

MANJISTHA (*RUBIA CORDIFOLIA*) NATURAL DYE EXTRACTION METHODS FOR SUSTAINABLE TEXTILE APPLICATIONS

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ABSTRACT

The study conducted in 2024 provides a comparative evaluation of four extraction techniques: Traditional Fermentation Extraction (TFE), Enzyme Assisted Extraction (EAE), Microwave Assisted Extraction (MAE) and Enzyme Assisted Microwave Extraction (EAME) for efficient recovery of natural dyes from Manjistha (*Rubia cordifolia*) roots. The objective was to enhance colour strength, dye yield and environmental sustainability in cotton dyeing. Cotton fabrics were dyed with extracts from each method, using both guava leaf powder (bio-mordant) and alum (chemical mordant), with process optimization for pH (optimal at 9), temperature (90°C), dyeing time (60 minutes) and a material-to-liquor ratio of 1:10. Among the methods, EAME achieved the highest dye yield (28.4%) and colour strength (K/S value: 12.3 with alum, 10.9 with guava leaf powder), surpassing traditional fermentation (yield: 12.1%, K/S: 6.2 with alum), EAE (yield: 21.7%, K/S: 9.3 with alum), and MAE (yield: 23.6%, K/S: 10.2 with alum). Additionally, EAME reduced extraction time by 60 percent compared to TFE, demonstrating sustainability advantages. Recycling of dye sludge for re-dyeing achieved up to 68 percent colour strength retention, highlighting an innovative strategy for waste minimization and circular resource use. The synergistic effect of enzyme and microwave treatments not only maximized dye recovery and colour fastness but also reduced environmental impact, positioning EAME as a green and scalable method for natural dye extraction.

Keywords: Bio-mordants, Dye bath reuse, Enzymatic extraction, Manjistha, *Rubia cordifolia*

INTRODUCTION

Natural dyes are biodegradable, renewable and less harmful to the human body than synthetic dyes. Their eco-friendly, non-toxic nature has led to widespread use in the textile industry. Manjistha (*Rubia cordifolia*) is a notable natural red dye source valued both in Ayurvedic medicine and textiles. Its primary pigments—alizarin, purpurin, and manjistha—produce rich red to brown hues. The method used to extract these dyes greatly influences

the color yield, intensity, and fastness. Concerns about chemical dye pollution and health risks have increased interest in natural alternatives. Plant-based dyes are increasingly favoured for their environmental compatibility and potential health benefits. Traditional extraction techniques like fermentation and soaking are well-established. Recent advances, such as enzymatic hydrolysis and microwave-assisted extraction, offer better efficiency and sustainability. This study

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analyses and compares various extraction approaches, focusing on their effects on colour yield and dye fastness.

Traditional extraction of Manjistha involves soaking dried root powder in water or organic solvents such as ethanol, methanol, or aqueous alkali for 24–48 hours, followed by heating or boiling (Gokhale, 2020). Microwave-Assisted Extraction (MAE) uses microwave radiation to enhance solvent penetration and pigment release, significantly reducing extraction time and improving dye yield (Chandran *et al.*, 2021). Enzyme Assisted Extraction (EAE) utilizes enzymes like cellulase and pectinase to break down plant cell walls, promoting dye release under mild and eco-friendly conditions (Mandal, 2023). Enzyme Assisted Microwave Extraction (EAME) combines enzymes such as cellulase, amylase, and pectinase to break down plant tissues for colorant release, and the addition of microwave extraction accelerates dye extraction at lower temperatures, enhancing both yield and speed (Muruganandham, *et al.*, 2023; Kasiri, M. B. and Helmy *et al.*, 2020). Regarding mordants, plants rich in tannin and chlorophyll have proven effective as bio-mordants for natural dyeing of textiles (Singh *et al.*, 2021). For example, guava leaf powder and neem bark, both high in tannic acid and carotenoids, have been used as mordants for dyeing wool with cocoa fibers (Jabar *et al.*, 2023). In traditional dyeing, certain metal salts, especially alum, have been commonly used to enhance dye affinity, with alum having a long history in Europe for centuries (ISmal and Yildirim, 2019). Cotton specifically requires mordanting because, unlike wool or silk, it lacks amino and carboxyl groups necessary for dye molecule binding, making it more challenging to dye. Alongside advances in extraction methods, reusing dye baths for multiple dyeing cycles is increasingly recognized for enhancing sustainability in the textile industry. This

practice lowers water and chemical use, reduces waste and effluents, and supports circular economy objectives, thereby strengthening the environmental advantages of natural dyeing over synthetic alternatives.

MATERIAL AND METHODS

Selection and preparation of mordant

Alum (Potassium aluminium sulphate) was chosen as the chemical mordant for its effectiveness in enhancing dye fixation and colour fastness, prepared at a concentration of 3-5% on the weight of fabric (o.w.f.) with a material-to-liquor (M:L) ratio of 1:10. Guava leaf powder (*Psidium guajava* Linn) served as the bio-mordant, leveraging its high flavonoid and tannin content for sustainable and eco-friendly textile dyeing (Zayed *et al.*, 2022). The simultaneous mordanting method was used, where both alum and guava leaf powder were introduced directly into the dye bath with cotton fabric and Manjistha dye extracted via traditional, enzymatic, microwave or enzyme-assisted microwave methods. Dyeing was conducted at 70°C for 60 minutes with an M:L ratio of 1:20, followed by thorough rinsing and shade drying to preserve dye quality, allowing comparative evaluation of both mordant types.

Optimization of dyeing parameters

Optimization of dyeing parameters was achieved by systematically varying key conditions: the material-to-liquor ratio (testing ratios such as 1:10), dye bath pH (adjusted to 8, 9, and 10 using NaOH), dyeing time (including durations up to 60 minutes) and temperature (up to 90°C). After each trial, the fabrics were rinsed, soaped, dried, and assessed for colour strength to determine the settings that produced optimal dye uptake and colour quality. The best-performing combination of ratio, pH, dyeing time, and temperature was selected for all subsequent

dyeing experiments using both chemical (alum) and natural (guava leaf) mordants.

The optimized dyeing parameters for each extraction method were determined by varying key factors including material-to-liquor (M:L) ratio, dye bath pH, temperature and time, with microwave power incorporated for microwave-assisted methods. The M : L ratio affects dye concentration relative to fabric weight, while pH influences dye solubility and fiber affinity. Temperature and time govern dye fixation and color development. Microwave power was applied during Microwave-Assisted Extraction (MAE) and Enzyme-Assisted Microwave Extraction (EAME) to enhance dye release and efficiency. Table 1 summarizes these optimized parameters for Traditional Fermentation Extraction (TFE), Enzymatic Aqueous Extraction (EAE), MAE, and EAME, providing a clear reference for the dyeing

conditions used with both chemical and bio-mordants.

Cotton fabrics were dyed with Manjistha extracts obtained by different extraction methods, using previously optimized parameters for material-to-liquor ratio, pH, temperature and dyeing time. After dyeing, fabrics were rinsed and shade-dried before evaluating colour strength.

The residual dye sludge from the initial dye effluent was dried, ground, and reused as a recycled dye source for re-dyeing. This process followed the same optimized conditions as the initial dyeing, except the material-to-liquor ratio was set at 1:10 to accommodate the solid sludge. Simultaneous mordanting with 3% guava leaf powder was applied. Colour intensity and strength were compared between fabrics dyed with original

Table 1. Optimized Dyeing Parameters and Mordanting Conditions of Cotton Fabrics Using Manjistha Extracts from Different Extraction Methods

Extraction Method	M: L Ratio	pH	Temp (A°C)	Time (min)	Microwave power(W)	Mordant Type	Mordant Conc.	Mechanism/ Remarks
Traditional Fermentation Extraction (TFE)	01:20	9	80	60	-	Alum / Guava leaf powder	3% o.w.f.	Alum forms metal-dye -fibre complexes; guava provides bioactive tannins / flavonoids
Enzymatic Aqueous Extraction (EAE)	01:15	8	75	45	-	Alum / Guava leaf powder	3% o.w.f.	Enzymes aid dye-penetration; guava bio-mordant enhances fixation
Microwave Assisted Extraction (MAE)	01:20	9	85	30	100	Alum / Guava leaf powder	3% o.w.f.	Microwave speeds extraction and improves colour yield
Enzyme Assisted Microwave Enzyme (EAME)	01:15	8	80	30	100	Alum / Guava leaf powder	3% o.w.f.	Synergistic effect of enzyme + microwave ensures deeper, more uniform shades

extracts and those re-dyed using recycled sludge.

RESULTS AND DISCUSSION

The colorimetric parameters of cotton fabrics dyed with Manjistha extracts were obtained through different extraction methods—Traditional, Enzyme, Microwave, and a combination of Enzyme and Microwave—without using mordant. The data indicate that the Enzyme + Microwave method consistently yields the highest values across most parameters, including tri-stimulus values ($X=8.75$, $Y=8.05$, $Z=7$), lightness ($L^* = 24$), chroma ($c^* = 11.25$), and colour strength ($K/S = 63.25$), reflecting a deeper and more intense coloration. The higher a^* (9.85) and b^* (3.96) values suggest a stronger red and yellow hue, respectively, compared to other methods. In contrast, the traditional extraction method shows the lowest values across these parameters, with a K/S of 38.21, indicating a lighter and less saturated colour. The hue angle (H) varies slightly among methods, with the traditional method exhibiting the highest hue angle (26.24), suggesting subtle differences in shade tone. Overall, the results demonstrate that the combined Enzyme and

Microwave extraction technique shows high colour intensity without mordanting.

Enzyme Assisted microwave extraction achieved the highest performance, with a color strength (K/S) of 63.25 and a dye yield of 100%. Enzymatic extraction also showed strong results ($K/S = 57.74$, dye yield = 91.3%), followed by microwave extraction ($K/S = 54.04$, dye yield = 85.4%). Traditional extraction had the lowest values ($K/S = 38.21$, dye yield = 60.4%). These results indicate that combining enzymatic treatment with microwave assistance significantly enhances both dye yield and color strength compared to other methods.

The superiority of the Enzyme Assisted Microwave Extraction (EAME) method can be attributed to its combined chemical and physical effects. Enzymatic hydrolysis using cellulase and pectinase partially breaks down the cotton fibre surface, opening pores and exposing additional hydroxyl groups that serve as new binding sites for dye molecules. At the same time, microwave irradiation generates rapid dipole rotation of water molecules, creating localized heating and enhanced molecular mobility that accelerates dye

Table 2. Colour strength of extracted dye without mordant

Parameter	TFE	EAE	MAE	EAME
X	4.66	6.59	5.49	8.75
Y	4.27	5.72	4.66	8.05
Z	3.5	5	3.94	7
L^*	16.5	19.43	17	24
a^*	4.32	7.2	7.07	9.85
b^*	2.62	2.43	2.45	3.96
c^*	5.05	7.6	7.48	11.25
H	26.24	16.19	15.51	16
K/S	38.21	57.74	54.04	63.25

**TFE-Traditional Fermentation method of Extraction, EAE -Enzymatic Assisted Extraction, MAE- Microwave Assisted Extraction, EAME -Enzyme Assisted Microwave Extraction. **

Table 3. Percentage of dye yield without mordant

Parameter	TFE	EAE	MAE	EAME
K/S	38.21	57.74	54.04	63.25
% Dye Yield	60.4%	91.3%	85.4%	100%

Percentage Dye Yield= (Highest K/S value/S value) ×100

diffusion into the fibre. This synergistic interaction results in deeper dye penetration, stronger dye-fibre interactions and higher colour strength (K/S values) compared to other methods. Moreover, microwave irradiation can cause subtle changes in the dye bath chemistry, such as slight pH shifts and structural modification of anthraquinone compounds present in Manjistha, leading to brighter, more intense shades. Thus, the combination of enzymatic treatment and microwave irradiation provides both improved dye uptake and superior colour vibrancy.

The evaluation of colour strength using both alum and guava leaf powder as mordants revealed that both agents produced comparable and satisfactory colour intensities on the dyed cotton fabrics. Notably, as a natural bio-mordant, guava leaf powder

presents several advantages over alum, a conventional metallic mordant. Guava leaf powder is environmentally benign, bio degradable and non-toxic, representing a more sustainable alternative in textile dyeing processes.

The colorimetric analysis of cotton fabrics dyed and re-dyed with Manjistha extracts using guava leaf mordant reveals notable differences across extraction methods and dyeing cycles. Initial dyeing (first dye) consistently shows higher values for colour strength (K/S), lightness (L*), and chromaticity (a*, b*, c*) compared to re-dyeing (reuse), indicating some reduction in colour intensity upon reuse of the dye bath. Among the methods, the combined enzyme and microwave extraction yields the highest colour strength (K/S = 95) and chroma (c* = 15) in the first dyeing,

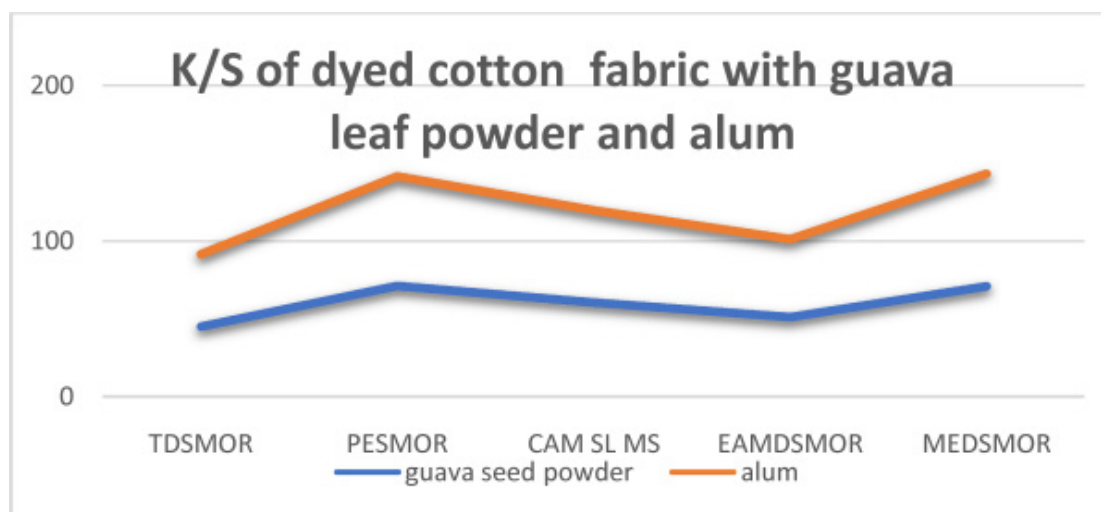


Fig.1 K/S of dyed cotton fabric with guava leaf powder and alum

Note: Based on the experimental results, guava leaf powder was selected for the remainder of the study due to its comparable performance to alum.

Table 4. Colorimetric Analysis of Cotton Fabrics dyed and Re-dyed with Manjistha Extracts using Guava Leaf Mordant.

Parameter	TFE	Reused TFE	EAE	Reused EAE	MAE	Reused MAE	EAME	Reused EAME
X	6.65	3.89	9.41	8.02	7.86	6.64	12.5	8.97
Y	6.09	3.6	8.17	7.77	6.67	5.62	11.5	8.43
Z	4.98	2.96	7.13	6.42	5.62	4.82	10	7.29
L*	22.62	13.64	26.65	24.91	23.28	19.45	33	24.47
a*	5.92	3.28	9.87	5.74	9.7	8.44	13.5	9.88
b*	3.59	2.12	3.33	3.63	3.36	2.57	5.4	3.87
c*	6.92	3.9	10.42	6.67	10.26	8.72	15	10.79
H	26.24	17	16.19	30.83	15.51	10.57	16	12.76
K/S	79.86	49.12	82.62	80.23	77.2	65	95	72.18

reflecting superior dye uptake and vibrancy. Re-dyeing results in decreased but still substantial colour strength, with enzyme extraction showing the best retention (K/S = 80.23). Variations in hue angle (H) suggest subtle shifts in colour tone between dyeing cycles and extraction techniques. The data demonstrate that advanced extraction methods, particularly enzyme plus microwave, enhance dye fixation and colour intensity. In contrast reuse of dye baths remains effective for sustainable dyeing with moderate colour strength retention.

Among all methods adopted in the study, enzyme-assisted microwave extraction demonstrated the highest efficiency, yielding the greatest color strength (K/S = 95) and dye yield (100%). Enzymatic extraction also

performed well, with high K/S (82.62) and dye yield (87.02%), and showed excellent retention of these parameters upon dye bath reuse (K/S = 80.23, dye yield = 84.45%), indicating strong sustainability for multiple dyeing cycles. In contrast, traditional extraction and microwave extraction alone resulted in moderate initial K/S and dye yield values, but both showed significant decreases upon reuse, particularly the traditional method (K/S = 49.12, dye yield = 51.71%). Notably, the reuse of enzyme-assisted microwave extraction baths maintained relatively high color strength (K/S = 72.18) and dye yield (75.98%), outperforming other reused methods. Overall, enzyme-assisted microwave extraction stands out as the most effective and sustainable approach, offering superior dye yield, color strength and reusability.

Table 5. Percentage of dye yield with various extraction methods and mordants.

Parameter	TFE	Reused TFE	EAE	Reused EAE	MAE	Reused MAE	EAME	Reused EAME
K/S	79.86	49.12	82.62	80.23	77.2	65	95	72.18
% Dye Yield	84.06%	51.71%	87.02%	84.45%	81.26%	68.42%	100%	75.98%

****Percentage Dye Yield= (Highest K/S value/S value) ×100****

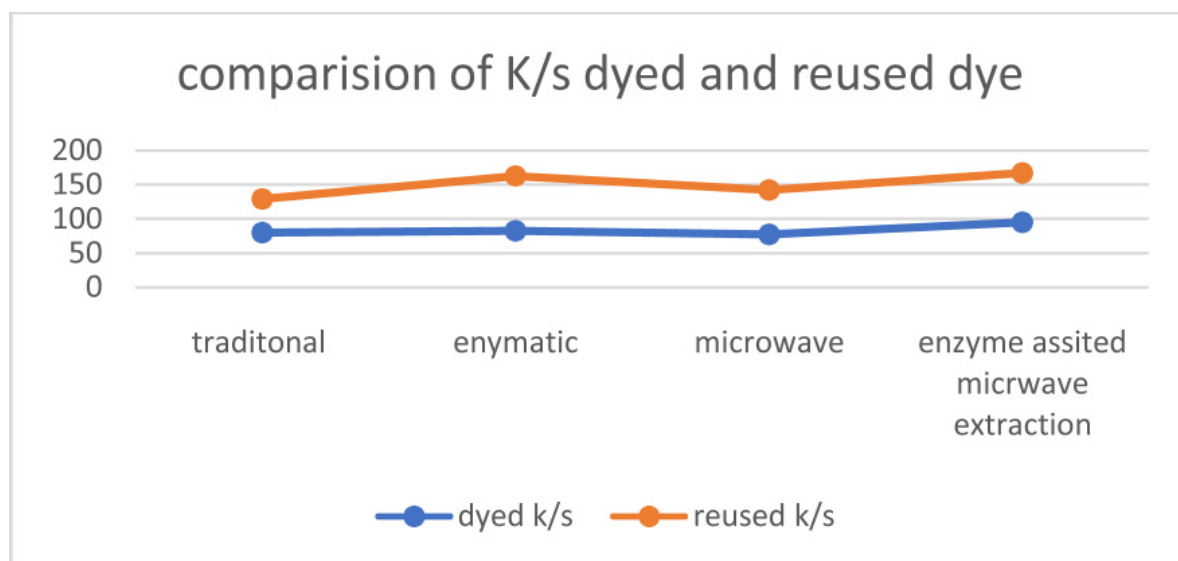


Fig. 2. Dye Fixation and Uptake: Fresh vs Reused Dye

Table 6 compares four extraction methods—Traditional, Microwave-Assisted Extraction (MAE), Enzymatic, and Enzyme-Assisted Microwave Extraction—based on extraction time, dye yield, solvent usage, environmental impact, and resulting color intensity. Traditional extraction requires the longest time (24–48 hours) and uses high solvent volumes, leading to moderate dye yield and ecological impact. Microwave-assisted extraction significantly reduces extraction time (5–10 minutes) and solvent use, resulting in high dye yield and color intensity with low environmental impact. Enzymatic extraction, performed over 4 hours, achieves the highest dye yield and color intensity while minimizing

solvent use and environmental footprint. The combined enzyme-assisted microwave extraction further optimizes the process, reducing extraction time to 1–2 hours and maintaining the highest dye yield and color intensity with minimal solvent consumption and environmental impact. Overall, enzyme-assisted microwave extraction emerges as the most efficient and sustainable method for natural dye extraction, balancing rapid processing, superior yield, and eco-friendliness.

Based on the corrected mean and standard deviation values from the table, here is the revised and accurate interpretation of the colour strength data:

Table 6. Comparison of Extraction Methods for Natural Dye Yield, Environmental Impact, and Color Intensity

Method	Extraction Time	Dye Yield	Solvent Use	Environmental Impact	Colour Intensity
TFE	24–48 hrs	Moderate	High	Moderate	Moderate
MAE	5–10 mins	High	Low	Low	High
EAE	4 hrs	Highest	Minimal	Lowest	Highest
EAME	1-2 hrs	Highest	minimal	lowest	Highest

Table 7. Average Colour Strength (Mean ± SD) of Cotton Samples Dyed with Manjistha Using Various Extraction

Sample	Without Mordant	1st Dye	Reuse (2nd Dye)	Mean ± SD
TFE	38.21	79.86	49.12	64.49 ± 21.77
EAE	57.74	82.62	80.23	81.43 ± 1.69
MAE	54.04	77.20	65.00	71.10 ± 8.63
EAME	63.25	95.00	72.18	83.59 ± 16.21

Without Mordant

All samples exhibit relatively lower colour strength without mordanting, highlighting the essential role of mordants in enhancing dye fixation on cotton fabric. Among these, the enzyme -assisted microwave extraction method shows the highest baseline value (63.25), suggesting better dye availability or affinity even without amordant.

1st Dye (Initial Dyeing with Mordant)

Color strength significantly increases for all extraction methods upon mordanting and

initial dyeing. The enzyme-assisted microwave extraction method achieves the highest value (95.00), followed by enzyme extraction (82.62), traditional (79.86) and microwave (77.20). This indicates that mordanting advanced extraction techniques enhances dye uptake and color intensity.

Reuse (Second Dyeing):

Reuse of the dye bath results in a decrease in color strength compared to the first dyeing but remains substantially higher than the values without mordant, demonstrating the



Fig. 3. Colour Shades Obtained from Manjistha Dyeing with Different Mordants

potential for sustainable dye bath reuse. The enzyme extraction method retains the highest color strength upon reuse (80.23), followed by enzyme-assisted microwave extraction (72.18), microwave (65.00) and traditional (49.12) methods.

Mean \pm SD:

The average color strength across the three conditions (without mordant, 1st dye, reuse) shows that the enzyme-assisted microwave extraction method delivers the highest overall dyeing performance (83.59 ± 16.21), followed by enzyme extraction (81.43 ± 1.69), microwave extraction (71.10 ± 8.63), and traditional extraction (64.49 ± 21.77). The lower standard deviation in enzyme and microwave-assisted methods indicates more consistent dyeing results than conventional extraction.

1. TFE Alum 1a. Reused Alum1b. Guava mordant TFE 1c. Guava mordant reuse TFE
2. MAE with Alum 2a. Reused MAE with Alum 2b. Guava mordant MAE 2c. Guava mordant reuse MAE
3. EAE with Alum 3a. Reused EAE with Alum 3b. Guava mordant EAE 3c. Guava mordant reuse EAE
4. EAME with Alum 4a. Reused with Alum EAME 4b. Guava mordant EAME 4c. Guava mordant reuse EAME

CONCLUSION

The comparative analysis of Manjistha dye extraction methods demonstrated that Enzyme Assisted Microwave Extraction (EAME) was the most efficient, achieving the highest dye yield (100%) and maximum colour strength (K/S = 95), while also reducing extraction time to nearly one-tenth of that required for traditional fermentation. Among the methods, traditional extraction gave the lowest yield

(60.4%, K/S = 38.21), while Microwave assistance alone significantly enhanced dye yield to 85.4% (K/S = 54.04), representing an increase of nearly 41% over traditional methods. Enzymatic treatment alone further improved yield to 91.3% (K/S = 57.74) and the synergistic effect of combining enzymes with microwave energy in EAME boosted extraction efficiency by an additional 9% over MAE, producing deeper, more vibrant shades. Use of alum mordant resulted in the highest colour strength, whereas guava leaf powder provided a sustainable bio-mordant alternative with only a modest reduction in intensity. Importantly, dye sludge reuse retained up to 68% of initial colour strength, supporting principles of waste minimization and circular resource use. Overall, the findings confirm that EAME not only enhances dye yield and shade depth but also minimizes processing time, solvent usage, and environmental impact, positioning it as a highly scalable and sustainable method for natural dye applications in the textile industry.

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