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RESEARCH ARTICLE

Comparative Natural Dyeing and Bioactive Finishing of Cotton with Three Plant Extracts under $FeSO_4$ Fixation

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ABSTRACT

Plant-derived colorants can combine textile coloration with bioactive finishing, but controlled comparisons among chemically distinct plant extracts on a common substrate remain limited. This study compared extracts from Lycium ruthenicum fruit, Broussonetia papyrifera fruit, and Rhodiola rosea root on a standardized plain-woven cotton substrate. Source-specific aqueous-ethanol extraction was followed by dyeing at an equal dry-extract concentration, and dyed fabrics were fixed with $FeSO_4$ for colour, fastness, and functional evaluation. Colour properties, washing/rubbing/light/perspiration fastness, ultraviolet protection factor (UPF), fabric antioxidant activity, and antibacterial activity against Staphylococcus aureus and Escherichia coli were evaluated. L. ruthenicum produced the deepest red-purple shade and the highest $FeSO_4$-assisted colour strength (K/S = 6.8), followed by B. papyrifera (4.6) and R. rosea(3.7). Under the common $FeSO_4$ fixation condition, the three extracts produced distinct colour and functional profiles, with L. ruthenicum showing the strongest overall performance. The antibacterial and antioxidant values represent the net response of the complete $FeSO_4$-fixed extract–cotton systems because separate extract-only and fixative-only controls were not included. These results support the potential of the tested plant sources for multifunctional cotton treatment while limiting the conclusions to the evaluated fixation route and initial performance conditions.

Keywords: anthocyanins; plant polyphenols; eco-friendly coloration; mordanting; bioactive finishing; ultraviolet protection; antioxidant fabric; antibacterial textile.

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1. Introduction

The textile industry is one of the industries with the highest water and chemical consumption in the world. It is a prime source of coloured industrial effluents in its dyeing and finishing processes. The amount of water, electrolytes, alkalis and fixing auxiliaries used in synthetic dyes is large. A good proportion of the dye does not penetrate the fibre. Rather, it is flushed out with the used liquor. Many of these dyes and their degradation products are chemically stable, not easily biodegraded and toxic to aquatic species. Untreated effluents can lead to water pollution, dissolved oxygen depletion and can be ingested by humans [1,2]. These pressures and the need for more stringent regulations and consumer demands for cleaner colors have sparked renewed interest in natural, plant-based dyes.

Natural dyes are desirable for a variety of reasons. They are biodegradable and from renewable biomass. The phytochemicals responsible for their colour (phenolics, flavonoids, anthocyanins,

tannins) can be biologically active. One bio-based treatment can thus be used to colour the fabric and also provide it with ultraviolet (UV) protection, antioxidant and antibacterial properties. This can be used to substitute for multiple synthetic finishing processes [3, 4]. Low substantivity and limited wash fastness are major disadvantages of many natural dyes on cotton. The use of metal-salt mordants is traditionally used to solve this problem. In the last decade, however, green fixatives such as tannin and bio-mordants (e.g. chitosan) have been promoted in place of metals because of concerns about metal discharge [5, 6].

Plant-extract bioactivity is affected not only by botanical source but also by solvent polarity, concentration, extract ratio, and physiological stage. Aqueous, ethanolic, and methanolic extracts have shown different antibacterial profiles, combined extracts can show concentration- and solvent-dependent antibacterial effects, response-surface optimization has shown that extract type, concentration, solvent, and target organism can interact significantly, and developmental stage can influence antioxidant and biological activity. These observations reinforce the need to report extraction variables precisely and to distinguish extract-derived activity from the independent effects of textile fixatives [38–41].

The three species were not chosen from the same botanical group, but from different groups of phytochemicals and possible textile uses. L. ruthenicum fruits were known to contain anthocyanin-rich visible chromophores and was thus chosen as a pigment dominant source. The fruit of B. papyrifera has been reported to possess phenolic, flavonoid and polysaccharide components that have antioxidant, and antibacterial properties. R. rosea root was found to contain literatures reported phenolics/glycosides and was chosen as an antioxidant rich source of phenolics/glycosides. They thus offer a structure-function comparison of an anthocyanin-rich fruit and a phenolic-polysaccharide fruit, both growing on the same cotton substrate, with a glycoside-rich root. These phenolic and glycosidic structures can form good bonds with mordanted fibre and also offer UV blocking and bioactive finishes [7–21].

The individual natural dyes, natural dyeing techniques using bio-mordants and multifunctional colour treatment of the textiles have been the subject of recent studies. The available studies use various plant materials, extraction solvents, fabric structures and test protocols, however, making it difficult to compare performances from different sources. There was no study found in the literature that directly compared the three sources, under the same conditions of cotton substrate, extraction using the same source-specific standardized extraction to an equal dry-extract concentration, dyeing, $FeSO_4$ fixation and testing. The current gap is the lack of a controlled comparison to determine: (i) the difference in shades and colour strength properties of all three extracts when using an equal amount of dry extract; (ii) the comparison of the conventional fastness properties of the three extracts with regards to a common fixation route; and (iii) whether the antioxidant, antibacterial and UV-protective properties can be transferred to cotton from each source.

Accordingly, this study compared the three extracts on one standardized cotton substrate under a common $FeSO_4$ fixation route. The principal novelty is the controlled comparison of three phytochemically distinct plant extracts on the same cotton substrate, with simultaneous evaluation of colour, conventional fastness, UPF, antioxidant activity, and antibacterial performance. Unlike studies that examine a single plant source in isolation, the present design evaluates how plant source determines both coloration and transferred functional performance under source-specific extraction standardized to an equal dry-extract concentration with identical dyeing, $FeSO_4$ fixation, and testing.

2. Materials and methods

2.1. Materials

Dried *Lycium ruthenicum* fruit, *Broussonetia papyrifera* fruit, and *Rhodiola rosea* root were purchased from a certified medicinal-plant supplier (north-west China). Scoured, bleached 100% cotton fabric (120 g/m², plain woven) was used as the textile substrate; cotton served as the sole substrate in this study. The reagents

were all of analytical grade (Sigma-Aldrich): ethanol, hydrochloric acid, citric acid, sodium carbonate, Folin–Ciocalteu reagent, gallic acid, quercetin, ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), DPPH, ABTS, Mueller–Hinton agar, and nutrient broth. Distilled water was used throughout.

Instrumentation comprised a UV–Visible spectrophotometer (Shimadzu UV-1900), a Fourier-transform infrared (FTIR) spectrometer with ATR (Bruker Tensor II), a spectrophotometric colour-measurement system (Datacolor 650), a UV transmittance analyser/UPF tester (Labsphere UV-1000F), a laundering (wash-fastness) tester, a crockmeter (rubbing-fastness tester), and standard microbiological incubation equipment.

2.2. Preparation and authentication of plant samples

Plant materials were authenticated by a qualified botanist; voucher specimens (LR-2024-01, BP-2024-02, and RR-2024-03) were deposited at the institutional herbarium. Samples were cleaned, oven-dried at 40 °C until constant mass, ground, and sieved through a 60-mesh screen to a uniform particle size. Powders were stored in airtight, light-protected containers at 4 °C to limit moisture uptake and pigment degradation prior to extraction.

2.3. Extraction of phytochemical dye compounds

Extraction parameters (ethanol concentration, pH, temperature, and time) were adapted from source-specific published procedures for each plant material because the target phytochemical classes differ in polarity and stability; they were not independently optimized in the present study. The resulting yield values are therefore descriptive of the selected procedures and are not interpreted as an intrinsic comparison of extraction efficiency among the three plants. To ensure a fair textile comparison, all recovered extracts were subsequently standardized on an equal dry-extract basis before dyeing, as described below. This source-specific approach follows established extraction practice: anthocyanin recovery from *L. ruthenicum* is favoured by acidified, lower-temperature aqueous-ethanol systems, whereas the phenolic and glycosidic colorants of *B. papyrifera* and *R. rosea* are recovered at the native, unadjusted pH of the solvent, so that imposing a single identical condition would bias recovery toward one phytochemical class [7,10,12,17].

2.3.1. Extraction of Lycium ruthenicum dye extract

Anthocyanin-rich extract was prepared by macerating *L. ruthenicum* powder in acidified aqueous ethanol (60% v/v, acidified to pH 2.0 with citric acid/HCl) at a solid-to-liquid ratio of 1:20 (w/v), 50 °C for 90 min, protected from light. The mixture was filtered and centrifuged (4000 × g, 15 min); the supernatant was concentrated under reduced pressure at 40 °C and stored at −20 °C in the dark.

2.3.2. Extraction of Broussonetia papyrifera fruit dye extract

B. papyrifera fruit powder was extracted under analogous conditions (70% v/v aqueous ethanol, 1:20 w/v, 60 ° C, 90 min, native pH ≈ 5); the phenolic-rich filtrate was clarified, concentrated, and stored as above.

2.3.3. Extraction of Rhodiola rosea root dye extract

R. rosea root powder was extracted in aqueous ethanol (60% v/v, 1:20 w/v, 60 °C, 90 min) to recover salidroside/rosavin- and flavonoid-containing colorant; the extract was filtered, concentrated, and stored as above. Extracts were either used directly as dye liquors at defined concentration or freeze-dried for gravimetric and phytochemical analysis.

After rotary evaporation, the dry-solids concentration of each concentrated extract was determined gravimetrically by drying a measured aliquot to constant mass. Before dyeing, each stock was diluted or reconstituted to an equal dry-extract-equivalent concentration of 40 g/L, so that the textile comparison was standardized on a dry-extract basis and was independent of the source-specific extraction yield. Concentrated extracts were stored in single-use, light-protected aliquots at −20 °C and thawed once immediately before analysis or dyeing without refreezing.

2.4. Determination of extraction yield and phytochemical content

Extraction yield was calculated on a dry-mass basis according to Equation (1):

$$\text{Extraction yield (\%)} = (m_{extract} / m_{powder}) \times 100 \quad (1)$$

where $m_{extract}$ is the mass of dried extract and m_{powder} is the mass of dried plant powder. Total phenolic content (TPC) was measured by the Folin–Ciocalteu method. It is expressed as mg gallic acid equivalents per gram (mg GAE/g) [22]. Total flavonoid content (TFC) was measured by the aluminium-chloride method and expressed as mg quercetin equivalents per gram (mg QE/g) [23]. Total anthocyanin content (TAC) is most relevant to *L. ruthenicum*. It was determined by the pH-differential method and expressed as mg cyanidin-3-glucoside equivalents per gram (mg C3G/g) [24]. Antioxidant capacity of the extracts was evaluated by DPPH and ABTS radical-scavenging assays [25,26]. Scavenging was calculated from Equation (2):

$$\text{Scavenging (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (2)$$

where $A_{control}$ and A_{sample} are the absorbances of the blank and the sample, respectively.

2.5. UV–Visible and ATR-FTIR characterization

2.5.1. UV–Visible spectral analysis

Extracts were scanned over 200–700 nm to locate characteristic pigment absorption bands, including the visible anthocyanin band of L. ruthenicum and the UV phenolic/hydroxycinnamic bands of all three sources.

2.5.2. FTIR analysis

FTIR spectra (4000–500 cm^{-1}) of the extracts and of undyed and dyed fabrics were recorded to identify functional groups associated with hydroxyl (O–H), carbonyl (C=O), aromatic (C=C), glycosidic, and phenolic structures, and to provide qualitative evidence of dye–fibre and dye–mordant interaction.

2.6. Textile pretreatment

Cotton fabric was scoured with 2 g/L non-ionic detergent and 2 g/L sodium carbonate at 60 °C for 30 min, rinsed to neutral pH, and air-dried. Samples were conditioned under standard atmosphere (20 ± 2 °C, 65 ± 2% RH) before dyeing.

2.7. $FeSO_4$ pre-mordanting and dyeing procedure

The experimental scope was restricted to a single $FeSO_4$ pre-mordanting route in order to permit a controlled comparison of the three plant sources under one constant fixation condition. Cotton specimens were pre-mordanted with $FeSO_4$ at 10% o.w.f. using a material-to-liquor ratio of 1:30 at 60 °C for 30 min. The specimens were subsequently dyed with the standardized plant extracts at an equal dry-extract concentration of 40 g/L, pH 4, and 80 °C for 60 min, followed by rinsing, neutralization, and drying. Only pre-mordanting was performed; meta-mordanting and post-mordanting were outside the experimental design. Mordant-free, alum and chitosan routes were not experimentally evaluated, and no comparative performance claims are made for these treatments.

2.8. Colorimetric evaluation

Reflectance was measured under illuminant D65/10° observer to obtain CIE L*, a*, b*, chroma (C*), and hue angle (h°). Colour strength was calculated from reflectance at λ_{max} using the Kubelka–Munk relationship, Equation (3) [30]:

$$K/S = (1 - R)^2 / 2R \quad (3)$$

where R is the decimal reflectance of the dyed sample at λmax, K is the absorption coefficient, and S is the scattering coefficient. Total colour difference (ΔE) relative to the undyed control was computed from the CIE L*a*b* coordinates.

2.9. Fastness testing

Colour fastness was evaluated using ISO/AATCC procedures and rated on the grey scale (1 = poor to 5 = excellent).

2.9.1. Washing fastness

Assessed per ISO 105-C06 (test A1S); colour change and staining on adjacent fabric were rated.

2.9.2. Rubbing fastness

Dry and wet rubbing (crocking) fastness assessed per ISO 105-X12 using a crockmeter.

2.9.3. Light fastness

Assessed per ISO 105-B02 against the blue-wool scale (1–8).

2.9.4. Perspiration fastness

Acid and alkaline perspiration fastness assessed per ISO 105-E04.

2.10. Functional textile performance

2.10.1. Ultraviolet protection factor

Spectral transmittance was measured across the UV-A (315–400 nm) and UV-B (280–315 nm) regions, and UPF was computed by the standard weighting (AS/NZS 4399), classifying samples as good (15–24), very good (25–39), or excellent (40–50+).

Because all specimens were cut from the same plain-woven cotton lot (120 g/m^2), UPF changes were interpreted as treatment-associated effects on a constant parent textile structure rather than as effects of fabric construction.

2.10.2. Antioxidant activity of dyed fabrics

Radical-scavenging activity of the dyed fabrics was determined by the DPPH assay on fabric extracts/discs and expressed as percentage scavenging.

2.10.3. Antibacterial activity

Activity against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) was assessed by agar-diffusion (zone of inhibition) and quantitative reduction (AATCC 100) methods. For the quantitative test, conditioned fabric specimens were inoculated with a known bacterial suspension and incubated; viable bacteria were then recovered, serially diluted, plated, and enumerated, with untreated cotton processed in parallel as the control. Antibacterial reduction was calculated as Reduction (%) = $100 \times (C - T)/C$, where C and T are the viable counts recovered from the untreated control and the treated specimen after the same contact period, and log reduction as $\log_{10}(C/T)$; on this basis a 96.4% reduction corresponds to approximately a 1.44-log reduction.

2.11. Statistical analysis

All measurements were performed in triplicate (n = 3) and reported as mean ± standard deviation. Continuous outcomes (extraction yield, phytochemical contents, K/S, CIELAB coordinates, UPF, antioxidant activity, and antibacterial reduction) were compared among the three plant sources by one-way ANOVA followed by Tukey-adjusted pairwise comparisons, after checking residual normality and variance homogeneity; significant pairwise differences ($p < 0.05$) are indicated in the tables by different superscript letters. Ordinal grey-scale fastness grades are reported as grades and are not analysed as continuous data. Statistical significance was set at $p < 0.05$. Analyses were performed in SPSS (v26) and OriginPro 2021.

3. Results

3.1. Extraction yield and visual characteristics of dye extracts

Extraction yields, extract colour, native pH, and apparent stability of the three dye liquors are summarised in Table 1. The anthocyanin-bearing L. ruthenicum liquor was strongly pH-responsive, while the B. papyrifera fruit and R. rosea root liquors presented amber-to-brown phenolic hues.

Table 1. Extraction yield and visual characteristics of the phytochemical dye extracts.

Dye source	Plant part	Yield (%)	Extract colour	Native pH	Stability note
L. ruthenicum	Fruit	22.4 ± 0.6[a]	Red–purple	3.4 ± 0.1	pH-sensitive; light-protect
B. papyrifera	Fruit	16.8 ± 0.5[b]	Amber–brown	5.2 ± 0.1	Moderately stable
R. rosea	Root	13.5 ± 0.4[c]	Pale brown	5.6 ± 0.1	Stable in the dark

Values are mean ± SD (n = 3). Different superscript letters in the Yield column indicate significant differences ($p < 0.05$). λmax values are reported in Section 3.3.

3.2. Phytochemical composition of the extracts

Total phenolic, flavonoid, and anthocyanin contents and antioxidant capacities are reported in Table 2. Consistent with their botany, L. ruthenicum dominated in anthocyanin content, whereas R. rosea root and B. papyrifera fruit contributed phenolic- and flavonoid-based activity.

Table 2. Phytochemical content and antioxidant activity of the natural dye extracts.

Dye source	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg C3G/g)	DPPH IC_{50} (mg/mL)	ABTS (%)
L. ruthenicum	38.6 ± 1.2[a]	21.4 ± 0.8[a]	14.8 ± 0.5[a]	0.42 ± 0.03[a]	89.3 ± 1.5[a]
B. papyrifera	27.9 ± 0.9[c]	15.7 ± 0.6[b]	n.d.	0.68 ± 0.04[c]	78.5 ± 1.8[c]
R. rosea	31.5 ± 1.0[b]	12.3 ± 0.5[c]	n.d.	0.55 ± 0.03[b]	82.1 ± 1.4[b]

Mean ± SD (n = 3). TPC, total phenolic content; TFC, total flavonoid content; TAC, total anthocyanin content; n.d., not detected. A lower DPPH IC_{50} indicates stronger activity; ABTS is radical scavenging at the test concentration. Different superscript letters within a column indicate significant differences ($p < 0.05$).

3.3. UV–Vis and FTIR characterization

Representative UV–Visible spectra of the three extracts are shown in Figure 1. L. ruthenicum displays a characteristic visible absorption band near 515–525 nm attributable to anthocyanins, alongside UV phenolic bands; B. papyrifera fruit and R. rosea root show predominantly UV bands (~275–325 nm) consistent with phenolic acids, hydroxycinnamic derivatives, and phenylethanoid/cinnamyl glycosides.

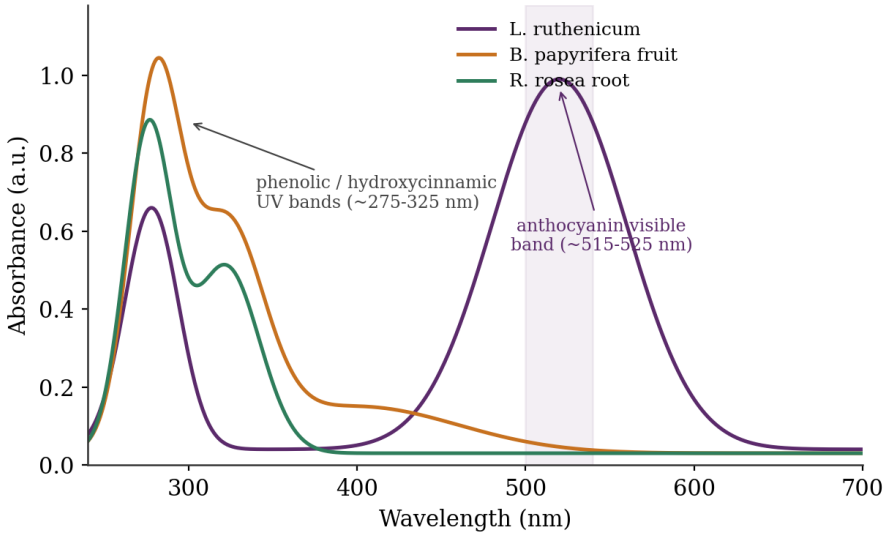

Figure 1. UV–Visible absorption spectra of the dye extracts from L. ruthenicum, B. papyrifera fruit, and R. rosea root.

FTIR spectra (Figure 2) of the extracts and dyed cotton show broad O–H stretching (~3300 cm^{-1}), C–H stretching (~2920 cm^{-1}), C=O (~1700 cm^{-1}), aromatic C=C (~1600/1515 cm^{-1}), and C–O (~1040 cm^{-1}) features. Shifts and intensity changes in the O–H, C=O, and C–O regions of dyed versus undyed fabric provide qualitative evidence of hydrogen bonding and dye–fibre/dye–mordant coordination.

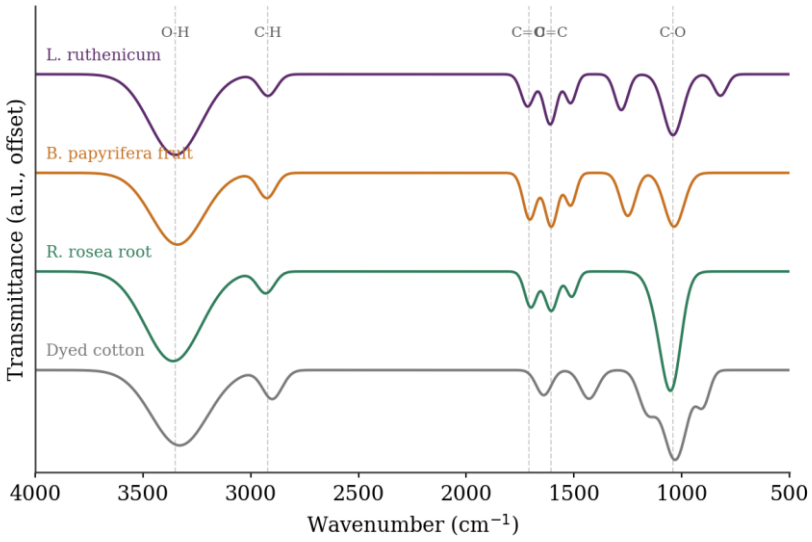

Figure 2. FTIR spectra of the three plant extracts and of dyed cotton fabric, with characteristic functional-group regions indicated.

3.4. Colour performance of dyed fabrics

CIE L*a*b*, chroma, hue angle, colour strength (K/S), and ΔE of the dyed fabrics are presented in Table 3. Across treatments, L. ruthenicum produced the deepest shades (lowest L*, highest K/S), consistent with its high pigment loading.

Under $FeSO_4$ fixation, L. ruthenicum produced the highest K/S value (6.8), which was 47.8% higher than that of B. papyrifera (4.6) and 83.8% higher than that of R. rosea (3.7). Its lower L* value of 38.2 confirmed the greatest shade depth. The positive a* and negative b* values produced a red-purple shade for L. ruthenicum, whereas the positive a* and b* values of B. papyrifera and R. rosea indicated warmer amber-brown shades. The corresponding ΔE values of 56.5, 44.0, and 34.7 confirmed that the visual difference from undyed cotton followed the same order as colour strength.

Table 3. Colorimetric properties of cotton dyed with the phytochemical-based natural dyes ($FeSO_4$-mordanted).

Sample	L*	a*	b*	C*	h°	K/S	ΔE
Undyed control	92.5 ± 0.3	−0.4 ± 0.1	2.1 ± 0.2	2.1	100.8	—	—
L. ruthenicum	38.2 ± 0.5	12.4 ± 0.3	−6.8 ± 0.2	14.1	331.3	6.8 ± 0.3[a]	56.5
B. papyrifera	52.6 ± 0.4	8.2 ± 0.2	18.4 ± 0.3	20.1	66.0	4.6 ± 0.2[b]	44.0
R. rosea	61.3 ± 0.4	5.1 ± 0.2	16.2 ± 0.3	17.0	72.5	3.7 ± 0.2[c]	34.7

Mean ± SD (n = 3) for L, a*, b*, and K/S; C*, h°, and ΔE are derived. K/S measured at λmax (Kubelka–Munk); ΔE relative to the undyed control. Different superscript letters in the K/S column indicate significant differences ($p < 0.05$).*

3.5. Colour development under $FeSO_4$ fixation

Under the common $FeSO_4$ fixation condition, the three plant extracts produced significantly different colour strengths and shades. The comparison in Figure 3 is therefore a comparison among plant sources under one constant fixation route and should not be interpreted as evidence that $FeSO_4$ outperformed mordant-free, alum or chitosan treatments, because those routes were not evaluated.

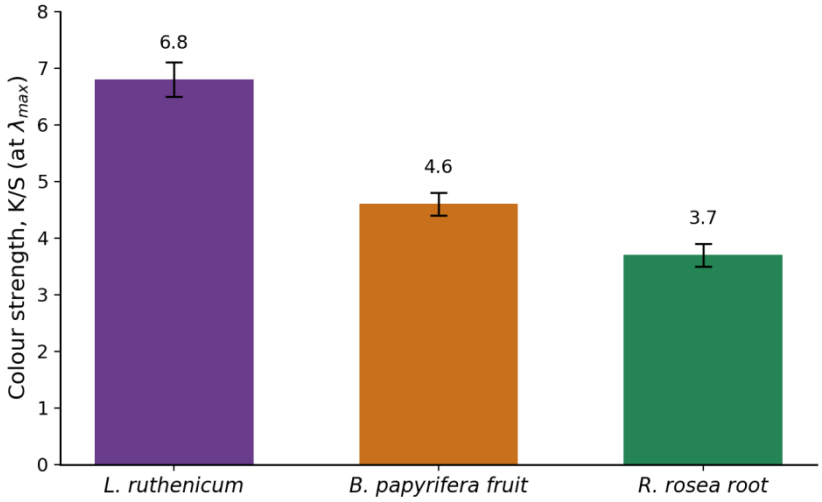

Figure 3. Colour strength (K/S) of the three plant extracts on cotton under $FeSO_4$ fixation, relative to the undyed control. Values are mean ± SD from three independently prepared and dyed fabric specimens per condition (error bars = SD).

3.6. Fastness properties of dyed textiles

Washing, rubbing, light, and perspiration fastness ratings are summarised in Table 4. The $FeSO_4$-fixed samples showed generally moderate-to-good fastness across all four tests, consistent with effective dye–fibre fixation; because the mordant-free and alum routes were not quantified, no direct comparison against an unmordanted baseline is drawn here.

Table 4. Fastness ratings of the naturally dyed cotton samples ($FeSO_4$-mordanted).

Sample	Wash (change/stain)	Rub (dry/wet)	Light	Perspiration (acid/alk.)
L. ruthenicum	3–4 / 4	4 / 3	3	3–4 / 3–4
B. papyrifera	4 / 4	4 / 3–4	3–4	4 / 4
R. rosea	4 / 4–5	4 / 4	4	4 / 4

Grey-scale ratings 1 (poor) to 5 (excellent); light fastness on the blue-wool scale 1–8. Determined per the ISO 105 series. The lower light fastness of L. ruthenicum reflects the known photosensitivity of anthocyanins.

3.7. UV-protective performance

UPF values for the undyed control and the three dyed fabrics are compared in Figure 4 and Table 5. Dyeing substantially raised UPF relative to the undyed control, with the phenolic/anthocyanin chromophores acting as UV absorbers; the highest protection was associated with the most deeply dyed samples.

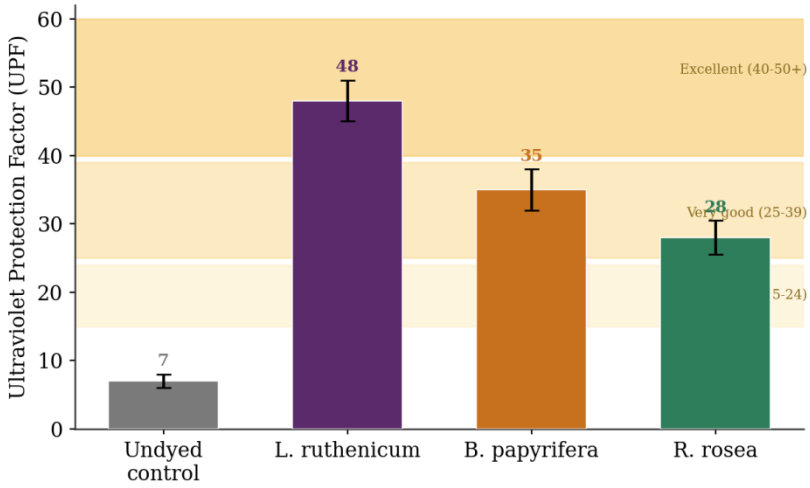

Figure 4. Ultraviolet protection factor (UPF) of the undyed control and the dyed cotton fabrics ($FeSO_4$ fixation), with UPF rating bands indicated. Values are mean ± SD from three independently prepared and dyed fabric specimens per condition (error bars = SD).

Table 5. UPF values and UV-A/UV-B transmittance of the treated fabrics ($FeSO_4$-mordanted).

Sample	UPF	UV-A T (%)	UV-B T (%)	Rating
Undyed control	7 ± 1^{d}	22.5 ± 1.1	16.8 ± 0.9	Not rated (<15)
L. ruthenicum	48 ± 3^{a}	3.2 ± 0.3	1.4 ± 0.2	Excellent (40–50+)
B. papyrifera	35 ± 3^{b}	5.1 ± 0.4	2.6 ± 0.3	Very good (25–39)
R. rosea	28 ± 2^{c}	6.4 ± 0.5	3.3 ± 0.3	Very good (25–39)

Mean ± SD (n = 3). T, transmittance; UPF and rating per AS/NZS 4399 (15–24 good, 25–39 very good, 40–50+ excellent). Different superscript letters in the UPF column indicate significant differences ($p < 0.05$).

3.8. Antioxidant and antibacterial activities of dyed fabrics

Table 6 reports the antioxidant and antibacterial performance of the complete $FeSO_4$-fixed extract–cotton systems. Because separate $FeSO_4$-only and extract-only control fabrics were not included, these values represent the net performance of the combined treatment and do not permit quantitative partitioning of the contributions of the plant extract, $FeSO_4$ and their interaction.

Table 6. Antioxidant and antibacterial performance of the complete $FeSO_4$-fixed extract–cotton systems.

Sample	DPPH scavenging (%)	S. aureus reduction (%)	E. coli reduction (%)
L. ruthenicum	82.6 ± 1.8^{a}	96.4 ± 1.2^{a}	89.7 ± 1.5^{a}
B. papyrifera	71.3 ± 1.6^{c}	91.8 ± 1.4^{b}	83.5 ± 1.7^{b}
R. rosea	76.5 ± 1.5^{b}	88.9 ± 1.6^{c}	80.2 ± 1.8^{c}

Mean ± SD (n = 3). Antibacterial reduction determined per AATCC 100; fabric antioxidant activity by DPPH. Activity is stronger against Gram-positive S. aureus than Gram-negative E. coli. Different superscript letters within a column indicate significant differences ($p < 0.05$).

4. Discussion

4.1. Phytochemical profiles and source-dependent colour development

The comparative results can be rationalised on the basis of the phytochemistry of the three sources. The high colour strength and red-purple hues of L. ruthenicum are consistent with the anthocyanin-rich composition reported for this species; anthocyanins are pH- and flavylium-dependent, so both the dyeing-bath pH and the metal centre of the mordant influence the final shade and depth. In particular, the shade darkens and shifts bathochromically in the presence of the iron(II) complex [7,10,11]. B. papyrifera fruit is a source of phenolic acids and flavonoids, while the spectral and antioxidant behaviour of R. rosea was interpreted in relation to the

phenylethanoid and cinnamyl glycosides previously reported for this species. These groups provide ortho-dihydroxy and carbonyl chelating sites that can coordinate with cellulose and mordant ions, but they absorb less strongly in the visible region than anthocyanins, which is consistent with their lower K/S values [12,17,19].

4.2. Fixation chemistry and dye–fibre interaction

$FeSO_4$ fixation increased dye uptake and fastness, consistent with the known function of metal mordants, which coordinate with both the dye and the fibre to anchor the colorant to cellulose [5,13]. The FTIR changes in the O–H, C=O, and C–O regions are consistent with hydrogen bonding and coordination between the polyphenolic dye and the cellulose–mordant system. Chitosan is discussed here as a metal-free fixation concept: rather than acting as a coordinating metal centre, its protonated amino groups can promote electrostatic attraction and additional hydrogen bonding, and biopolymer-based fixatives have been reported as environmentally motivated alternatives that may also contribute antibacterial character [27–29,31,32]. Because the present colour and fastness data were obtained under $FeSO_4$ fixation, a direct performance comparison between chitosan and metal salts was not made in this study and is identified as future work. A schematic of the proposed dye–Fe(II)–cellulose coordination and hydrogen-bonding interactions is shown in Figure 5.

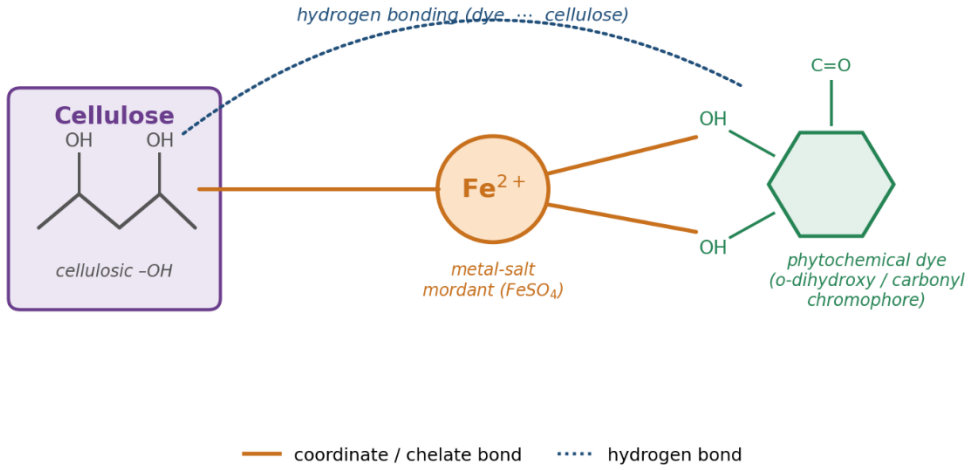

Figure 5. Proposed schematic of the dye–Fe(II)–cellulose interaction. The Fe(II) centre supplied by $FeSO_4$ coordinates simultaneously with the o-dihydroxy/carbonyl groups of the phytochemical chromophore and with cellulosic hydroxyl groups (coordinate/chelate bonds), while direct hydrogen bonding between the dye and cellulose provides additional fixation. The diagram is a qualitative mechanistic illustration consistent with the FTIR evidence and is not derived from structural determination.

Chitosan, alum and mordant-free routes were considered relevant alternative fixation strategies on the basis of the literature, but they were not part of the experimental matrix. Consequently, the present results should be interpreted as a plant-source comparison under $FeSO_4$ fixation rather than as a comparative evaluation of mordants. A factorial comparison of fixation route and plant source is required before conclusions can be drawn regarding the relative performance of metal and bio-based fixation systems.

4.3. UV-protective, antioxidant and antibacterial performance of the complete treated systems

The $FeSO_4$-fixed extract–cotton systems showed measurable UV-protective, antioxidant and antibacterial performance, in agreement with earlier reports of natural-dye UV-protective and bioactive cotton finishing[20,21,33]. The highest values occurred for *L. ruthenicum*, which also produced the greatest colour strength and contained the highest measured anthocyanin content. This parallel pattern is compatible with a contribution from retained phenolic and anthocyanin constituents, but it does not establish that these constituents were solely responsible for the observed functionality.

The experimental design included untreated cotton as the textile reference but did not include separate $FeSO_4$-only or extract-only control fabrics. The respective contributions of the plant extract, the iron fixative and possible extract–fixative interactions therefore cannot be quantitatively separated. Accordingly, the antibacterial and antioxidant results are interpreted as properties of the complete $FeSO_4$-fixed dyeing system rather than as isolated effects of the dye extract or mordant. The stronger reduction against *S. aureus* than against *E. coli* may be associated with differences in bacterial-envelope structure [13,28], but mechanistic attribution requires dedicated component controls.

The present results demonstrate system-level functionality under the tested treatment conditions. Future work should include untreated cotton, $FeSO_4$-only cotton, extract-only dyed cotton and combined $FeSO_4$–extract treatments to distinguish individual and interactive contributions.

To relate these findings to recent literature, Table 7 compares the present $FeSO_4$-fixed results with recently reported cotton-dyeing studies. The colour strength obtained here (K/S 3.7–6.8) is of the same order as that reported for cotton dyed with *Justicia schimperiana* leaf extract under metal-salt mordanting (K/S ≈ 3.28) [42], and lower than the deep shades reported for *Diospyros mollis* extract and a benchmark reactive dye on cotton (K/S 18.52 and 19.36, respectively) [44]. The antibacterial reductions recorded here (88.9–96.4% against *S. aureus*) are comparable to, or exceed, values reported for other naturally dyed cottons, such as 85.25% for *Bixa orellana*–dyed cotton [43] and up to 99.99% for *Diospyros mollis*–dyed cotton [44]; notably, in the same comparative study the conventional reactive dye showed no measurable antibacterial activity and its ultraviolet protection was lower than that of the natural dye [44]. These comparisons support the central premise of this work — that plant-extract dyeing can confer antibacterial and ultraviolet-protective functionality that conventional reactive dyeing does not, even where the absolute colour strength of the natural system is more moderate — while a matched experimental commercial-dye control remains necessary before any claim of industrial equivalence.

Table 7. Comparison of the colour strength, ultraviolet protection, antibacterial activity, and wash fastness of the present $FeSO_4$-fixed cotton with recently reported natural- and reactive-dyed cottons. n.r., not reported in the cited source; ZOI, zone of inhibition.

Dye system (substrate, fixation)	K/S	UPF	Antibacterial reduction (%)	Wash fastness	Ref.
Present study — *L. ruthenicum* / cotton ($FeSO_4$)	6.8	48	96.4 (*S. aureus*)	3–4 / 4	This study
Present study — *B. papyrifera* / cotton ($FeSO_4$)	4.6	35	91.8 (*S. aureus*)	4 / 4	This study
Present study — *R. rosea* / cotton ($FeSO_4$)	3.7	28	88.9 (*S. aureus*)	4 / 4–5	This study
Justicia schimperiana leaf / cotton (metal-salt mordant)	3.28	n.r.	Active (ZOI; *S. aureus, E. coli*)	Reported	[42]
Bixa orellana seed / cotton (no mordant)	n.r.	Enhanced	85.25 (*Staphylococcus*)	n.r.	[43]
Diospyros mollis / cotton (natural extract)	18.52	Superior to reactive	99.99 (*S. aureus, E. coli*)	High	[44]
Reactive dye / cotton (conventional benchmark)	19.36	Lower than natural	None	High	[44]

4.4. Practical considerations and constraints

Several practical constraints apply when interpreting these results. Anthocyanin pigments are sensitive to pH, light, and heat, which can limit long-term and light fastness [10]. Plant material is known to vary from batch to batch, which complicates standardisation. Metal-salt mordants are effective but may introduce the

environmental burden that bio-mordants are intended to reduce [5]. Scale-up, effluent characterization, and life-cycle assessment are still required before industrial use.

4.5. Limitations and future perspectives

The findings should be interpreted within the defined experimental scope. First, the study compared three plant sources under a single $FeSO_4$ pre-mordanting condition. Mordant-free, alum and chitosan routes were not evaluated, and the results therefore do not establish the comparative superiority of one fixation system over another. Second, separate $FeSO_4$-only and extract-only control fabrics were not included. The antioxidant and antibacterial outcomes consequently represent the net performance of the complete $FeSO_4$-fixed extract–cotton systems and cannot be partitioned into independent extract, fixative or interaction effects.

Third, source-specific retention of functional performance and UPF after repeated laundering was not resolved; the reported fastness grades should not be interpreted as quantitative evidence of long-term retention of antioxidant, antibacterial or UV-protective functionality. Fourth, UV–Visible and FTIR analyses provide class-level evidence of chromophores and functional groups but do not identify individual compounds. References to anthocyanins, salidroside, rosavin and phenolic acids are therefore based on the reported phytochemistry of the corresponding species and require confirmation by HPLC-DAD or LC–MS/MS.

Finally, the plant materials originated from one commercial batch, and no matched commercial dye was included in the experimental design. The study therefore establishes comparative performance within the tested lots and processing conditions but does not demonstrate batch-to-batch reproducibility, industrial equivalence or environmental superiority. Future research should address these issues through factorial fixation-route comparisons, component controls, independent plant batches, compound-level profiling, source-specific laundering assessment and matched commercial benchmarking. Process-intensification routes such as ultrasound- and microwave-assisted extraction may also be explored to improve colourant yield and dye uptake with lower energy and water use [34–37].

5. Conclusion

This study compared three phytochemically distinct plant extracts on a standardized cotton substrate using equal dry-extract loading, identical dyeing conditions and one common $FeSO_4$ pre-mordanting route. Under these controlled conditions, plant source determined shade depth and the measured functional profile. L. ruthenicum produced the highest colour strength, UPF and antibacterial reduction, whereas B. papyrifera and R. rosea produced lighter shades with moderate functional performance. The antioxidant and antibacterial results represent the net behaviour of the complete $FeSO_4$-fixed extract–cotton systems and cannot be assigned independently to the plant extract or iron fixative. The study therefore demonstrates source-dependent performance under a defined fixation condition rather than comparative superiority among mordants. Additional fixation routes, component controls, source-specific functional durability, independent plant batches and compound-level characterization are required before industrial equivalence or environmental superiority can be concluded.

Data Availability

No external dataset was used in this study. All data generated and analysed during this study are included in this article. Additional information is available from the corresponding author upon reasonable request.

Author contributions

Conceptualization, ML; methodology, MK; software, ML; validation, ML; formal analysis, CA; investigation, ML; resources, ML; data curation, ML; writing—original draft preparation, ML; writing—

review and editing, ML; visualization, ML; supervision, ZJ; project administration, ML; funding acquisition, MF. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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