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MACERATION-BASED EXTRACTION AND SPECTROSCOPIC CHARACTERISATION OF *Sorghum bicolor* LEAVES EXTRACT AS A NATURAL DYE ON COTTON FABRIC

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ABSTRACT

This study investigates the extraction, characterisation, and application of natural dyes derived from *Sorghum bicolor* leaves as an eco-friendly alternative to synthetic dyes in textile colouration. The dye was extracted using ethanol-based maceration and purified via gravity column chromatography, with thin-layer chromatography (TLC) employed for component monitoring. Phytochemical screening confirmed the presence of flavonoids, tannins, and alkaloids bioactive compounds known for their colourant and antioxidant properties. UV-Vis and FTIR spectroscopy further validated the presence of chromophoric and phenolic functional groups, suggesting the suitability of the extract for dyeing applications. The dye was applied to cotton fabrics, and colour fastness was evaluated with and without ferrous sulfate mordanting. At a 4% dye concentration, both mordanted and unmordanted fabrics recorded a Blue Wool Scale (BWS) rating of 5. However, at 8%, mordanted fabrics achieved a higher rating of 6, indicating improved light fastness. This enhancement is attributed to the formation of stable dye-metal complexes, which increase UV resistance and dye-fibre binding. Mordanted fabrics also exhibited better wash fastness due to the formation of insoluble dye–metal–fibre complexes that reduce dye leaching. Rubbing fastness was moderate for both treatments, with more colour transfer observed under wet conditions.

Overall, *Sorghum bicolor* leaves extract shows strong potential as a sustainable natural dye source, offering acceptable fastness properties and aligning with environmentally responsible textile practices.

Keywords: Natural dye, Maceration, Characterisation, Sorghum *bicolor* leaves, Cotton fabric

INTRODUCTION

As humanity transitions from the Information Age to the Environmental Age, there is a growing interest in sustainable technologies and materials, including natural dyes derived from plants, animals and minerals [1]. Unlike synthetic dyes, which were once widely adopted for their vivid colours and ease of production but are now recognised for their potential carcinogenic and allergenic properties, natural dyes are valued for being eco-friendly, non-toxic and often possessing additional medicinal benefits [2]. This resurgence of interest in natural dyes is particularly evident in regions with strict environmental regulations and among consumers who prioritize health and sustainability [3].

Among the many plant species being explored for natural dye production, *Sorghum bicolor* L. stands out as a promising candidate. Sorghum is a versatile crop, widely cultivated in arid and semi-arid tropical regions, and is recognised for its nutritional richness, containing minerals, proteins, vitamin E, bioactive compounds (such as phytosterols and policosanols), and high fibre content [4]. Beyond its role as a staple food and animal feed, sorghum is a rich source of phenolic acids (ferulic, tannic, and p-

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coumaric acids), flavonoids (including luteolin, apigenin, catechin gallate, and epigallocatechin), tannins, and lipids compounds that contribute not only to its nutritional value but also to its potential as a natural dye [5]. These bioactive constituents are associated with antioxidant, antibacterial, anticancer, and cardioprotective effects, further enhancing the appeal of sorghum-based dyes for applications in textiles, food, and pharmaceuticals [5-6].

The extraction of natural dyes from *Sorghum bicolor* leaves can be accomplished through various methods, but maceration is particularly favoured for its simplicity and gentle processing conditions. Maceration involves soaking sorghum plant material such as leaves, stems, or grains in a solvent (commonly water or ethanol) at ambient or slightly elevated temperatures for a specified period [7]. This method allows the dye compounds to diffuse into the solvent without the risk of thermal degradation that can occur with more aggressive techniques, such as Soxhlet extraction. Although Soxhlet extraction is efficient and yields highly concentrated dye solutions [8], maceration is better suited for preserving the integrity of heat-sensitive bioactive compounds in sorghum, ensuring that both the colour and potential health benefits are retained [9].

Characterisation of the extracted dyes is a crucial step in the development and application of natural dyes. UV-Visible (UV-Vis) spectroscopy is widely used to analyse the absorption spectra of dye solutions, providing valuable information about the presence of chromophores, dye concentration, and colour properties [1]. This study investigates the extraction, spectroscopic characterisation, and potential application of *Sorghum bicolor* leaves as a natural dye source. Evaluating the quality and potential colour yield of sorghum-derived dyes on various textile substrates is essential, and this is where analytical techniques play a crucial role. Fourier-transform infrared (FTIR) spectroscopy, in particular, is employed to identify key functional groups present in the dye extracts, including those linked to phenolic compounds, flavonoids, tannins, and other bioactive molecules responsible for the colour and functional properties [2].

MATERIALS AND METHODS

Chemicals and reagents

Laboratory grade ferrous sulphate ($FeSO_4$) was used as mordant while a diluted solution (2 g/L) of sodium carbonate (Na_2CO_3) was used to adjust the pH of the dye solution to 7. Reference detergent A (ECE phosphate-free standard detergent powder wfk-Testgewebe GmbH) soap (5 g/L) was used for the wash fastness test. Hydrochloric acid (37% fuming HCl), chloroform, methanol, glacial acetic acid, ethanol, Wagner's reagent (potassium iodide and iodine crystal) and sulfuric acid, all of which were of analytical grade and obtained from Merck (Darmstadt, Germany) were utilised in this study.

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Plant material

Sorghum bicolor leaves were collected as discarded plant waste from households in Boher, Nasarawa State. The plant was authenticated by the Botanical Survey, University of Calabar, Nigeria (2024/CAL/HRB 54). The material was carefully rinsed with distilled water to eliminate surface impurities, ensuring it was not crushed during the process. After cleaning, the samples were air-dried in a shaded area over several weeks to preserve delicate colour compounds that might degrade under heat [10]. Once thoroughly dried, the plant material was ground into a fine powder using a manual blender and stored at room temperature.

Extraction method

The extraction was carried out using a slightly modified method from Jack *et al.* [11]. Dried powdered *Sorghum bicolor* leaves (500 g) were soaked in 2.0 L of ethanol at room temperature (~25 °C) for 72 hours with intermittent stirring. The mixture was then filtered to collect the first extract. The leftover plant material was soaked three more times under the same conditions, each with fresh ethanol, to ensure complete extraction. All the filtered extracts were combined to get the final ethanol extract.

Column chromatography

The ethanolic extract (800 mg) underwent gravity column chromatography on silica gel (24 g, 60-80 mesh), which was packed using the wet method with chloroform to ensure uniform flow and minimise air bubbles. The column was eluted with a gradient of chloroform-ethyl acetate, increasing in polarity from 100:0 to 70:30 (v/v), and subsequently switched to chloroform-methanol for higher-polarity elution as needed. Fractions of 40 mL each were collected (fractions 1-97), with careful attention to numbering continuity (e.g., Fraction A (1-23), B (24-39), C (40-55), D (56-71), E (72-81), and F (82-97)). Fractions were monitored by TLC using silica gel 60 F254 plates developed in chloroform-methanol (9:1, v/v) and visualised under UV light at 254 and 365 nm. Based on similar TLC profiles, fractions were pooled into six groups (A-F). Fraction B (fractions 24-39) showed a single spot with a Rf value of 0.64 in the above solvent system, indicating purity and homogeneity. This fraction was subsequently characterised using UV-Vis and FTIR spectroscopy.

Phytochemical screening tests

The phytochemical analyses for the presence of alkaloids, flavonoids, glycosides, terpenoids, tannins and steroids were performed following standard methodologies outlined by Ndukwe *et al.* [12].

UV-Vis-NIR spectral analysis

The optical absorption properties of the dye were analysed using a Shimadzu UV-3101PC scanning spectrophotometer (400-800 nm range). All measurements were carried out using 10-mm quartz cells at 25±2 ℃, with

ISSN 0795 - 22066

instrument calibration ensuring baseline stability.

FTIR spectral fingerprinting

Molecular characterisation was performed via Perkin-Elmer Spectrum 100 FTIR and DTGS detector. Samples (5 mg dry powder) were analysed in transmission mode under controlled humidity. Spectrum was derived from 64 scans at a resolution of 2 cm^{-1} resolution (3500-1000 cm^{-1} range) using strong apodisation provided functional group identification

Dyeing procedures

Dyeing was carried out using a standardised method [13], maintaining a consistent fabric-to-dye liquor ratio of 1:10. A 0.25% aqueous dye solution was prepared, and pH levels were adjusted based on the dye source using sodium hydroxide or hydrochloric acid to reach either alkaline (pH 9–10) or acidic (pH 3–4) conditions. The dye bath was gradually heated to 100 °C and held for an hour before cooling to 60 °C. Fabrics were then removed, rinsed, and air-dried.

Evaluation of colour fastness properties

The wash fastness of the samples was evaluated in accordance with ISO 105-C10[14]. To determine rubbing fastness, the procedure outlined in ISO 105-X12 [15] was followed. Colour fastness to daylight is a critical parameter for consumer satisfaction; therefore, testing was conducted according to the ISO 105-B01 [16] standard method. For light fastness assessment, dyed fabric specimens measuring 1×3 cm were exposed to ultraviolet (UV) light using a Xeno tester for 30 hours. The test conditions included a temperature of 30°C, relative humidity (R.H.) of 65%, and an irradiation dose of 980 kJ/m². The degree of fading was evaluated by comparing the exposed specimens to the blue wool standard, under the ISO guidelines.

RESULTS AND DISCUSSION

This section outlines the results of the extraction, characterisation, and dyeing processes using *Sorghum bicolor* leaves extract, as well as evaluating its colour fastness. The extraction efficiency, chemical profile, and spectral data (UV-vis and FTIR) were used for this purpose, followed by an assessment of dyeing effectiveness and fastness.

Phytochemical screening and TLC-based characterisation of Sorghum bicolor leaves extract

Phytochemical screening provides a scientific foundation for selecting plant materials as potential source of natural dyes. In this study, crude extract from *Sorghum bicolor* leaves was obtained via maceration, resulting in a complex mixture subjected to further purification. Gravity column chromatography enabled the separation of the extract into distinct fractions, which were subsequently analysed using thin-layer chromatography (TLC) to monitor and characterize individual components. Phytochemical screening of these purified fractions indicated the presence of key secondary metabolites such as flavonoids,

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tannins, and alkaloids, compounds widely known for their pigmentary properties and potential to enhance the stability and functionality of natural dyes. This systematic approach allowed for a focused assessment of the constituents contributing to dye potential. As shown in Table 1, the phytochemical profile of TLC fraction B aligns with previous findings by Ali *et al.* [17] and Ojo *et al.* [18], confirming the presence of alkaloids, flavonoids and tannins in *S. bicolor* leaves. This consistency strengthens the reliability of the current results and underscores the applicability of these compounds.

Table 1: Phytochemical groups present in *S. bicolor* leaves

Phytochemical group	TLC Fraction B (Isolated Dye)
Alkaloids	+
Cardiac Glycosides	-
Flavonoids	+
Steroids	+
Tannins	+
Terpenoids	-

Key: + Present, - Absent

Spectroscopic characterisation of natural dye

The UV-VIS and FTIR spectroscopic analyses of the isolated natural dyes revealed distinct optical and chemical features that indicate their suitability for textile applications. The UV-VIS spectrum displayed a prominent absorption peak at 402 nm, suggesting the presence of conjugated chromophores, likely due to $\pi \rightarrow \pi^*$ electronic transitions (Figure 1). These characteristics are typical of colour-imparting compounds such as flavonoids or anthocyanins. FTIR analysis further validated these findings, showing notable peaks at ~3839 cm^{-1} (O–H stretching of phenolic –OH groups), ~2929 cm^{-1} (C–H stretching of aliphatic chains), ~1654 cm^{-1} (C=C stretching of aromatic rings), and ~1600–1500 cm^{-1} (C=O stretching and aromatic skeletal vibrations) (Figure 2). C=O stretching and aromatic skeletal vibrations are consistently reported in the literature for plant-based dyes [19-20-21]. Correspondingly, FTIR studies on dyes extracted from *Curcuma longa*, onion skin, and *Hibiscus* species have shown comparable patterns, confirming the presence of flavonoids, phenolic-OH groups, and aromatic structures as principal components [19-20]. Additional peaks around 1252–1000 cm^{-1}, corresponding to C–O stretching vibrations, have also been reported in studies on pomegranate peel and henna extracts, further confirming the presence of polyphenolic chromophores such as flavonoids and anthocyanins [19-20]. These spectroscopic results indicate that the natural dyes possess the

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necessary molecular properties for effective use as eco-friendly textile colourants.

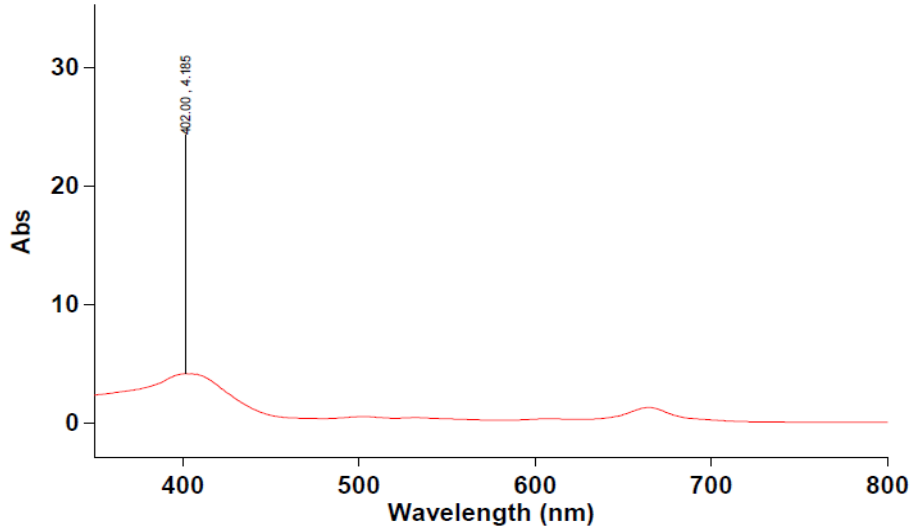

Fig. 1: UV-visible spectrum of *Sorghum bicolor* leaves

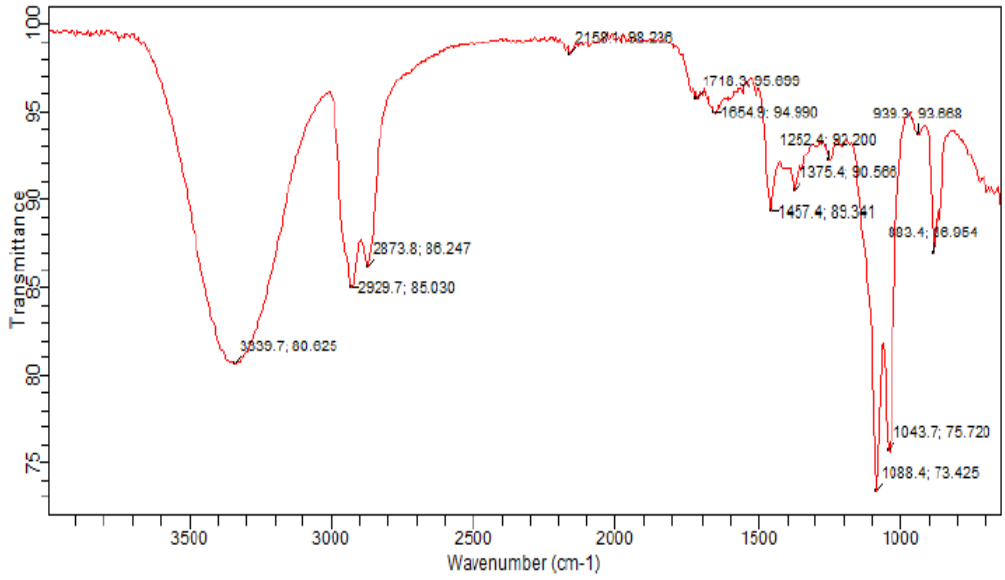

Fig. 2: FTIR spectrum of *Sorghum bicolor* leaves

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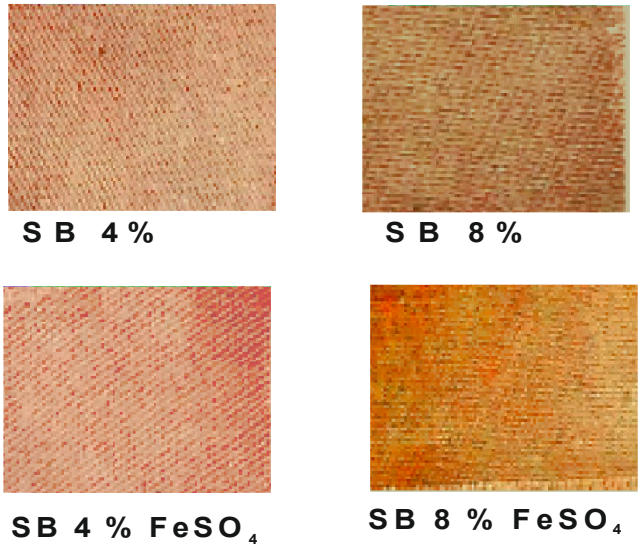

Fig. 3: Visual Comparison of Mordanted and Unmordanted Fabrics Dyed with Sorghum *bicolor* Leaves

Colour fastness to light

The light fastness data (Table 2 and Figure 3) show that at a 4% owf (on-weight-of-fibre) dye concentration, both mordanted and unmordanted cotton fabrics recorded a Blue Wool Scale (BWS) rating of 5, indicating good resistance to photofading. However, when the dye concentration was increased to 8%, a noticeable improvement was observed in the mordanted fabric, which achieved a rating of 6 (very good), while the unmordanted fabric maintained a rating of 5. These results were consistent across replicates, with the mordanted 8% sample yielding an average fastness of 6 ± 0.3 over three separate trials.

This enhanced performance at higher concentrations in the mordanted samples can be attributed primarily to the formation of stable dye-metal complexes [22]. The ferrous sulphate mordant likely interacts with the hydroxyl and carbonyl functional groups of phytochemicals such as flavonoids and tannins found in the TLC fraction B of *Sorghum bicolor* extract, leading to the formation of coordination complexes. These complexes anchor more strongly to the cellulose structure of cotton, particularly within amorphous regions, thereby enhancing the dye's resistance to photodegradation [1]. The increase in dye concentration from 4% to 8% leads to greater dye uptake, contributing to a denser population of chromophores on the fibre surface. This enhances the fabric's optical density, increasing its ability to absorb and scatter ultraviolet (UV) radiation [23]. As a result, less light penetrates the dye fibre matrix, which helps protect both the dye and the fibre from photo-induced oxidative damage. This "inner-filter" effect is more pronounced at higher dye concentrations, provided that the additional dye molecules are properly fixed to the fibre. The bioactive compounds in *Sorghum bicolor* leaves, including flavonoids, tannins, and alkaloids confirmed by phytochemical screening further contribute to the photostability of the dyed

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fabric. These phytochemicals are known to possess strong UV-absorbing properties due to their extended conjugated π-systems and phenolic hydroxyl groups [2]. Moreover, their antioxidant properties can quench reactive oxygen species (ROS) generated by UV exposure, further reducing the degradation of dye molecules. When complexed with Fe^{2+}, these compounds become more photostable, as the metal ions help stabilize their excited states and suppress photo-oxidative degradation pathways. Interestingly, the unmordanted fabric did not exhibit an improvement in light fastness at 8% concentration. This could indicate a saturation point in dye uptake where additional dye molecules are only loosely bound to the fibre or remain on the surface. These loosely attached molecules may act as photosensitizers, initiating oxidative degradation of the fixed dye and fibre under UV exposure, thereby counteracting any potential benefits from higher concentration.

Colour fastness to washing

The wash fastness results of the dyed cotton samples revealed significant differences between the mordanted and unmordanted fabrics (Table 3 and Figure 3). Unmordanted cotton fabric exhibited poor wash fastness at 4% dye concentration, with noticeable colour fading during laundering. This is attributed to weak, reversible interactions, primarily hydrogen bonding and ionic attractions, between the dye molecules and the cellulose fibres. These non-covalent interactions are easily disrupted in aqueous detergent solutions, leading to dye leaching from the fibre surface and resulting in lower wash fastness ratings. In contrast, mordanted cotton demonstrated improved performance, achieving a good wash fastness rating of 3 for colour change. This indicates relatively better dye retention during washing, which is noteworthy given cotton's known sensitivity to laundering conditions such as agitation, pH, and temperature. The enhanced resistance to fading can be attributed to the formation of insoluble dye-metal-fibre complexes. Specifically, ferrous ions (Fe^{2+}) introduced during mordanting can form coordination bonds with flavonoid-based pigments present in the *Sorghum bicolor* extract, as well as with functional groups on the cotton fibre, particularly carboxyl and hydroxyl groups. These metal-dye-fibre complexes are more stable and less susceptible to hydrolysis or mechanical detachment, thereby reducing dye loss during laundering.

Colour fastness to rubbing

The dyed cotton fabrics exhibited poor to fair dry rubbing fastness, with ratings typically ranging from 1 to 2 on the grey scale (1–5) at 4% dye concentration for both mordanted and unmordanted samples (Table 4 and Figure 3). These low ratings indicate a noticeable degree of colour transfer onto adjacent test cloths, particularly in darker shades. Such transfer is common with natural dyes that lack covalent bonding to the fibre surface and are instead retained through weaker non-covalent interactions or coordination with mordants. Although dye-metal-fibre complexes formed during mordanting improve overall dye fixation, some surface-bound pigments remain

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susceptible to mechanical abrasion under dry conditions. In wet rubbing tests, fastness ratings dropped further, often to 1, for both mordanted and unmordanted fabrics indicating significant colour transfer. This reduction can be attributed to the swelling of cotton fibres in water, which increases fibre porosity and softens the fabric structure. The resulting mechanical agitation facilitates the detachment of loosely bound dye molecules, particularly those not deeply anchored within the fibre matrix. Even though iron-flavonoid complexes are largely water-insoluble, they may be dislodged from the fibre surface when the cotton is in a swollen state, leading to increased dye transfer during wet rubbing.

Table 2: Colour Fastness to Light for *S. bocolor leaves*

Sample code	Ferrous sulfate Mordant	Control Unmordanted
	Cotton	Cotton
	Colour Change	Colour Change
SB 4 %	5	5
SB 8 %	5	6

Note: SB-dye concentration, 1-very poor, 2-poor, 3-fair. 4-moderate. 5-good, 6-very good, 7-excellent, 8-outstanding

Table 3: Colour Fastness to Wash for *S. bocolor leaves*

Sample code	Ferrous sulfate Mordant	Control Unmordanted
	Cotton	Cotton
	Colour Change	Colour Change
SB 4 %	3	1
SB 8 %	4	2

Note: SB-dye concentration, 1-poor, 2-fair, 3-good. 4-very good. 5-excellent

Table 4. Colour fastness to rubbing for *S. bocolor leaves*

Ferrous sulfate Mordant				Control Unmordanted
Sample Code	Cotton Dry rubbing	Cotton Wet rubbing	Cotton Dry rubbing	Cotton Wet rubbing
SB 4 %	1-2	1	1-2	1
SB 8 %	5	4	4	3

Note: SB-dye concentration, 1-poor, 2-fair, 3-good. 4-very good. 5-excellent

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CONCLUSION

The study confirmed that *Sorghum bicolor* leaf extracts contain bioactive dye compounds such as flavonoids, tannins, and alkaloids, making them viable natural dyes for textile applications. Spectroscopic analyses (UV-Vis and FTIR) supported these findings. The use of ferrous sulfate as a mordant significantly improved the dye's performance on cotton fabrics, enhancing both light and wash fastness. Mordanted samples, especially at 8% dye concentration, showed very good resistance to photofading (Blue Wool Scale rating of 6) due to the formation of stable dye-metal complexes. These complexes anchor the dye more effectively and protect against photodegradation and washing. In contrast, unmordanted samples showed lower durability. Overall, the study highlights the potential of *Sorghum bicolor* leaves as a sustainable dye source and emphasizes the critical role of mordanting in achieving durable coloration.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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