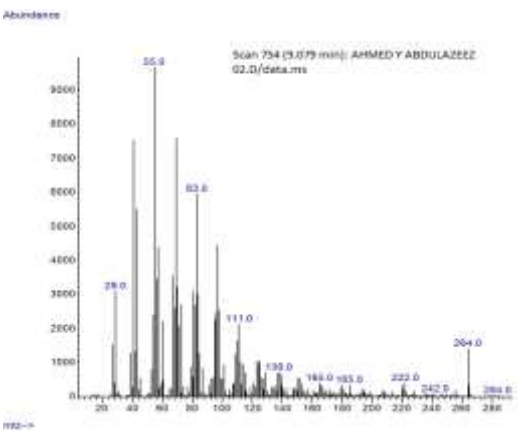


FTIR results Dyes synthesis

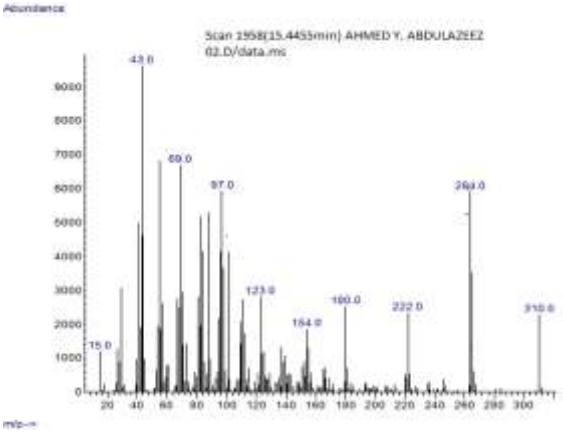


FTIR results of Dye Extracts

Table 3: shows the GCMS results of Indigo dyes



GCMS RESULTS OF INDIGO DYE EXTRACT



GCMS RESULTS OF INDIGO DYE EXTRACT

The elucidation of the dyes structures using GCMS shows the corresponding fragments present in the indigo dyes with the molecular value of 264 at the spectral close to the theoretical value of 262, the differences may be due to the existence of isotopes within the structures, therefore, this value obtained from the GCMS reflect the results from the two samples.

Table 8 shows the results of Acute toxicity test on Indigo dyes

S/NO	PARAMETERS	CONTROL2ml/kg	Indigo 2000mg/kg
1	Alertness	Normal	Normal
2	Touch and pain	Normal	Normal
3	Food and water intake	Normal	Normal
4	Tremors/epilepsy/sedation/coma	Not observed	Not observed
5	Gripping/corneal/Righting reflex	Normal	Normal
6	Salivation, Urination	Normal	Normal
7	Skin color and Pupils	Normal	Normal
8	General physique	Normal	Normal

9	Temperature	Normal	Normal
10	Faces consistency	Normal	Normal
11	Depressed	Not observed	Not observed
12	Restlessness	Not observed	Not observed
13	Abdominal constriction	Normal	Normal
14	Mortality	Not observed	Not observed

The table 8 shows the results of acute toxicity test on indigo dyes (synthesized and extracted). The acute test was carry out using up and down method at the oral limit dose of 2000mg/kg of indigo dyes samples which caused no death in the rats (mice). No significant changes were observed in the wellness parameters used for the evaluation of acute oral toxicity as shown in table 8, furthermore, no death was observed from the two samples of indigo thus establishing their safety in use. All the behavioral and other visual observations were to be normal for the 2000mg/kg of indigo dyes.

CONCLUTIONS

The results of extraction show that indigo dye was obtained and the possible resultant of dark blue colored from the solution reflects that, indigo vat dyes is formed.

The results from characterization techniques using UV-Spectroscopy shows that the extract has a Wavelength of 555.00 (nm) at Abs of 0.305 while the synthesed indigo has 600.00(nm) at abs of 0.534 which shows that all the values were within the visible region and that indicate the formation of indigo blue, the FTIR results show the values obtained correspond to the peaks on the indigo dye structure with the appearance of N-H(3041.5-3250.2), C=O (1707.1-1796.2), C=C(1610.2-16065), C-H (2878.0-2922.0), groups were found to be present in the structure. The GCMS results confirm the molecular structure of the indigo with the value at 264 on both extracted and synthesis dye being existence of isotopes. The acute toxicity shows the non-toxic of indigo dyes as all the parameters tested were found to be normal and mortality rate was not observed. The results demonstrate that the indigo dye has good fastness to color yield.

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