Enhancing the Value of Indigo-Blue Dyes from Lonchocarpus Cyanescens Leaves

William Owusu Oduro
Council for Scientific and Industrial Research
Institute of Industrial Research
P.O. Box LG 576, Legon
Accra, Ghana

Festus Addo-Yobo
Research & Innovation Centre
Accra Polytechnic
P. O. Box 561, Barnes Road
Accra, Ghana

Abstract

The parameters for the extraction of an indigenous dyestuff from Lonchocarpus cyanescens leaves and its spectroscopic characterization are discussed. The extraction was achieved by cold maceration in alkaline $Na_2S_2O_4$ solution. The IR spectra identified the presence of the following active functional groups; υ(O-H) and υ(C-O) stretching of primary and secondary alcohols as well as υ(N-H) and υ(C-N) stretching of an aromatic amine. These functional groups are similar to those associated with synthetic indigo dyes used in dyeing denim jeans. Refinement of the crude dyestuff is achieved by precipitation with acid leaving the noxious principles in solution. The variation of yield of dye with alkalinity is consistent with the dye existing as an insoluble ketal form in the leaves which is reduced with hydrosulfite to a sparingly soluble leuco form. This leuco form is converted to a more soluble salt species by addition of base.

Keywords: Vegetable indigo dye, solvent extraction, hydrosulfite usage, spectroscopic characterization

Introduction

Dyeing dates back since antiquity, to the development of textiles and art forms (Bechtold, Turcanu, Ganglberger, and Geissler, 2003). Strip woven clothes manufactured from cotton and vegetable dyes are an ancient African art form in West Africa which dates back to at least the 10th century (http://bawabawa.com/Daboya/Daboya.htm). *Lonchocarpus cyanescens Benth*, also commonly known as; West African wild indigo, Garra, Wulim, or Yuroba indigo (Ayensu, 1978) is one of the frequently used vegetable dyes, especially by the cottage industry in Daboya in the Northern region of Ghana (http://bawabawa.com/Daboya/Daboya.htm.). Though vegetable dyes generally have poor fastness to wash and light (Cristea, and Vilarem, 2006), Garra is still widely used because it is one of the few vegetable vat dyes which are fast to wash and light (http://bawabawa.com/Daboya/Daboya.htm.and http://www.indigopage.com/herbal/indigofera.htm).

The traditional method of extraction of the dyestuff from the plant material and the dyeing of the cotton fabric involves a one-pot in situ process. This makes the cloth acquire distinct strong scent which according to the indigenes has therapeutic properties for morning sickness resulting from pregnancy and also improves the health of sickly new born babies when they are wrapped around with Garra dyed textiles (http://bawabawa.com/Daboya/Daboya.htm.). This smell is however, inimical to the value of the final product.
Natural dyes and fabrics dyed with them are fast becoming a preferred brand on the dyes and textiles market (Ali, Hussain and Nawaz, 2009). This new paradigm is generally borne out of the increasing concern of the carcinogenic properties of some of these synthetic dyes (Guinot, et. al., 2009) especially the azo dyes which account for about 50% of all commercial dyes (Kroschwitz, 1993, Hueper, 1969 and Gregory, 1991).

It is scientifically verified that phytoconstituents like polyphenols, coumarins, flavonoids and plant sterols are powerful antioxidants (radical scavengers), function as antimutagenic agents (Goyal, et. al., 2009; Cristea, and Vilarem, 2006) as well as show antimicrobial properties (Baliarsingh, et. al., 2012). Hence the use of herbal coloring agents, not necessarily as a pure naturally occurring compound but in combination with other natural products co-extracted with the active ingredient, makes these dyes sustainable materials with biodegradable properties and hence health and environmental benefits. These characteristics make them niche products for special markets (Bechtold, et. al., 2003). Vegetable dyes are very attractive in their use as textile dyes, instigating a rise in market value of products dyed with them.

The technologies for extracting indigo dyes from their vegetable source in West Africa remain very rudimentary. This normally involves the ashing of the leaves of *Parkia biglobosa* and addition of water to form a leaching bath from which the colors are extracted (Polakoff, 1982 and Moeyes, 1993). In most instances, dyeing is done in situ hence the dye yield is poor; the dye's shelf life is limited by that of the leaves which is short. The quality of the products is below internationally acceptable standards as smelly substances and plant material with little or no effect on the chromaticity of the dye are introduced onto the fabric to be dyed. The noxious principles in these indigo dyed fabrics make them very unattractive outside the domain of cultural jurisdiction and the rudimentary process does not allow the dye to be packaged and sold as vegetable dyes.

It is therefore, essential to study the relation between the noxious principles and the indigo color of the vegetable dye as well as the optimum extraction conditions of the extraction bath. The chromaticity of a compound is influenced by the functional groups in the molecular structure which to a large extent determines its hue. Synthetic indigo dye used in dyeing denim jeans and jackets (structure shown in Figure 1) has chromatic features determined by the conjugated unsaturated functional groups (Kroschwitz, 1993).

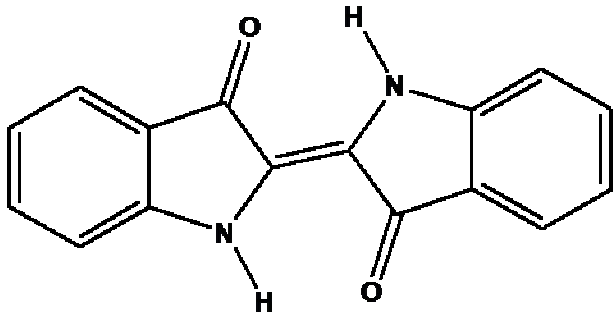

Figure 1: 2, 2-Bis (2, 3-dihydro-3-oxoindolyliden) the active ingredient in synthetic indigo dyes used in dyeing denim Jeans and Jackets.

To date very little literature has been published concerning optimizing the extraction processes as well as elucidating some of the structural features of dyes from these indigenous Ghanaian cottage industries in order to improve the traditional practice. Some work however, has been done on documenting traditional extraction techniques of dyes obtained from Asian sources and their storage (Polakoff,1982 and Moeyes, 1993). Thus the task of improving the quality of traditional dyes obtained from Africa is even more urgent. Studies into the extraction optimization processes of vegetable dyes in China, (Wang,& Jia, 2007, Liu, et.al., 2008) and other parts of the world (Bechtold, et. al., 2006) have helped improve indigenous vegetable dyes making them hot commodities on the international textile market.

The objectives of this research paper are; to refine vegetable indigo dyes from *Lonchocarpus cyanescens* with the aim of eliminating the noxious principles associated with the traditional dyeing process, to observe the chromatic features of the refined fractions by UV-VIS spectroscopy and to elucidate key functional groups present in the dye by IR spectroscopy. Since hydrosulfite and caustic are critical cost components and can cause environmental concerns in the extraction of vat dyes (Ali, Hussain. and Nawaz, 2009), we determine their optimum concentrations in the leaching bath.

Methods

Sample preparation

The fresh leaves of Garra were harvested, pounded, fermented, rolled into balls and dried by the local textile dyers of Daboya in the Northern region of Ghana. The average weight of each ball of fermented leaves is about 37.0 g. The dry balls of Garra were milled to particle sizes that go through a sieve of aperture size 630 μm. The dry powder was sealed in a labeled polythene bag.

Extracting Indigo-blue dyes from plant leaves

Preliminary results showed that reducing by hydrosulfite without making the solution alkaline led to negligible yields. In a typical extraction process, 30.0 g of powdered Garra leaves was weighed into an extraction batch stir tank. 600 ml of freshly prepared aqueous solution of concentration 0.1 M sodium hydroxide and 0.1 M sodium hydrosulfite was added and stirred continuously for 2 h using an overhead mechanical stirrer. The extraction mixture was filtered using a Buchner funnel with Whatman No 1 filter paper, the residue washed with aliquots of deionized water to recover extracted dye and the total filtrate volume made up to 1000 ml. Each of three, 100 ml portions of the resulting liquor was acidified using 3 drops of 12 M hydrochloric acid to precipitate the dye before recovering the precipitate by centrifuging at a speed of 4400 rev/min.

The dark blue precipitate at the bottom of the centrifuge bottle was dried and weighed. The dye yield from the vegetable source was determined gravimetrically and computed using Equation 1.

$$Percentage\ \ dye\ yield = \frac{mass\ of\ \ dried\ \ precipitate}{mass\ of\ \ plant\ leaves} \times 100 \quad \text{--- (1)}$$

50 ml of the yellowish brown supernatant, which has the characteristic strong pungent smell, was stored in a refrigerator at 5 ^{o}C. The structural features of the different fractions were analyzed by UV-VIS and IR spectroscopy.

Optimizing the extraction conditions

Various experiments were conducted for the extraction of natural dye from Garra leaves in distilled water as well as in aqueous solutions of sodium hydrosulfite at 0.05 M, 0.10 M, 0.15 M, 0.20 M and 0.25 M concentrations with sodium hydroxide concentrations of 0.05 M and 0.10 M and material to solvent ratio (M:S) of 1:20 at room temperature (27 ^{o}C). To study the kinetics of the extraction at room temperature, 1.5% wt/wt NaOH and 1.5% wt/wt $Na_2S_2O_4$ in deionized water was used as solvent system with M:S of 1:20. 0.5 ml aliquots of the impregnated liquor were sampled with a hypodermic syringe from the extraction tank at different time durations, for 5 min, 10 min, 15 min 20 min., 25 min., 30 min., 35 min., 90 min., 120 min., 150 min., 180 min. and 210 min. topping up each time a sample is taken with 0.5 ml of the solvent system. The aliquot sample is then diluted to a 25 ml volume with deionised water before analyzing using the UV-Vis spectrophotometer at λ_{max} of 500 nm.

Measurement of extraction yield using UV-Vis spectroscopy

To establish the calibration curve between the concentration of dyestuff leached into solution and UV absorbance, a stock solution of 1.0 g of extracted Indigo-blue dye dissolved in a 100 ml of freshly prepared aqueous solvent system containing 1.5% wt/wt NaOH and 1.5% wt/wt $Na_2S_2O_4$ in deionized water was prepared. The stock concentration of the indigo-blue dye was serially diluted in the solvent system to obtain dye solutions at 0.04 g/100ml, 0.0192 g/100ml, 0.0096 g/100ml, 0.0048 g/100ml and 0.00224 g/100ml concentrations. The wavelength at the maximum absorbance (λ_{max}) of the stock dye solution was determined by performing a UV-VIS spectrum scan with Beckman DU 640 spectrophotometer with the 1.5% wt/wt sodium hydroxide and 1.5% wt/wt sodium hydrosulfite solution as blank. Figure 2, a calibration curve indicating spectrophotometric absorbance plotted as a function of known concentration of extracted vegetable indigo dye at wavelength of 500 nm, shows significant linearity of response to concentration.

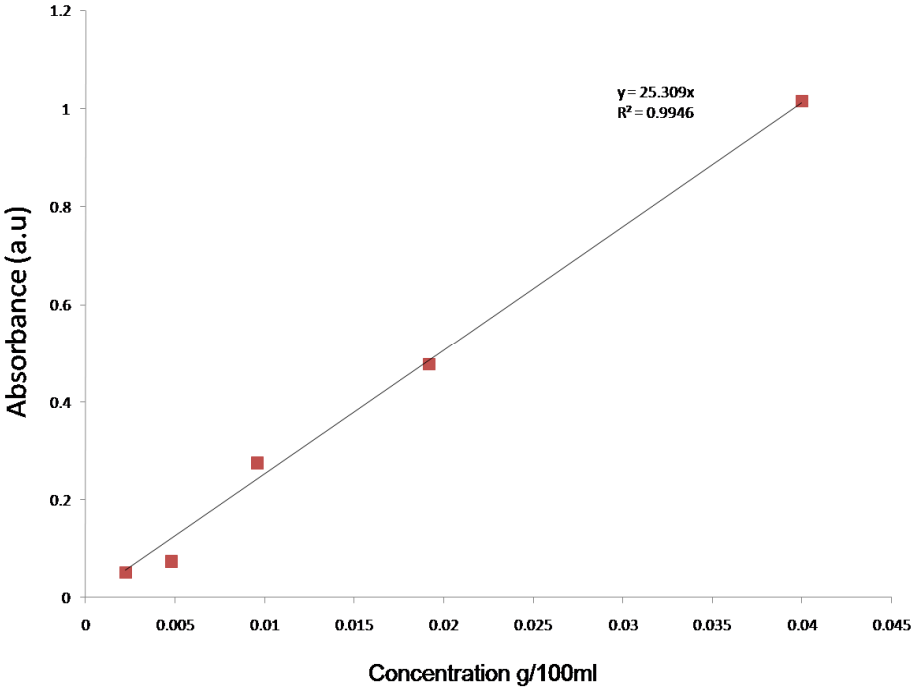

Figure 2: Calibration curve demonstrating the linearity of the relationship between the absorbance at λ_{max} 500 nm and the concentration of the dye over the range of interest.

Results and discussion

Infrared spectroscopic studies of extracted indigo dyestuff from Lonchocarpus cyanescens

The characterization of the dye extract using a Perkin Elmer 1330 infrared spectrophotometer at a resolution of 4 cm^{-1} with an average of 4 scans to elucidate the functional groups present shows clearly as observed in Figure 3a, a characteristic υ(O-H stretching) at between 3565 – 3165 cm^{-1}. The corresponding υ(C-O stretching) of primary and secondary alcohol functional groups are depicted by the sharp absorbance at *ca* 1050 cm^{-1} and 1100 cm^{-1} respectively. This establishes that the O-H stretching is not due to trailing O-H of the caustic or water molecules used in the extraction process but associated with an alcohol group on the dye molecule (Pavia, Lampman, and Kriz, 2000)

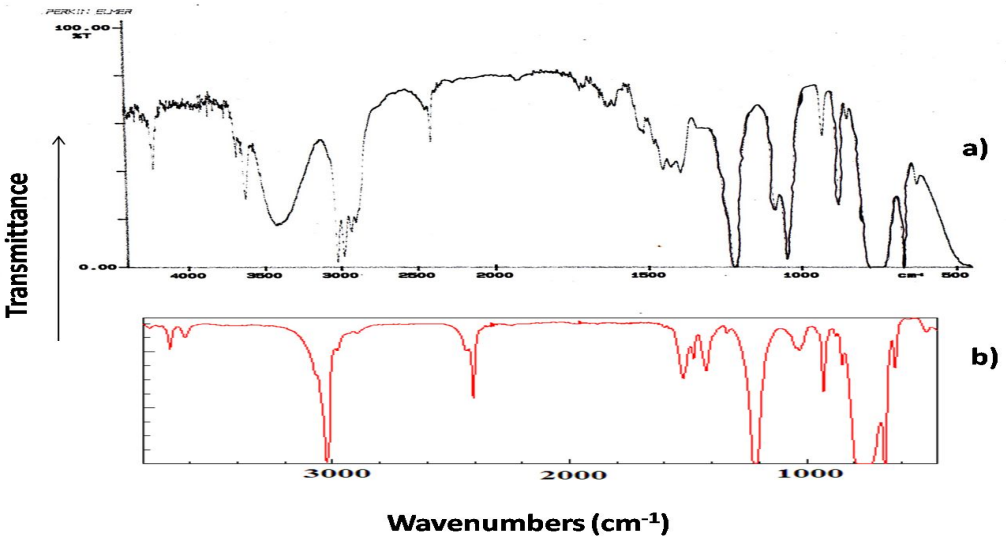

Figure 3: Infrared spectra at a resolution of 4 cm^{-1} of a) extracted crude vegetable indigo dye from *L. cyanescens* in Chloroform and b) neat chloroform.

The υ(N-H stretching) of an aromatic amine group is observed at 3610 cm^{-1}. The corresponding υ(N-H bending) of the primary amine absorbs between 1608-1583 cm^{-1} and the υ(C-N stretching) of the primary aromatic amine is shown between 1342-1379 cm^{-1}. The strong absorbance peaks at 1208 cm^{-1} and 800-700 cm^{-1} in Figure 3a is from chloroform (compare with Figure 3b, spectrum for neat chloroform). The sharp absorbance at *ca* 3020 cm^{-1} shows the υ(C-H stretching) of an aromatic compound with the corresponding υ(C=C) absorbed at 1440 cm^{-1} (Pavia, et. al., 2000). Clearly, the structural elucidation using the IR spectroscopy demonstrates a primary aromatic amine and an alcohol as the main functional group features of the raw indigo natural dye. Further elucidation of the structure of the vegetable indigo dye would require NMR spectroscopy which was not available.

These structural features are very similar to the synthetic indigo dyes used in dyeing denim jeans and jackets which structure is depicted in Figure 1 (Kroschwitz, 1993). Taking into cognizance the fact that the functional groups present in a compound determines its chromatic properties through the absorption of light of a specific maximum wavelength (λ_{max}) which permits electron transition in the form of $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ from lower to higher energy levels and the subsequent transmittance of the remainder of the electromagnetic spectrum, it will not be unusual to have of similar functional groups exhibiting similar hue.

The presence of noxious compounds is the main drawback to the indigenous technology of using the indigo dyes extracted from the Garra leaves. To investigate the functional groups responsible for the foul smell and to see if it can be removed from the crude indigo dye without damaging the hue, the crude dye was placed on a silica chromatographic column and eluted with (solvent system). Figure 4a shows the IR spectra of the column refined indigo dye. Comparison of the brightly colored blue fraction eluted from the column chromatograph dissolved in acetone with a neat acetone IR spectrum as shown in Figure 4, demonstrates a distinctively intense and sharp absorbance around 3100 cm^{-1} which is associated with υ(=C-H stretching) of a aromatic compound shown on Figure 4a.

The corresponding out-of-plane deformation υ(-C-H) of the aromatic compound is absorbed at *ca* 757-714 cm^{-1}. The intensity of the υ(=C-H stretching) suggests the structure of a polynuclear aromatic compound.

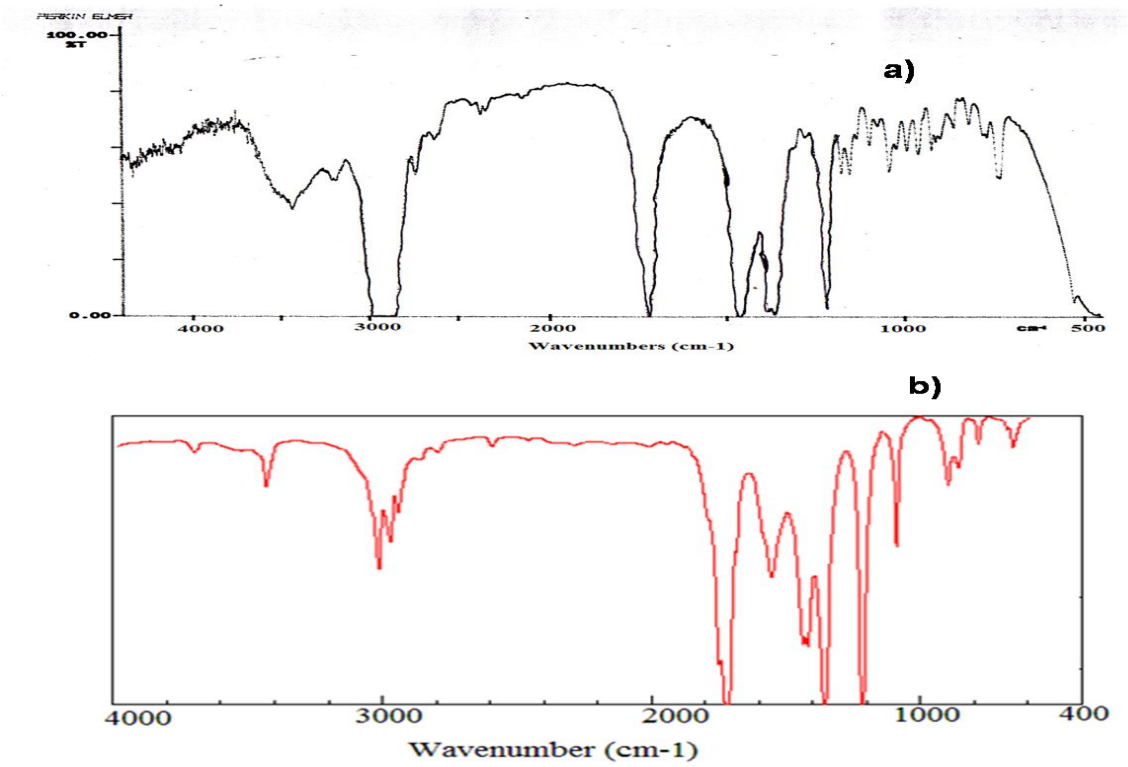

Figure 4: Infrared spectra at a resolution of 4 cm^{-1} of a) silica column refined vegetable indigo dye from *L. cyanescens* in acetone and b) neat acetone.

The broad peak 3700-3200 cm^{-1} is indicative of an O-H stretching of an alcohol. The absence of the primary amine group from the structural features of the refined indigo vegetable dye (cf Figure 4a and Figure 3a) and the associated removal of the noxious smell suggest that the foul smell could be a property of the primary amine which is the active constituent of the noxious principle in the crude indigo dye extract. A significant finding in this work is that the noxious principle could be isolated by swinging the pH of the crude extract solution to the acid region using few drops of 12.0 M HCl. The indigo dye precipitates from the solution with the yellowish green liquor supernatant giving the very pungent smell.

UV-visible spectroscopic studies of extracted dyestuff from *Lonchocarpus cyanescens*

UV-Vis spectroscopic studies of the separate components of the crude vegetable dye are shown in Figure 5

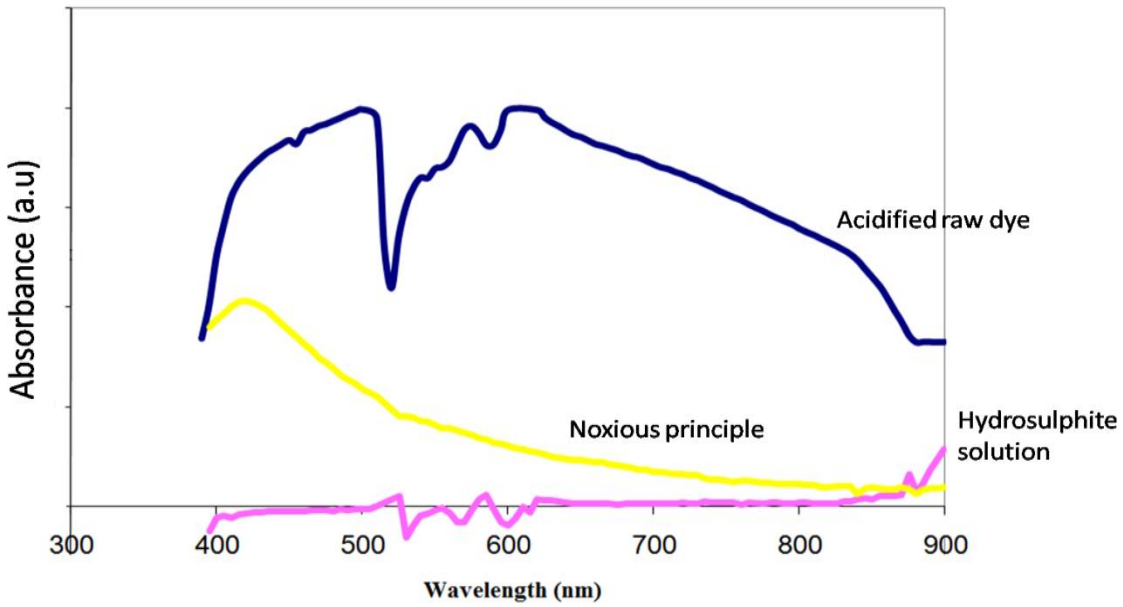

Figure 5: Spectra of the precipitated vegetable indigo dyestuff re-dissolved in alkaline hydrosulfite solution, the yellowish brown solution containing the noxious principles and the control alkaline hydrosulfite solution at a wavelength between 400 nm and 900 nm (visible spectrum).

Though UV spectroscopy suggests very minimal information about the structure of the dye, (Harris, 1995 and Pavia, et. al., 2000) it gives a valuable insight into the hue of the color of the vegetable dye and the contribution of the associate compounds to color contrast (Furniss,. et. al., 1978 and Bohren, & Clothiaux, 2006). Table 1 lists the hue of light transmitted at a given wavelength of absorbance.

Table 1: The colors of light at characteristic absorbance wavelength

Color	Wavelength (nm)
Violet	450-400
Indigo	420-450
Blue	450-490
Green	490-560
Yellow	560- 590
Orange	590-635
Red	635-700

The hydrosulfite solution does not show any significant activity around the wavelength of electromagnetic spectrum (visible region) scanned and therefore, can be inferred that it does not interfere much in the chromaticity of the dye extracted. The maximum absorption (λ_{max}) by the component of the dye with the noxious principle is 420 nm which corresponds to the wavelength of indigo light. The complementary color which is transmitted and hence visible to the eye is the yellowish color of the noxious extract according to the RGB color model (Bohren, and Clothiaux, 2006).

Similarly, dominant wavelength (λ_{max}) absorbed for the acidified dye precipitate is at 500 nm and 610 nm. This is equivalent to light absorbed around the green and orange region of the visible spectrum respectively hence, its complementary color transmitted will be red and blue which color combination results in violet/indigo to the visible eye (Bohren, and Clothiaux, 2006). It can therefore, be inferred that the noxious principle can be isolated from the vegetable dye extract without injuring the hue of the dye. Comparing the above spectroscopic results with similar studies in the literature (Kawagoe, and Robinson, 1981) lead one to suggest that the three known forms of the indigo dye; the ketal insoluble form (I), the sparingly soluble leuco form (II), and the soluble salt (III) of the dye might be represented schematically as in Figure 6.

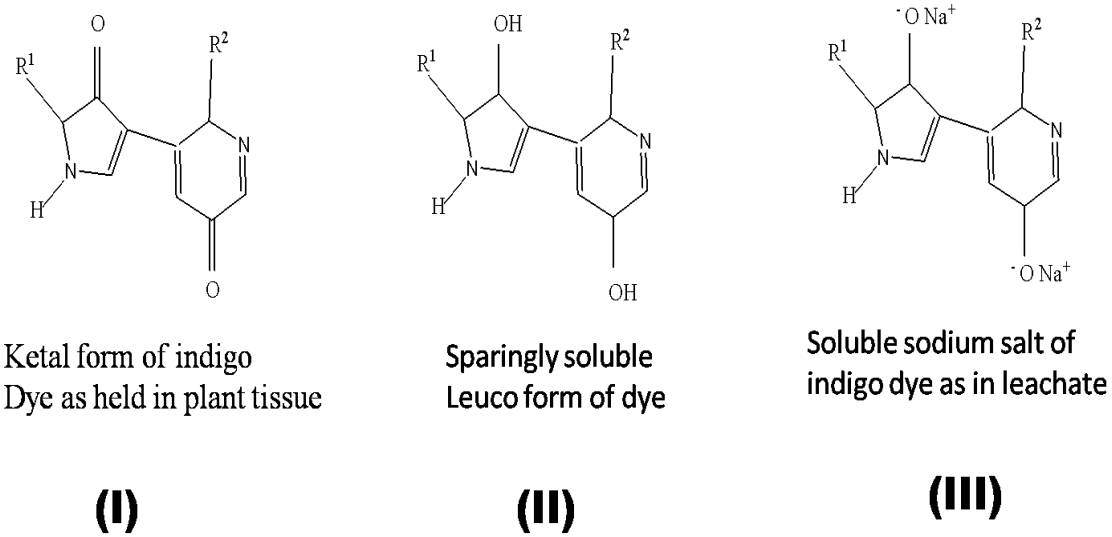

Figure 6: A postulation of the various chemical species of the vegetable indigo dye form as held in the plant tissue and in solution with R^1 and R^2 representing a different (alkyl and or aryl) substituent.

The effect of hydroxide concentration on yield of crude dye

Increasing the concentration of sodium hydroxide results in a corresponding increase in the yield of crude indigo dye extracted from the plant tissue from an optimum of 12% for a NaOH concentration of 0.05 M to 20.5% for a NaOH concentration of 0.1 M as shown in Figure 7.

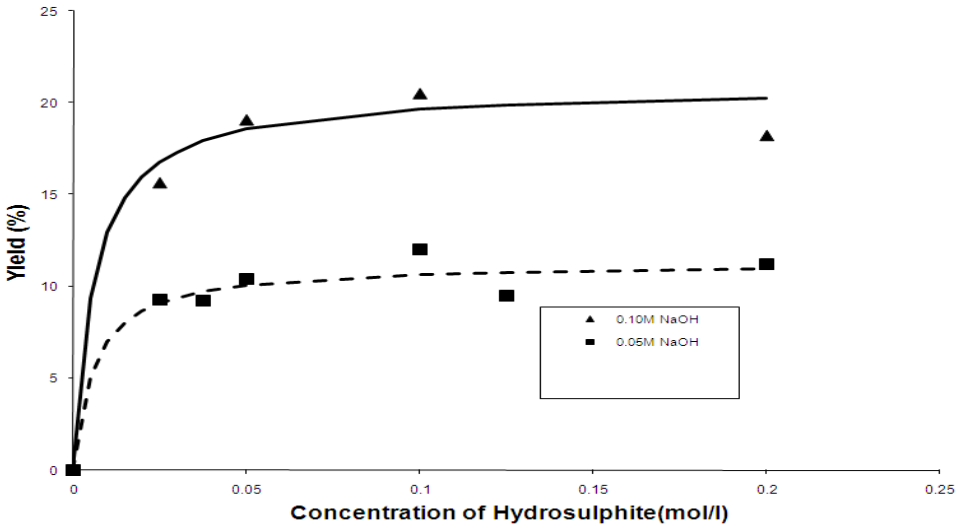

Figure 7: the yield of dye extracted from plant material as a function of hydrosulfite and caustic concentration in a cold maceration extraction set-up with a material: liquid ratio of 1:20.

Averagely, the yield increases by about 8% as the concentration of NaOH in the extraction solvent system doubles from 0.05 M to 0.1 M. The shift in equilibrium of dye species from the solid phase in the plant tissue to the aqueous phase with increasing pH suggests that the indigo dye in the plant material has a protonic species in its molecular structure which is sparing soluble in water (Kawagoe, and Robinson, 1981). Its dissociated product however, is soluble hence, the availability of hydroxide ions shifts the equilibrium towards dissociated products (oxide salt) as illustrated by the scheme in Figure 6.

The effect of hydrosulfite concentration on yield of crude dye

The yield of crude dyestuff increases steeply with increasing hydrosulfite concentration in the extracting solvent up to about 0.025 M as shown in Figure 7. The yield however, increases less steeply with subsequent increases in hydrosulfite concentration. This is probably due to two factors; the finite amount of dye in the plant material which becomes less available to be reduced by the hydrosulfite and also the hydrosulfite concentration in the reaction becomes depleted as the reaction progresses. The effect of the $Na_2S_2O_4$ and NaOH on the yield of the dye indicates clearly that the solubilization of the dye from the *Lonchocarpus cyanescens* leaves is very similar to that in vat dyes which ordinarily is initiated by the reduction of the insoluble dye species in the plant tissue into soluble leuco forms which are sparingly soluble (c.f. Figure 6).

Effect of extraction time on yield of dyestuff

In a solvent extraction system, the extraction efficiency is dependent on the capacity factor which is directly proportional to the partition coefficient (Harris, 1995). The capacity factor reflects the retention of the solute in the solid phase and is time related. To establish the optimum extraction time the yield of dyestuff from plant material was measured as a function of time as depicted in Figure 8. It is apparent from Figure 8 that the extraction is saturated after 90 min.

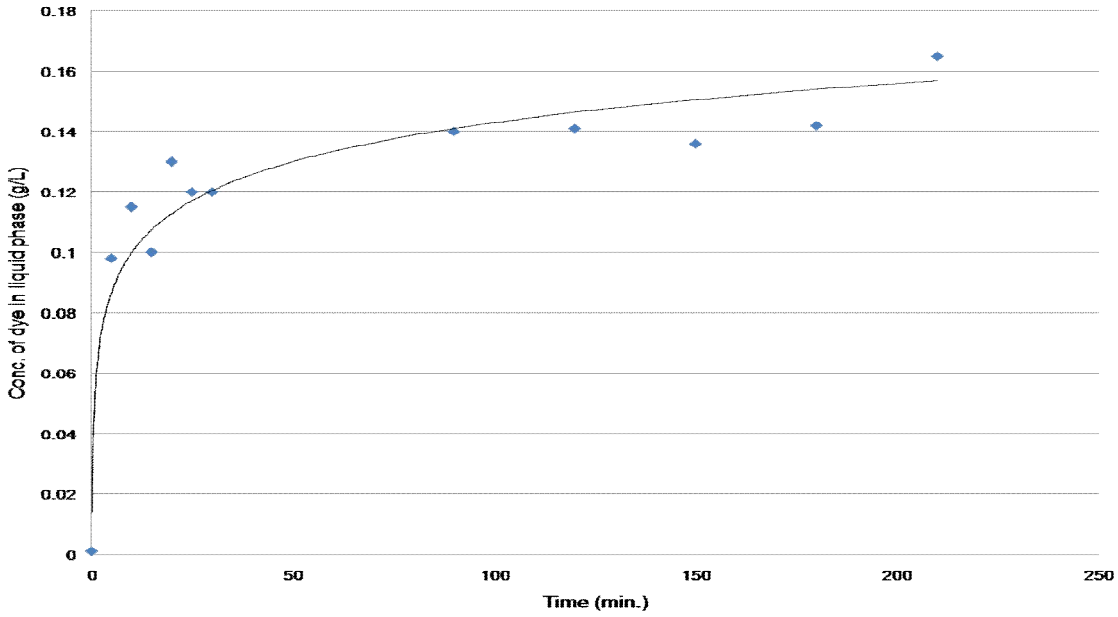

Figure 8: A kinetic study of the extraction of dyestuff from the plant material into solution in a batch system with material liquid ratio of 1:20 show the extraction is near saturation in 90 min.

Conclusion

The extraction of indigo dyestuff by cold maceration from the fermented leaves of *Lonchocarpus cyanescens* using 0.10 M $Na_2S_2O_4$ and 0.10 M NaOH gives a maximum yield of 20.5% wt/wt. The crude dyestuff can be refined through precipitation to remove the noxious principles by acidifying with concentrated hydrochloric acid. Infrared spectroscopic studies show that the main functional groups in the crude indigo dye are polyaromatic amine and alcohol groups which are consistent with synthetic indigo dyes. The noxious principle is probably a Volatile primary amines and their removal enhance the value of the dyestuff. The indigo dye is insoluble in water when in the leaves as it is present in a ketal form.

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