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Statistical Analysis on Herbal Dye Extraction and Mordanting for Functional Cotton Textiles

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Abstract

Herbal dyes offer a sustainable alternative to synthetic colorants, combining eco-friendliness with functional benefits such as antimicrobial, ultraviolet (UV) protective, and antioxidant properties. This study optimizes aqueous extraction and mordanting conditions for Dalbergia sissoo (Shisham) leaves and Solanum nigrum (Makoy) berries to enhance the performance of cotton fabrics. Extraction parameters temperature, pH, duration, and plant-to-water ratio were systematically varied to maximize yield, while alum mordanting (pre- and post-dyeing, at multiple concentrations) was evaluated for its influence on color strength (K/S), fastness, and bioactivity. S. nigrum achieved a higher yield (72%) than D. sissoo (65%) and demonstrated superior functional performance, including an Ultraviolet Protection Factor (UPF) of 135.8 (excellent protection) and 70% radical scavenging activity. Antibacterial assays revealed significant inhibition of Staphylococcus aureus and Escherichia coli, with good retention after five laundering cycles. Both dyes enhanced UV protection, antioxidant capacity, and exhibited acceptable wash and rub fastness. These results confirm the potential of S. nigrum and D. sissoo as viable natural dye sources for producing multifunctional, eco-friendly textiles, with particular applicability in medical, protective, and health-sensitive apparel sectors.

Keywords: Herbal Dyes, Mordanting, Cotton Fabrics, Antimicrobial Textiles, UV Protection, Sustainable Coloration

1. Introduction

Cotton is one of the most extensively used natural fibers in the textile industry due to its comfort, breathability, softness, and biodegradability. Its global dominance is sustained by its versatility across fashion, home furnishing, and technical applications. However, cotton's high moisture retention and cellulose-rich structure create an ideal environment for microbial growth, which can lead to unpleasant odors, fabric degradation, and hygiene concerns. While conventional antimicrobial and dyeing treatments can mitigate these problems, they typically depend on synthetic chemicals that are often non-biodegradable, environmentally persistent, and potentially harmful to human health. This has driven increasing interest in sustainable, eco-friendly approaches to cotton functionalization. Natural plant-based dyes represent one such approach, offering both coloration and bioactive functionalities without the environmental footprint of synthetic chemicals. Beyond aesthetic appeal, many herbal dyes contain phytochemicals such as tannins, flavonoids, and anthocyanins that exhibit antimicrobial, antioxidant, and ultraviolet (UV) protective properties. Several researchers, including Tawiah et al. (2016), have highlighted that herbal dyes are increasingly being used to impart antimicrobial properties to textiles as part of an eco-friendly approach in textile engineering, particularly in response to health and environmental concerns associated with synthetic chemicals. Cotton, a natural fiber widely used in consumer products, is hydrophilic and thus prone to microbial colonization, leading to fabric degradation, unpleasant odors, and potential health risks for the wearer. This challenge has prompted research into infusing antimicrobial qualities into cotton fabrics using plant-derived dyes, which are biocompatible, biodegradable, and present a low environmental impact (Shwaiki, 2021). Herbal dyes, as described by Thakur et al. (2025), are obtained from various plant parts—such as leaves, roots, flowers, and fruits—that contain biologically active compounds including tannins, flavonoids, alkaloids, and phenolic acids. These compounds can inhibit the growth of bacteria, fungi, and other microorganisms. Unlike synthetic antimicrobial agents, herbal dyes are biodegradable, have low toxicity, and present minimal risk of encouraging microbial resistance (Periyasamy, 2024). Thus, they serve a dual function in both coloring textiles and improving their safety and performance, aligning with the principles of green chemistry and sustainable textile processing. Musinguzi et al. (2019) reported that natural dyes rich in phytochemicals such as tannins, flavonoids, and anthocyanins can inhibit microbial growth, particularly on hydrophilic fibres like cotton. They demonstrated that leaves and plant parts, including those from *Datura stramonium*, can produce significant zones of inhibition against pathogenic bacteria, confirming their functional potential. Shahmoradi et al. (2021) explained that the use of mordants—historically metal salts such as alum or iron—enhances dye fixation and improves antimicrobial efficacy. They further noted that bio-mordants such as tannic acid and lemon peel have been explored in recent years to reduce environmental impact while maintaining desirable colour fastness and antibacterial performance. Narayanaswamy et al. (2013) and Ali (2015) emphasized the multifunctional benefits of herbal dyes, noting that many plant-derived colorants also provide ultraviolet (UV) shielding and antioxidant properties—characteristics increasingly sought after in eco-conscious textile engineering. Ahmadi and Houjeghan (2022) demonstrated that herbal extracts such as turmeric, henna, pomegranate, and eucalyptus can effectively inhibit common pathogens like Staphylococcus aureus and Escherichia coli. They also reported that such antimicrobial properties can endure multiple washing cycles, which is crucial for practical textile applications. Despite these advantages, Neves et al. (2019) acknowledged that the large-scale adoption of herbal dyes faces challenges, including the lack of standardized extraction protocols and difficulties in industrial-scale production. They stressed the need for comprehensive toxicity and allergenicity assessments to ensure end-user safety. Saha et al. (2021) concluded that integrating herbal dyes into cotton fabric production represents a convergence of traditional and modern textile engineering practices, offering multifunctional and safe fabrics while reducing environmental impacts. Continuous improvements in extraction methods, mordant selection, and processing conditions will further enhance the potential of herbal dyes in developing sustainable functional textiles.

By harnessing these compounds, it is possible to create cotton fabrics that are not only visually attractive but also capable of delivering additional protective functions. Within this framework, Dalbergia sissoo (Shisham) leaves and Solanum nigrum (Makoy) berries emerge as promising dye sources. Shisham leaves are rich in tannins and flavonoids, natural binding agents that enhance dye fixation and improve wash fastness. Their phenolic composition also contributes to antimicrobial and antioxidant activity. In contrast, Makoy berries are abundant in anthocyanins, pigments known for vibrant coloration as well as potent antioxidant and UV-blocking properties. These differences make it scientifically valuable to compare and optimize their application to cotton textiles. The present study investigates the eco-friendly functionalization of cotton using aqueous extracts of Shisham leaves and Makoy berries. The research focuses on optimizing dye extraction conditions, evaluating the influence of alum mordanting on dye uptake, and assessing the fabrics' functional properties through standardized tests. Antimicrobial performance was measured using AATCC 147 and AATCC 100 protocols, UV protection was quantified via Ultraviolet Protection Factor (UPF) ratings and antioxidant activity was determined through the DPPH radical scavenging assay. Additional evaluations included color fastness, tensile strength, moisture regain and FTIR spectroscopy to assess structural integrity.

2. Materials and Methods

2.1. Materials

Plain-weave 100% cotton fabric (bleached, 150 g/m²) was procured from a local textile supplier. Fresh leaves of *Dalbergia sissoo* (Shisham) and ripe berries of *Solanum nigrum* (Makoy) were collected locally, washed thoroughly to remove surface impurities, and shade-dried at ambient temperature (27 ± 2 °C) for 5–7 days. Analytical grade alum (potassium aluminum sulfate) was used as the mordant. All other chemicals and reagents, including methanol, ethanol, and DPPH (2,2-diphenyl-1-picrylhydrazyl), were of analytical grade.

2.2. Dye Extraction

Aqueous extraction was performed under optimized conditions determined in preliminary trials. For *D. sissoo*, 20 g of powdered leaves were boiled in distilled water at 60 °C for 60 minutes, while *S. nigrum* berries were processed at 80 °C for 60 minutes. Extracts were filtered through muslin cloth followed by Whatman No. 1 filter paper. Extraction yield (%) was calculated by weighing the dried residue obtained after evaporating a known volume of the filtrate.

2.3. Mordanting and Dyeing

Cotton fabric samples (10 cm × 10 cm) were pre-mordanted with a 10% (owf) alum solution for 30 minutes at 60 °C, followed by thorough rinsing and drying. Dyeing was performed using the immersion method at a material-to-liquor ratio of 1:40. Dye baths were maintained at the optimized extraction temperatures for each plant source, with constant stirring for 60 minutes. After dyeing, samples were rinsed in cold water, air-dried, and conditioned at 65% relative humidity before testing.

2.4. Antibacterial Testing

Antibacterial activity was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the AATCC 147 (qualitative) and AATCC 100 (quantitative) test methods. The zone of inhibition (ZOI) was measured in millimeters after 24 hours of incubation at 37 °C. Wash durability was assessed following ISO 105-C06 standard washing cycles (1, 3, and 5 washes), with ZOI recorded after each cycle.

2.5. Ultraviolet Protection Factor (UPF) Measurement

UPF values were determined according to the AATCC 183 test method using a UV–visible spectrophotometer. Fabrics were classified into protection categories as per AS/NZS 4399:2017 standards.

2.6. Antioxidant Activity

Antioxidant potential was assessed using the DPPH radical scavenging assay. Dyed fabric swatches (0.5 g) were extracted in methanol, and absorbance was measured at 517 nm. Radical scavenging activity (RSA, %) was calculated using:

$$RSA(\%) = \frac{A_c - A_s}{A_s} * 100$$

where A_c is control absorbance and A_s is sample absorbance.

2.7. Color Strength and Fastness Testing

Color strength (K/S) values were measured using a spectrophotometer in the CIELAB color space. Color fastness to washing, rubbing, and light was assessed according to ISO 105-C06, ISO 105-X12, and ISO 105-B02, respectively.

3. Statistical Analysis

Experimental data were compiled from replicate measurements where available and expressed as mean ± standard deviation. For antibacterial activity, the zone of inhibition (ZOI) values against *Staphylococcus aureus* and *Escherichia coli* were compared across wash cycles. The percentage retention of activity was calculated relative to the unwashed (cycle 0) fabric. Statistical analyses were performed using IBM SPSS Statistics (version 20) and Microsoft Excel.

Descriptive Statistics

	Mean	Std. Deviation	N
Staphylococcus_aureus	13.5000	2.88675	4
Escherichia_coli	12.2500	2.50000	4

Correlations

		Staphylococcus_aureus	Escherichia_coli
Staphylococcus_aureus	Pearson Correlation	1	.993**
	Sig. (2-tailed)		.007
	N	4	4

Escherichia_coli	Pearson Correlation	.993**	1
	Sig. (2-tailed)	.007	
	N	4	4

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson's correlation analysis was performed to evaluate the relationship between antibacterial performance against *Staphylococcus aureus* and *Escherichia coli*. Results indicated an extremely strong, statistically significant positive correlation (r = 0.993, p = 0.007), suggesting that fabrics exhibiting higher inhibition zones for *S. aureus* also showed correspondingly higher inhibition zones for *E. coli*. This high correlation implies that the antimicrobial compounds present in both Shisham and Macoy dyes exert a broad-spectrum inhibitory effect, with similar relative effectiveness against both Gram-positive and Gram-negative bacteria. In the unwashed state, Macoy-dyed cotton fabric demonstrated superior antibacterial performance, producing inhibition zones of 17 mm against *Staphylococcus aureus* and 15 mm against *Escherichia coli*. In contrast, Shisham-dyed fabric exhibited inhibition zones of 14 mm and 13 mm, respectively. This enhanced activity in the Macoy treatment is likely attributable to its higher concentration of bioactive compounds, such as anthocyanins and alkaloids. After five laundering cycles, both treatments retained measurable antibacterial efficacy, although reductions were observed. For Shisham-dyed fabric, the inhibition zones decreased to 10 mm (*S. aureus*) and 9 mm (*E. coli*), while Macoy-dyed fabric maintained larger zones of 13 mm and 12 mm, respectively. These findings indicate that laundering moderately reduces antibacterial potency but does not eliminate it, particularly in the case of Macoy dye. Overall, the results confirm the durable antimicrobial functionality of herbal dye-treated cotton fabrics, with Macoy consistently outperforming Shisham. Such wash-resistant bioactivity supports their potential use in hygiene-sensitive and healthcare textile applications where sustained microbial protection is essential. The result supports the hypothesis that the phytochemical constituents such as tannins, flavonoids, and anthocyanins are effective across different bacterial cell wall structures.

The pH stability of dye extracts from *Dalbergia sissoo* and *Solanum nigrum* was evaluated by measuring their absorbance across pH levels 3 to 10. For both dyes, maximum absorbance occurred under strongly acidic conditions (pH 3), with values of 0.65 for *D. sissoo* and 0.70 for *S. nigrum*, indicating the highest color intensity and stability at this pH. As pH increased from 3 to 10, a steady decline in absorbance was observed for both extracts, suggesting reduced pigment concentration or increased degradation in neutral to alkaline conditions. At pH 10, the lowest absorbance values were recorded—0.48 for *D. sissoo* and 0.51 for *S. nigrum*. Overall, these findings confirm that both dyes are more stable in acidic environments and progressively lose stability in alkaline media. Notably, *S. nigrum* consistently exhibited higher absorbance than *D. sissoo* across all pH levels, reflecting greater resistance to degradation. This highlights the importance of pH control during dyeing with natural dyes to ensure maximum color preservation and stability.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	D_sissoo	.5713	8	.05463	.01931
	S_nigrum	.6175	8	.06364	.02250

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	D_sissoo & S_nigrum	8	.996	<.001

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	D_sissoo - S_nigrum	-.04625	.01061	.00375	-.05512	-.03738	-12.333	7	<.001

Paired Samples Effect Sizes

			Standardizer[a]	Point Estimate	95% Confidence Interval Lower	95% Confidence Interval Upper
Pair 1	D_sissoo - S_nigrum	Cohen's d	.01061	-4.360	-6.688	-2.021
		Hedges' correction	.01122	-4.122	-6.322	-1.910

a. The denominator used in estimating the effect sizes.
Cohen's d uses the sample standard deviation of the mean difference.
Hedges' correction uses the sample standard deviation of the mean difference, plus a correction factor.

The paired samples t-test revealed a statistically significant difference in absorbance between *Dalbergia sissoo* (M = 0.5713, SD = 0.05463) and *Solanum nigrum* (M = 0.6175, SD = 0.06364) across the same pH levels, $t(7) = –12.333$, $p < 0.001$, with *S. nigrum* exhibiting on average 0.046 higher absorbance than *D. sissoo*. The 95% confidence interval for the mean difference (–0.05512, –0.03738) indicated a consistent advantage for *S. nigrum* at all tested pH levels. A very strong positive correlation between the two dyes' absorbance values ($r = 0.996$, $p < 0.001$) suggested that both respond similarly to changes in pH, though *S. nigrum* maintained greater stability. The effect size was exceptionally large (Cohen's $d = –4.360$; Hedges' $g = –4.122$), confirming that the difference is not only statistically significant but also of substantial practical importance. These results demonstrate that while both dyes follow the same trend of decreasing absorbance with increasing pH, *S. nigrum* consistently retains higher color intensity and greater resistance to degradation across the pH range.

4. Conclusion

Optimized extraction and mordanting markedly improved the color strength and functional attributes of cotton fabrics dyed with herbal extracts. Statistical analysis of pH stability data showed that *S. nigrum* maintained significantly higher absorbance than *D. sissoo* across pH levels ($t(7) = –12.333$, $p < 0.001$, Cohen's $d = –4.360$), indicating superior color stability and greater resistance to degradation. Consistent with these findings, *S. nigrum* also outperformed *D. sissoo* in dye yield, UV protection, antioxidant capacity, and antimicrobial activity. Alum mordanting at 6% emerged as the most effective, offering an optimal balance between aesthetic appeal and functional performance. These results confirm the feasibility of producing high-performance, eco-friendly textiles from plant-based dyes with minimal chemical inputs. Scaling up such processes, particularly in rural areas where plant resources are abundant, could support small- and medium-scale textile enterprises, reduce environmental pollution, promote biodiversity, and strengthen sustainable value chains in the fashion sector. Future research should include larger sample sizes and explore alternative bio-mordants to maximize both environmental and economic benefits.

References

- Ahmadi, Z., & Gholami Houjeghan, F. (2022). Assessment of antibacterial, antimicrobial, and colorimetric properties of cotton and woolen yarns dyed with some plant extracts. *Textile & Leather Review*, *5*(4), 463–483. https://doi.org/10.31881/TLR.2022.43
- Ali, S. (2015). Sustainable functional finishes for natural fibers using bioactive plant extracts. *Journal of Natural Fibers*, *12*(6), 531–542. https://doi.org/10.1080/15440478.2014.984046
- Musinguzi, A., et al. (2019). Antimicrobial activity of cotton and silk fabrics dyed with *Datura stramonium* (Jimson weed) plant leaf extracts. *African Journal of Microbiology Research*, *13*(12), 667–674.
- Narayanaswamy, V., Ninge Gowda, K. N., & Sudhakar, R. (2013). Dyeing and color fastness of natural dye from *Psidium guajava* on silk. *Journal of Natural Fibers*, *10*(3), 257–270. https://doi.org/10.1080/15440478.2013.797948
- Neves, M. I. L., & Landim, A. P. M. (2019). Trends and challenges in the industrialization of natural colorants. *Journal of Textile Science and Engineering*, *9*(2), 1–8. https://doi.org/10.4172/2165-8064.1000389
- Periyasamy, A. P. (2024). Recent advances in the remediation of textile-dye-containing wastewater: Prioritizing human health and sustainable wastewater treatment. *Sustainability*, *16*(2), 495. https://doi.org/10.3390/su16020495
- Saha, J., Chakraborty, S. S., Rahman, A., & Sakif, R. (2021). Antimicrobial activity of textiles from different natural resources. *Journal of Research in Environmental and Earth Sciences*, *7*(1), 32–44.

- Shahmoradi Ghaheh, F., Moghaddam, M. K., & Tehrani, M. (2021). Comparison of the effect of metal mordants and bio-mordants on the colorimetric and antibacterial properties of natural dyes on cotton fabric. *Coloration Technology, 137*(6), 689–698. https://doi.org/10.1111/cote.12569
- Shwaiki, L. N. (2021). A study on the application of synthetic antimicrobial peptides derived from plants for the reduction of yeast spoilage in food [Doctoral dissertation, University College Cork]. University College Cork Repository.
- Tawiah, B., Badoe, W., & Fu, S. (2016). Advances in the development of antimicrobial agents for textiles: The quest for natural products. *Fibres & Textiles in Eastern Europe, 24*(3), 136–149. https://doi.org/10.5604/12303666.1207833
- Thakur, P., Sahu, M., Sonwani, H. P., & Singh, S. (2025). Phytochemical active compound: Its biological sources and uses of herbal plants containing dye properties – Review. *Cuestiones de Fisioterapia, 54*(1), 453–495. https://doi.org/10.17705/1Q202554