



**Research Article**

**EXTRACTION OF NATURAL INDIGO DYE FROM *INDIGOFERA ARRECTA* AND ITS APPLICATION ON COTTON FABRICS**

Ukanah S.P.\*<sup>1</sup>, Salisu Z.M.<sup>1</sup>, Shittu U.M.<sup>1</sup>, Gadimoh S.<sup>1,2</sup>, Joseph O. Odey<sup>3</sup>, Suleiman M.A.<sup>1</sup>, Gero M.<sup>1</sup>, Sulaiman I.A.<sup>1</sup>, Umar I.S.<sup>1</sup>, Oddy-Obi I.C.<sup>1</sup>, Ilyasu A<sup>1</sup>, and Daniel D<sup>1</sup>.

<sup>1</sup>Department of Textile Technology, National Research Institute for Chemical Technology, Zaria, Nigeria.

<sup>2</sup>Department of Textile Technology, College of Science and Technology, Kaduna Polytechnic, Kaduna, Nigeria.

<sup>3</sup>Department of Pure and Applied Chemistry, Faculty of Physical Sciences, University of Calabar, Calabar, Nigeria.

\*Corresponding author's Email: [pendo4success@gmail.com](mailto:pendo4success@gmail.com), [doi.org/10.55639/607.02010091](https://doi.org/10.55639/607.02010091)

**ARTICLE INFO:**

**Keywords:**

*Indigofera arrecta*,  
Enzymatic hydrolysis,  
Indigotin,  
Vat dyeing,  
Chromophore.

**ABSTRACT**

Indigo dye is an historically important natural pigment that is traditionally obtained from plants of the *Indigofera* genus. In this study, indigo dye was extracted from *Indigofera arrecta* leaves via enzymatic hydrolysis and followed by alkaline treatment to release indigotin which is the principal chromophore. The extraction yielded approximately 0.236 wt % crude indigo based on fresh leaf mass as determined from a single extraction experiment. The dye was purified and characterized using UV-visible and FTIR spectroscopy to confirm the presence and structural integrity of indigotin. The dye exhibited a maximum absorption wavelength at 617 nm and was applied on cotton fabric through a conventional vat dyeing technique. Its dyeing performance was assessed based on dye uptake and colour fastness properties, including resistance to washing, rubbing, and light exposure. The wash fastness showed ratings between 4 and 5 for both colour change and staining of undyed adjacent fabric. Rubbing fastness results indicated ratings of 3–4 under wet conditions and 4 under dry conditions, while light fastness showed a rating of 6 on the blue wool scale. These results demonstrate that *Indigofera arrecta* is a viable alternative source of natural indigo, with promising dyeing performance and potential for sustainable textile applications.

**Corresponding author:** Ukanah S.P Email [pendo4success@gmail.com](mailto:pendo4success@gmail.com)

Department of Textile Technology, National Research Institute for Chemical Technology, Zaria, Nigeria

**INTRODUCTION**

Colour has been an integral part of human life from the Stone Age through to the contemporary digital era, significantly contributing to the aesthetic quality of the environment (Bhuyan and Saikia, 2004). It plays a crucial role in everyday life, influencing areas such as

clothing, interior decoration, and visual communication (Yusuf *et al.*, 2017). Prior to the late eighteenth century, all colouring agents were obtained exclusively from natural sources such as plants, lichens, insects, and mollusks but with the advent of the Industrial Revolution in the nineteenth century, the

production of dyes shifted towards chemical synthesis (Holme, 2006).

Based on origin, dyes are broadly classified into natural dyes and synthetic dyes. While the natural dyes are biodegradable and environmentally friendly because they are obtained from natural sources, the synthetic dyes are produced through chemical synthesis and offer a wide range of bright, consistent colours with superior fastness properties over the natural dyes, but with toxic byproduct which poses health and environmental concern (Lizamoni and Smita, 2020).

Amongst the wide range of natural dyes receiving increasing international attention, indigo is distinguished as one of the earliest known and most widely utilized dyes in commercial history. It originates from a colourless glucoside of the enol form of indoxyl, commonly known as indican (Laitonjam and Wangkheirakpam, 2011). Owing to its long-

standing historical significance, indigo continues to be an important industrial dye, largely driven by the sustained demand for indigo-coloured denim fabrics (Garcia-Macias and John, 2004). Natural indigo is obtained from several plant sources amongst which is *Indigofera arrecta*, where it exists as precursor compounds that require enzymatic hydrolysis and oxidation to form the insoluble indigo pigment. However, despite growing interest in natural indigo as an environmentally friendly alternative to synthetic dyes, its application is associated with inherent challenges arising from multistep bioconversion which result in low and variable dye yields, thus, limiting application in large scale. Also, the poor solubility of indigo in water necessitate its chemical reduction to soluble leuco compound before application which further complexes the entire process. Figure 1 shows the chemical structures of indigo and its corresponding leuco form.

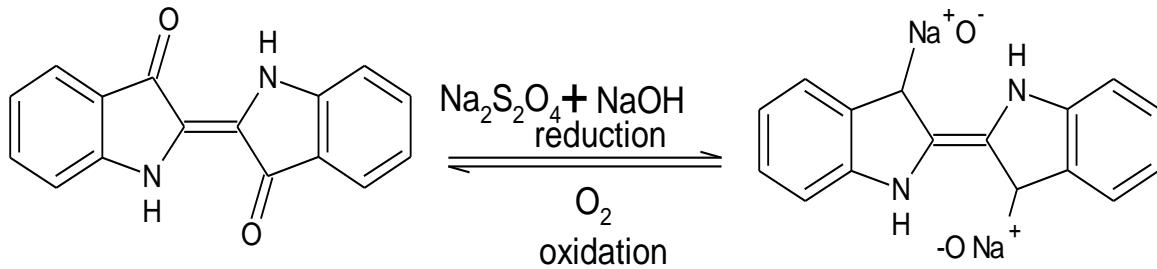


Figure 1: Chemical structure of indigo and its sodium leuco indigo

*Indigofera arrecta*, commonly referred to as Bengal indigo or Java indigo, is a leguminous plant belonging to the family Fabaceae. It is widely distributed across tropical and subtropical regions of Africa and Asia and serves as one of the major botanical sources of natural indigo dye. The species is a rapidly growing shrub that flourishes in warm climates and well-drained soils. *Indigofera arrecta* has been cultivated primarily for its high indigotin content, which is obtained from its leaves through fermentation processes.

However, despite the traditional use of *Indigofera arrecta* as a natural indigo source, there is scanty literature with no quantitative data on indigo dye yield from fresh leaves and enzymatic hydrolysis during fermentation. This study addresses these gaps by reporting extraction yield based on fresh leaves of *Indigofera arrecta* and also discussing the biochemical basis of indigo formation.

## MATERIALS AND METHODS

### Materials and Equipment

Sodium hydrogen sulphite (Hydros), caustic soda, ammonium hydroxide, sodium carbonate, acetic acid,

and *N,N*-dimethylformamide (DMF) were purchased from Sigma Aldrich, Germany. The chemicals were of analytical grade and were used without further purification. Plain weaved cotton fabric was also obtained from terminus market, Jos, Plateau State, Nigeria. UV-Visible Spectrophotometer (UV-2500PC Series), Fourier Transform Infrared Spectrophotometer (Agilent technology), Light Fastness Tester Xenon Arch Light Fastness Tester, Laboratory dyeing machine were used for the study.

### Plant materials

The fresh leaves of *Indigofera arrecta* were obtained from the research farm of Textile Technology Department at National Research Institute for Chemical Technology, Zaria, Nigeria. The leaves were washed with fresh water to remove dust and sands. The sample was conditioned under laboratory conditions of 25 - 30 °C and 65 ± 5% relative humidity for 24 h prior to weighing.

### Extraction of Indigo Dye

Fresh *Indigofera arrecta* leaves were soaked in distilled water for 5 days under ambient laboratory

temperature of 25 – 30 °C in a ratio of 1: 4 w/v to allow fermentation, and aqueous ammonia was added to adjust the pH to 11.0. The pH was monitored with a calibrated digital pH meter ( $\pm 0.05$  pH units) throughout the fermentation process. The solution was filtered to separate the leaves from the filtrate, and the filtrate was oxidized using an aquarium type air pump with air flow rate of 1.0 L/min. in a 5 L beaker to supply gentle and continuous aeration suitable for indigo formation for 1 h. The solution was left standing in an open air over a period of 24 h to allow the fine particles of the dye settle at the bottom. It was then filtered using vacuum pump and the residue which is the desired dye was oven dried at 105 °C to obtain the indigo dye powder.

#### Purification of Extracted Indigo Dye

The extracted indigo dye was purified by recrystallization following established procedures for vat dye purification (Hunger, 2003). Crude indigo was suspended in a minimal volume of hot *N*, *N*-dimethylformamide (DMF) and heated to obtain a homogeneous deep-blue solution. The mixture was filtered while hot to remove insoluble materials. Purification was achieved by slowly adding 8 volumes of cold water to the filtrate with vigorous stirring, resulting in the precipitation of indigo as fine crystalline solids. The product was isolated by vacuum filtration, washed thoroughly with water, and dried at 105 °C to afford purified indigo dye.

#### Scouring of Cotton Fabric

The purchased cotton fabric was scoured following the method reported by Vankar *et al.*, 2009 with slight modification. The fabric was boiled for 30 min. in a solution containing 2 g/l sodium carbonate and 2 g/l non-ionic detergent, keeping the material to liquor ratio at 1:40 o.w.f. The scoured cotton fabric was thoroughly washed with warm water, neutralized with 1 g/l acetic acid and rinsed again using distilled water.

#### Dyeing of cotton with indigo dye

The indigo dye bath was prepared according to the method reported by Nizar *et al.*, 2008 with slight modification. Indigo dye (1.0 g) was dispersed in 200 mL water, and sodium hydroxide 1 ml of 50% (w/v) corresponding to 2.5 g/l NaOH, was added to a total dyebath volume of 200 ml to adjust the pH to 11. Sodium hydrogen sulphite (1.0 g) was added gradually under stirring, and the solution was maintained at 50–55 °C for 20–30 min. until the solution decolorized from blue colour of indigo to greenish-yellow of leuco-indigo. Previously treated cotton fabric was introduced into the vat and dyed at a liquor ratio of 50:1 o.w.f. for 20 min. and exposed to air for

oxidation. After dyeing, the cotton fabric was rinsed, washed with a mild detergent solution and dried at room temperature for 24 h.

#### Characterization of the Extracted Dye

The purified dye was taken for the UV-Visible spectrophotometry and FTIR spectroscopy for their functional group detection and melting point determination to ascertain their heat stability.

#### UV-visible spectroscopy

The pure dye was analysed for its maximum absorption wavelength i.e.  $\lambda_{\text{max}}$ . The UV-visible spectra were measured in the range of 350–800 nm using UV-2500PC Series

#### FT-IR spectroscopy

The Infrared Spectrum was obtained using Agilent technology to find the functional groups present in the purified dye.

#### Melting/Decomposition point determination

The melting point of indigo was determined using a digital melting point apparatus. A small amount of dried sample was loaded into a sealed capillary tube and heated at a rate of 2 °C/min. The temperature at which decomposition occurred was recorded and reported as the decomposition point.

#### Determination of Colour Fastness Properties

**Wash fastness:** The wash fastness test of the dyed sample was carried out according to the standard procedure specified by International Standards Organization (ISO 3). The composite samples were treated with a solution containing 5 g/L detergent, 2 g/L sodium carbonate at liquor ratio 50:1 for 30 min. at 60 °C. The change in colour of the tested samples and the degree of staining of the adjacent undyed fabrics were evaluated using grey scale.

**Rubbing fastness:** Rubbing fastness of the dyed cotton fabric was evaluated according to ISO 105-X12 using a crockmeter under dry and wet conditions. Colour transfer was assessed using the grey scale for staining.

**Light fastness:** The light fastness was tested according to ISO 105-BO2:2014 test method. The dyed fabric was exposed to xenon arc lamp for 48 hours alongside the blue wool standard fabrics at standard testing conditions of 60% RH and black standard temperature of 45 °C. The light fastness was assessed by comparing the fading of the specimen with that of blue wool standard.

## RESULTS AND DISCUSSION

#### Extraction of indigo dye from *Indigofera arrecta*

The extraction of indigo dye from *Indigofera arrecta* leaves was carried out following enzymatic hydrolysis and aerobic oxidation pathway which is characteristic of indigo dye yielding plants. Freshly harvested leaves

of *Indigofera arrecta* were soaked in water and maintained under mildly alkaline conditions for 120 h, during which the endogenous  $\beta$ -glucosidase enzymes naturally present in the leaves facilitated the hydrolysis of indican (indoxyl- $\beta$ -D-glucoside) to indoxyl. The fermentation liquor gradually developed a pale yellow-green coloration, consistent with the

accumulation of soluble indoxyl. Upon subsequent aeration, a rapid colour transition to deep blue was observed as indoxyl underwent oxidative coupling to form insoluble indigo. The resulting indigo particles aggregated and settled readily, enabling efficient separation via decantation and filtration.

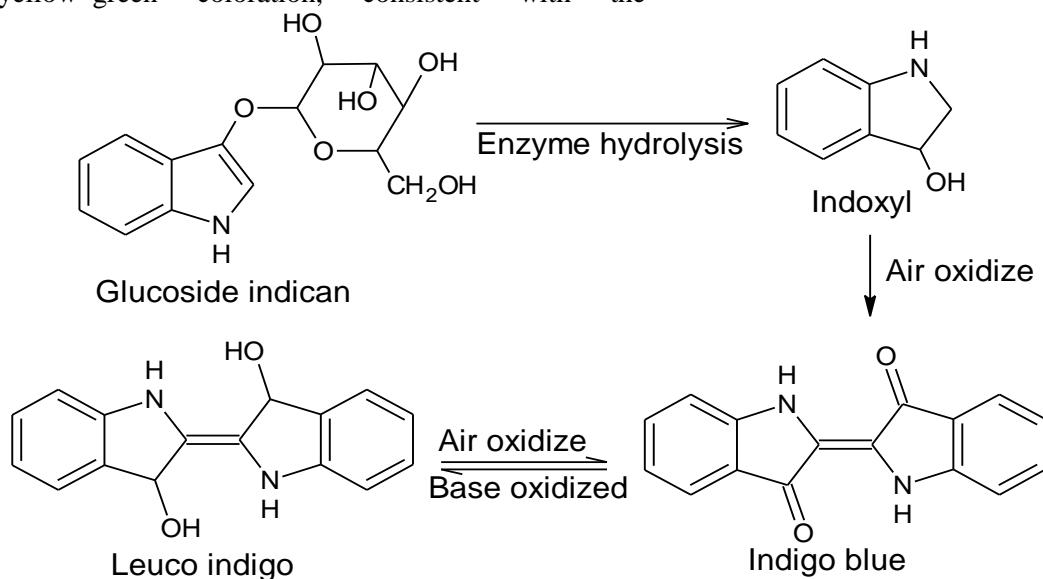


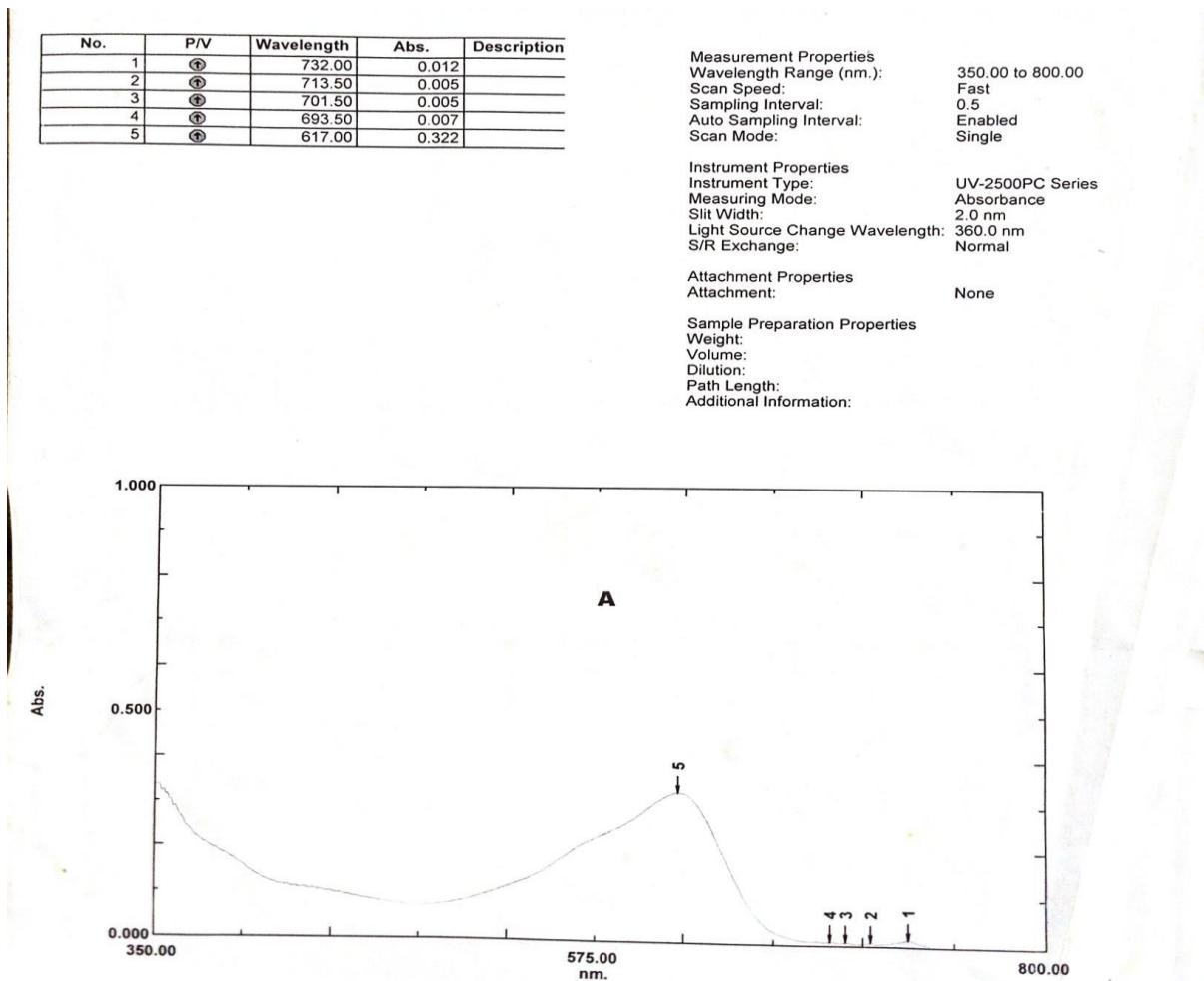
Figure 2: Reactions of indigo dye production process (Frignani *et al.*, 2024, Nguyen *et al.*, 2019).

The yield obtained was 0.236% (w/w fresh leaf) indicating a relatively low pigment content, which is characteristics of natural dyes (Ukanah *et al.*, 2020). In comparison with *indigofera tinctoria* which is most widely cultivated plant for natural indigo production in Asia and around the world, typically provides a higher yield of approximately 0.4% (Bhattacharyya 2010). This difference highlights the superior dye-bearing characteristics of *Indigofera tinctoria* and reinforces its long standing prominence in natural indigo cultivation and processing. However, *Indigofera arrecta* represents a promising alternative natural source of indigo dye, particularly within African region where the species is widely distributed and readily cultivated. Its potential for sustainable dye production is significant; however, the quantity and quality of indigo obtained from the plant may vary considerably. Generally, yields obtained from natural dyes are small (Ukanah *et al.*, 2020) and influenced by multiple factors such as extraction method employed, local climatic conditions, soil fertility, and the physiological maturity of the plant at the time of harvest etc. Also, environmental parameters such as

sunlight exposure, rainfall patterns, etc. affect the concentration of indigo precursors within the leaves. Optimization of these variables, in addition to refined extraction procedure, can significantly enhance dye recovery and improve the viability of *Indigofera arrecta* as a competitive natural indigo source.

#### UV-Visible absorption spectrum

Figure 3 shows the UV visible absorption spectrum of the extracted indigo dye. The UV-vis spectrum was recorded in DMF using a path length of 1 cm quartz cuvette, slit width of 2 nm over a wavelength range of 350 – 800 nm and a dye concentration of 10 mg/L. The absorption maximum ( $\lambda_{\max}$ ) was measured and found to be 617 nm which is characteristic of the  $\pi \rightarrow \pi^*$  electronic transition associated with the highly conjugated indigo chromophore. This result is in agreement with the findings of Chanayath *et al.*, 2002; Pathak and Madamwar, 2009, Vanquelin *et al.*, 2024 etc. which confirm the presence of the oxidized indigoid form. The strong absorption in the visible region arises from the extended delocalization of electrons across the bis-indole framework, which imparts the distinctive deep-blue coloration of the dye.

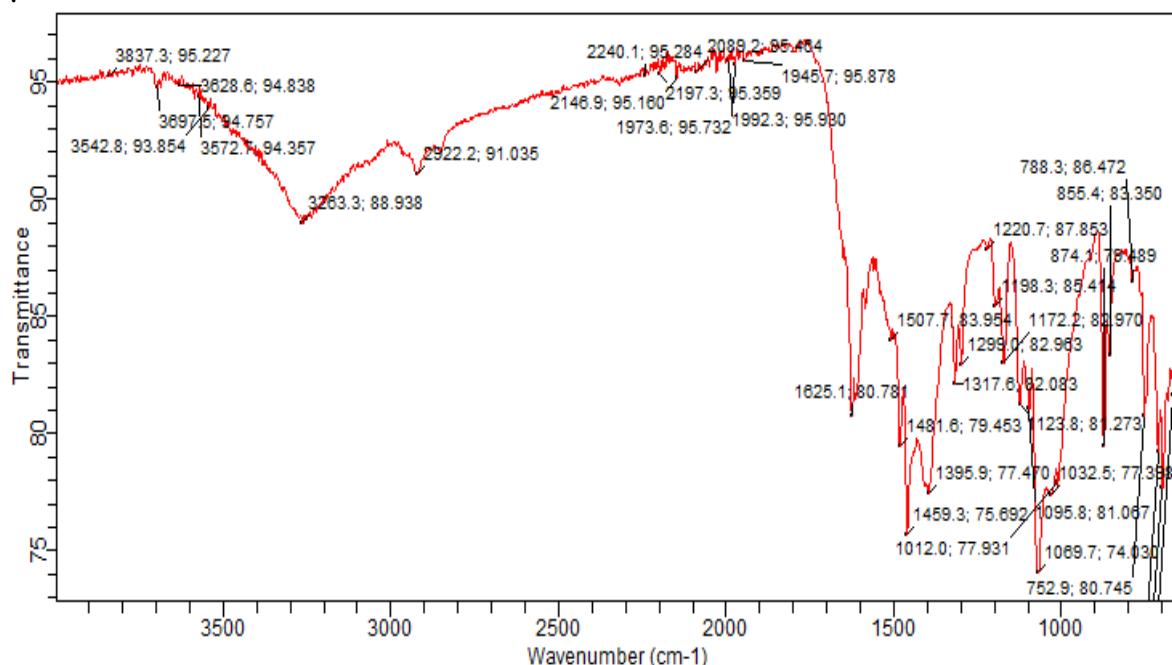


**Figure 3:** UV-visible spectrum of Indigo dye

#### FTIR Analysis of the Extracted Dye

Figure 4 shows the Fourier transform infrared (FTIR) spectrum of the extracted dye. The FTIR spectrum was recorded in the range 4000 - 650  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ . This was employed to confirm the presence of characteristics functional groups associated with the indigoid compound, thus, unravelling the chemical identity of the dye extracted from *Indigofera arrecta* leaves. Figure 4 shows the spectrum of the extracted dye. The absorption peak observed at 3263.3  $\text{cm}^{-1}$  is attributed to N-H stretching vibrations arising from the secondary amide functionalities present in the indigo molecular structure. The absorption peaks observed at 2922.2  $\text{cm}^{-1}$  was due C-H stretching of alkanes while absorption peaks arising from C=O stretching vibrations was observed at 1625.1  $\text{cm}^{-1}$  which correspond to the conjugated carbonyl groups, thus, confirming the presence of the indigo chromophore. The absorption band observed near 1507.7  $\text{cm}^{-1}$  is assigned to C=C stretching vibrations within the aromatic ring system, while peaks in the

region of 1459.3–1481.6  $\text{cm}^{-1}$  are associated with N-H bending vibrations. These bands further support the presence of indigoid structures in the extracted dye. The absorption bands appearing in the region 1012.0–1395.9  $\text{cm}^{-1}$  are regions attributed to the overlapping between C–N and C–O stretching vibrations. While the C–N stretching specifically appears at 1029–1200  $\text{cm}^{-1}$ , and C–O appears around 1080–1350  $\text{cm}^{-1}$ , but because of the overlap and close proximity of their vibrational modes, definitive assignment to single functional group is difficult and as such, these bands are interpreted as arising from the contributions of both the C – N and the C – O groups, which is consistent with the molecular structure of indigo. The absorptions peaks below 800  $\text{cm}^{-1}$  are associated with out-of-plane C–H bending vibrations of substituted aromatic rings. The overall spectral profile is in agreement with the findings of Chanayath *et al.*, 2002; Pathak and Madamwar, 2009; and Vanquelin *et al.*, 2024 thus, affirming the extraction of the target dye from *Indigofera arrecta*



**Figure 4:** FTIR spectrum of extracted Indigo dye

#### Melting/Decomposition Point of Indigo

The indigo dye sample did not exhibit a melting transition or visible decomposition up to the maximum operating temperature of the melting point apparatus (300 °C). The melting point therefore exceeds 300 °C (>300 °C), consistent with the known high thermal stability and decomposition behaviour of indigo.

#### Dyeing Mechanism of Indigo on Cotton Fabric

Dyeing of cotton fabric with indigo dye extracted from *Indigofera arrecta* was carried out via vat dyeing technique which necessitate the reduction of insoluble indigo dye to soluble leuco compound to allow diffusion into the cellulose matrix and subsequent oxidation to regenerate the insoluble indigo within the fabric. The reduction which was carried out under alkaline condition (NaOH/Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>), was confirmed by the characteristic yellow-green colouration of the dye-bath indicating successful conversion of insoluble

indigo dye to soluble leuco compound (Tayade and Adivarekar, 2014). In its reduced state, indigo becomes water-soluble and exhibits affinity for cellulose due to enhanced molecular mobility and hydrogen-bonding interactions and upon immersion of the cotton fabric, the leuco-indigo diffused into the amorphous regions of the cotton fabric, facilitated by swelling of cellulose under alkaline conditions. Exposure of the dyed fabric to air resulted in oxidative regeneration of indigo, producing the characteristic blue colouration. The insoluble indigo formed during oxidation became physically entrapped within the cellulose microfibrillar structure of the fabric.

#### Colour Fastness

The colour fastness properties of cotton fabrics dyed with indigo dye extracted from *Indigofera arrecta* were evaluated with respect to washing, rubbing, and light exposure and the results are shown in Table 1.

**Table 1:** Results of colour fastness of dyed cotton fabric

Dyed Sample	Rubbing Fastness	Wash Fastness	Light Fastness
Cotton Fabric	Wet	Dry	Colour Change Degree of staining

3-4

4

4

5

6

The wash fastness of the dyed cotton fabrics exhibited good to very good resistance to colour change and staining with a rating of 4 and 5 respectively as seen in Table 1. The good wash fastness is attributed to the insoluble nature of the indigo pigment after oxidation within the fabric and its entrapment within the fabric structure, thus, resulting to resistance to washing (Degani *et al.*, 2015). Rubbing fastness was carried out using crock metre and the result of the fastness rating were 3-4 and 4 for the wet rubbing and dry rubbing respectively. While the results of the dry rubbing indicate minimal colour transfer during the mechanical abrasion, the wet rubbing showed a result slightly lower and this may be attributed to inadequate washing after dyeing that leaves residual dye on fabric surface. The result of the light fastness testing revealed good photo-stability with a rating of 6 on the blue wool scale after prolonged exposure. The observed photo stability reflects the high conjugation and molecular stability of the indigo chromophore.

## CONCLUSION

This study examined the laboratory scale extraction, characterization, and application of indigo dye derived from *Indigofera arrecta*. The successful formation of indigo was confirmed by UV-Vis and FTIR analyses, and its application to cotton fabric was demonstrated using a conventional vat dyeing process. The study is limited by its laboratory scale scope, the absence of detailed kinetic study and the lack of evaluation of cost-effectiveness for large scale applications. Accordingly, while the results provide baseline data supporting feasibility under controlled conditions, further investigations involving process optimization, pilot-scale trials, and economic viability assessment are required before conclusions regarding industrial or large-scale textile applications can be drawn.

## REFERENCES

Bhattacharrya, N. (2010). Natural Dyes for Textiles and their Eco-friendly Applications (1st ed.). New Delhi: IAPL Publications.

Bhuyan, R. and Saikia, D.C. (2004). Natural dyes: Present scenario in North East India. *Colourage LI* (5): 27-32.

Chanayath, N., Lhieochaiphant, S., and Phutrakul, S. (2002). Pigment Extraction Techniques from the Leaves of *Indigofera tinctoria* Linn. and *Baphicacanthus cusia* Brem. and Chemical Structure Analysis of their major components. *CMU J.*, 1: 149-160

Degani, L., Riedo, C., and Chiantore, O. (2015). Identification of Natural Indigo in Historical Textiles by GC-MS. *Analytical and Bioanalytical Chemistry*, Volume 407(6), pp. 1695–1704.

Ding, Y. and Freeman, H.S. (2017). Mordant dye application on cotton: Optimisation and combination with natural dyes. *ColorationTechnology*, Vol. 133 No. 5, pp. 369-375.

Frignani, E., D'Eusonio, V., Grandi, M., Pigani, L., and Roncaglia, F. (2024). A Continuous Extraction Protocol for the Characterisation of a Sustainably Produced Natural Indigo Pigment. *Life* 14, 59.

García-Macías, P. and John, P. (2004). Formation of Natural Indigo Derived from Woad (*Isatis tinctoria L.*) in relation to Product Purity. *Journal of Agricultural and Food Chemistry*. 52:7891-7896.

Holme, I. (2006). Sir William Henry Perkin: A review of his life, work and legacy. *Colouration Technology* 122, 235–25.

Hunger, K., (2003). Industrial Dyes: Chemistry, Properties and Applications, copy right WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. ISBN 3-527-30426

Isma'il, Ö.E. and Yıldırım, L. (2019). Metal mordants and biomordants, in the Impact and Prospects of Green Chemistry for Textile Technology, Elsevier, pp. 57-82, doi:10.1016/B978-0-08-102491-1.00003-4.

Janhom, S., Watanesk, R., Watanesk, S., Griffiths, P., Arquero, O. and Naksata, W. (2006). Comparative study of Lac dye adsorption on cotton fibre surface modified by synthetic and natural polymers. *Dyes and Pigments*, Vol. 71 No. 3, pp. 188-193.

Laitonjam, W.S. and Wangkheirakpam, S.D. (2011). Comparative study of the major components of the indigo dye obtained from *Strobilanthes flaccidifolius* Nees and *Indigofera tinctoria* Linn. *International Journal of Plant Physiology and Biochemistry* Vol. 3(7), pp. 108-116.

Lizamoni, Chungkrang and Smita, Bhuyan. (2020). Natural Dye Sources and its Applications in Textiles: A Brief Review. *Int. J. Curr. Microbiol. App. Sci.* 9(10): 261-269. doi: <https://doi.org/10.20546/ijcmas.2020.910.034>

Nguyen, T.K.O., Marcelo, P., Gontier, E., and Dauwe, R. (2019). Metabolic Markers for the Yield of Lipophilic Indole Alkaloids in Dried Woad

Leaves (*Isatis tinctoria L.*). *Phytochemistry* 163, 89–98.

Nizar, M., Kechida, M., and M'henni, M. (2008). New process of dyeing cotton textile by indigo with borohydride: effect of concentration of the reducing agent. *International Journal of Applied Chemistry*, 4,1–14.

Pathak, H. and Madamwar, D. (2010). Biosynthesis of indigo dye by newly isolated naphthalene-degrading strain *Pseudomonas sp.* HOB1 and its application in dyeing cotton fabric. *Applied Biochemistry and Biotechnology*, 160, 1616–1626.

Tayade, P.B. and Adivarekar, R.V. (2014). Extraction of Indigo dye from *Couroupita guianensis* and its application on cotton fabric. *Fashion and Textiles* 1:16. doi:10.1186/s40691-014-0016-3.

Ukanah, P.S., Gadjimoh, S., Salisu Z.M., Suleiman, M.A., Sulaiman, I.A., Chibuzo-Anakor, N.C., Umar, I.S., Lawal, O.M. and Oddy-Obi, I.C. (2020). Influence of Selected Mordants and Mordanting Techniques on Eco-Friendly Dyeing of Cellulosic Fabric using Natural Flavonoid Dye Extracted from Onion Outer Scale. *Nigerian Journal of Textiles (NJT)*, vol. 6.

Vauquelin, R., Juillard-Condat, L., Joly, N., Jullian, N., Choque, E. *et al.*, (2024). Study of Woad (*Isatis tinctoria L.*)-Extracted Indoxyl Precursors Conversion into Dyes: Influence of the Oxidative Media on Indigo Recovery Yields and Indigo/Indirubin Ratio Measured by HPLC-DAD Method. *Molecules*, 10.3390/molecules29204804. hal-04731728

Yusuf, M., Shabbir, M. and Mohammad, F. (2017). Natural Colorants: Historical, Processing and Sustainable Prospects. *Nat Prod Bioprospect*, 7(1): 123–145.