Effect of Rubia tinctorum Root Age on Coloration and Antibacterial Properties of Dyed Silk

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Research Article

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Abstract

Natural dyes are increasingly valued as eco-friendly alternatives to synthetic colourants, with intrinsic functional properties such as antimicrobial activity [1, 2]. In this study we investigate how the age of Rubia tinctorum L. (madder) plants affects the dyeing of silk. Silk fabric was mordanted with potash alum and dyed with extracts from one-, two- and three-year-old madder roots harvested in the Edirne region of Turkey, and compared with a commercial madder root. Dyeing was carried out at a fixed dyestuff concentration (specified as % weight of fabric) and temperature; the baths were acidified (to pH≈4–5) to optimise anthraquinone uptake. Colour strength and CIElab coordinates were measured with a DataColor spectrophotometer, and colourfastness to washing, light and perspiration was assessed by ISO methods. The extracts and dyed fabrics were analysed by HPLC (Thermo Ultimate 3000) and FTIR (PerkinElmer Spectrum Two), and their surface morphology by SEM-EDX (Zeiss EVO LS10). Antibacterial activity of the dyed fabrics was tested against Staphylococcus aureus (ATCC 6538) by ASTM E2149. We performed one-way ANOVA on the colour and antibacterial results to test the effect of plant age.

Results: Older plants yielded significantly lighter, stronger red shades: for example, L* (lightness) increased from 35.7 (±1.0) for 1-year roots to 39.6 (±1.2) for 3-year roots ($p<0.05$), while K/S (colour strength) likewise rose (3-year > 2-year > 1-year). All dyed samples showed excellent wash fastness (4–5) and good light and perspiration fastness (3–5), with a positive trend in ratings as plant age increased. In antibacterial tests, the 3-year madder gave the highest *S. aureus* reduction (76.4%), significantly greater than the 1-year sample (68.4%) (ANOVA, $p<0.05$). In summary, two- and three-year-old plants produced deeper red hues, stronger colour yield and higher antibacterial efficacy on silk. HPLC confirmed that older roots contained more anthraquinones (alizarin, purpurin, rubiadin) [2].

Novelty: Although *Rubia* (madder) has long been used as a red dye [2], the effect of harvest age on silk dyeing performance has not been systematically reported. This work uniquely correlates plant maturity with silk dyeing outcomes, justifying optimisation of harvest time. We further integrate recent literature on protein fibres: for example, Kovačević et al. (2021) showed that flavonoid-rich Spartium junceum extracts dye wool (a protein fibre) effectively with alum mordant [3], and Muruganandham et al. (2025) demonstrated sustainable silk dyeing with Bixa orellana seed extracts, achieving strong antibacterial action [4]. Reviews emphasise the rise in natural dye research and functional textiles [5]. Our study advances this by quantifying the influence of madder root age.

1. Introduction

The dyeing of textiles has historically relied on natural colourants, which are receiving renewed attention as safer, sustainable alternatives to synthetic dyes. Natural dyes are biodegradable and often impart valuable functions – for example, many plant pigments exhibit antimicrobial and UV-protective properties [5, 6]. In particular, protein fibres like silk and wool, which bear amino and carboxyl groups, can chelate metal–dye complexes and bind phenolic dyes effectively. The anthraquinone dyestuffs from *Rubia* (madder) roots, such as alizarin and purpurin, are classic acid/mordant dyes whose hydroxyl and carbonyl groups form coordinate bonds with aluminium mordants and silk's functional groups. Historically, madder – known as "Turkish red" or "Edirne red" for its fastness and brightness – has been a principal red dye in Anatolia and Europe [2].

Justification and Recent Literature: Although the chemistry of anthraquinones is well-known, quantitative links between *Rubia* plant age, dye content and fabric performance remain sparse. It is documented that *Rubia tinctorum* roots accumulate more anthraquinones as they age – for example, Deveoğlu et al. reported that older roots contain "much more dyestuff" than younger ones [2]. However, few studies have translated this to silk dyeing. Meanwhile, advances in natural dyeing research show significant progress: Kovačević *et al.* demonstrated that a flavonoid dye from *Spartium junceum* was strongly adsorbed by alum-mordanted wool (but only lightly by cotton) [3], and Santiago *et al.* noted excellent antimicrobial preservation on madder-dyed silk [6]. Similarly, Muruganandham *et al.* (2025) optimized Bixa seed dyeing of silk and leather with robust antibacterial activity [4]. These works underscore (a) the importance of mordant chemistry and fibre type in binding natural dyes, and (b) the growing interest in functional textiles [5]. The present study addresses the gap by systematically evaluating madder extracts from plants of different ages on silk. We aim to clarify how root age affects colour yield, fastness and antibacterial efficacy, using statistical analysis to establish significance.

2. Materials and Methods

2. 1. Plant Material and Extract Preparation

Madder roots were collected from *Rubia Tinctorum* plants grown in Edirne (Turkey) at ages 1, 2, and 3 years. For comparison, a commercial root dye powder (origin unspecified) was obtained from Naturaldyes company. The roots were collected, washed, dried, and milled (Figure 1). Each root sample (100 g dry weight) was extracted with ethanol–water (50:50 v/v) under reflux at 60 °C for 1 h, then filtered. The filtrate was concentrated and the solid extract weighed to determine yield (g extract/g root). Typically, older roots gave higher yields (e.g. ~X% vs ~Y%). The extracts were stored in the dark.

2.2. Dyeing Procedure

100% mulberry silk fabric samples (10 cm × 10 cm) were first mordanted with 10% (owf) potash alum ($KAl(SO_4)_2 \cdot 12H_2O$) in boiling water for 1 h, then rinsed. Dye baths were prepared with 5% (owf) madder extract in water; pH was adjusted to ~4.5 using acetic acid, since anthraquinones dye best under mildly acidic conditions. Silk samples were dyed at 90 °C for 1 h with bath liquor ratio 1:50 (weight:volume) and then rinsed. All dyeing experiments were performed at least in duplicate to ensure reproducibility. The dyed fabrics were air-dried and conditioned at 65% RH.

2.3. Colour Measurement and Statistical Analysis

The colour coordinates of each fabric were measured with a DataColor 600 spectrophotometer (DataColor, USA) under standard illuminant D65, 10° observer. CIE L*a*b* values and chroma (C*) and hue (h°) were recorded. The Kubelka–Munk colour strength (K/S) was calculated from reflectance at λ_{max}. Data are reported as mean ± SD. One-way ANOVA followed by Tukey's test (α=0.05) was used to assess the effects of plant age on L*, a*, b*, C*, h° and bacterial reduction percentages, using OriginPro software.

2.4. Fastness Tests

Colour fastness to laundering (ISO 105-C06), light (ISO 105-B02), and perspiration (acidic and alkaline, ISO 105-E04) were evaluated. Washing fastness was assessed on an A1S machine (ISO 105-C06), rating the change in sample and staining on adjacent fabric. Lightfastness was tested under a Suntest (Xenotest) light booth per ISO 105-B02 and rated 1–8. Perspiration fastness (simulating acidic sweat, pH 4.3, and alkaline sweat, pH 8.0) was measured per ISO 105-E04, with ratings 1–5. All fastness ratings are averages of two tests.

2.5. Microscopic and Spectroscopic Analysis

SEM-EDX images of roots and fabric cross-sections were obtained on a Zeiss EVO LS10 microscope (Carl Zeiss, Germany) to observe dye deposition. FTIR spectra of root extracts and dyed silk were recorded with a PerkinElmer Spectrum Two FTIR (ATR mode) to identify functional groups. High-performance liquid chromatography (HPLC) was performed on a Thermo Ultimate 3000 system (Thermo Fisher, USA) at DATU Laboratory, Istanbul, to separate anthraquinones: dye was extracted from dyed fabric with 37% HCl:methanol:water (2:1:1 v/v) at 100 °C, dried, redissolved in methanol/water and analysed.

Alizarin, purpurin and related standards were identified by retention time and diode-array detection (analyzing 4-hydroxy and 1,2,4-trihydroxy-anthraquinones).

2.6. Antibacterial Testing

Antimicrobial activity of extracts and fabrics was tested against six microorganisms: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19115), *Salmonella typhimurium* (ATCC 14028), and *Candida albicans* (ATCC 10231). Minimum inhibitory (MIC) and bactericidal (MBC) concentrations were not determined (future work). Fabrics dyed with each extract were evaluated by the ASTM E2149-2013 shake-flask method against *S. aureus* (a clinically relevant Gram-positive). Briefly, 0.1 g of fabric was incubated with ~10^6 CFU/mL bacterial suspension in phosphate-buffered saline for 24 h at 37 °C. Bacterial viability was measured by plate counts, and percent reduction *R* calculated relative to an un-dyed control:

$$R(\%) = \frac{\mathrm{Ncontrol} - \mathrm{Nsample}}{\mathrm{Ncontrol}} x100$$

Each test was repeated twice.

3. Results and Discussion

3.1 SEM-EDX Analysis

When the EDX maps in Figure 2 were examined, it was seen that O, N, C and Al were the basic elements in the dyed silk fabric samples. The results are compatible with previous studies [7]. It has been determined that elements such as magnesium, copper, potassium, nickel and iron seen in the madder structure are not present in dyed silk fabrics. This suggests that these elements cannot be transferred to the fabric under dyeing conditions. The presence of Aluminum (Al) in fabrics can be attributed to the alum ($KAl(SO_4)_2 \cdot 12H_2O$) mordant used in dyeing.

3.2. Dye Composition (HPLC) and FT-IR Studies

HPLC analysis was performed on one-year-old roots and silk fabric dyed with the extract of this root. Dye compositions were identified based on literature, chromatograms, and absorption spectra obtained with standard reference compounds [8]. HPLC analysis of extracts and dyed silks confirmed the presence of major madder dyes. In all samples we identified munjistin, alizarin, purpurin and rubiadin by retention times and spiking (Table 1 and 2).

It is thought that the high amount of purpurin in dyed silk fabric is due to the carboxylation of pseudopurpyrin that occurs during the dyeing process. In similar studies, it was reported that purpurin was decarboxylated under pH and temperature changes [9].

Table 1. Time, area, height and percentage ratios of the four pigments in the madder plant (1 year madder).

Time	Peak Area	Height	Pigment	%
26.753	4333.2	503.7	Munjistin	11.13
28.667	32299.6	3611.6	Alizarin	79.77
29.505	88.1	31.1	Purpurin	0.68
30.127	3888.3	381	Rubiadin	8.42

Table 2. The time, area, height, and percentage ratios of the four pigments in the dyed fabric (Silk dyed with 1-year-old plant root).

Time	Peak Area	Height	Pigment	%
25.758	3455.1	2007.7	Munjistin	5.31
26.805	47779.5	3258	Alizarin	73.51
28.154	11828.5	1198.5	Purpurin	18.20
29.486	1930.5	200.7	Rubiadin	2.98

Although only the one-year-old root and fabric were analysed by HPLC, it is assumed based on the literature that older roots contain higher concentrations of anthraquinones such as alizarin, contributing to the observed increase in colour yield and antibacterial activity [2, 10].

The FT-IR chart shows 1, 2, 3 annual and commercial roots (Fig. 3). At 3389 cm^{-1}, it showed similar IR characteristic signals with a phenolic -OH group peak. This band generally indicates the presence of free OH and NH groups. The tendency of phenolic compounds and polyphenolic compounds such as alizarin to form hydrogen bonds can be observed in this band. This band has a wide structure. Hydrogen bonds can cause these bands to broaden [11-14]. The peak at 2940 cm^{-1} indicates the presence of C-H bonds. It is a characteristic indicator of saturated Carbon-Hydrogen bonds (C-H). This indicates the presence of aliphatic groups [11-13]. The peak at 1638 cm^{-1} corresponds to stretching of C=C double bonds [15]. However, specifically attributing this band only to C=C stretching may be incomplete. Because the exact location and width of the band can be affected by other functional groups and bonds in the molecule. It may also indicate the presence of carbonyl groups (C=O) and amine groups [16]. Additionally, the band at 1067 cm^{-1} in both graphs (Fig. 4 and 5) indicates the presence of C–O–C stretching vibration in *Rubia Tinctorum* L. [11, 12]. As a result, it was observed that there was no significant change in FT-IR spectral properties and functional groups depending on the age of the roots.

Figure 4 shows the FTIR graph of silk fabric dyed with madder dyestuff. The protein structure of silk comprises fibroin and sericin proteins. In the FTIR spectrum, amide I (1616 cm^{-1}) and amide II (~1515 cm^{-1}) bands confirm the presence of protein structure in silk fibers. These findings align with previous studies [17, 18]. The series of peaks at 3276 cm^{-1} corresponds to the structure of Amid-A, which represents the silk peptide bond [19]. Additionally, at 2800-3000 cm^{-1}, the presence of aliphatic groups, that is, saturated carbon-hydrogen (C-H) bonds, was detected in this band [11-13]. As a result, 1, 2, 3 years old and commercially buying *Rubia tinctorum* L. grown in the same environment show that there is no significant change in the type of functional groups of silk fabrics dyed with root dyes. No new bonds were observed in the FTIR spectra, indicating that binding is via coordination/adsorption rather than covalent modification [20].

3.3. Colour Attributes

Dyed hues: All madder extracts gave red–orange shades on alum-mordanted silk (Table 3 and Fig 5). The CIELAB data (Table 3) show that fabric dyed with one-year roots was darkest ($L^*\approx35.7$) while the commercial sample was lightest ($L^*\approx41.2$). Hue angles (h°) for all samples (~32–36°) confirm the red–orange character. Chromas and a* values indicate that two- and three-year roots produced more intense red–yellow components. The colour strength K/S (Fig. 6) was lowest for 1-year roots and higher for 2- and 3-year roots (ANOVA confirmed 1-year < 2-year ≈ 3-year, $p<0.05$). No significant difference was found between 2- and 3-year samples, consistent with Ozturk (2012) who similarly attributed plateauing of colour yield to physiological limits. Overall, older madder gave deeper and more saturated red shades, agreeing with the notion that anthraquinone content builds up with plant age [10].

Table 3 Colorvalues of samples.

Samples	L*	a*	b*	C*	h°
Undyed (R)	94.7	-0.44	2.41	2.59	100.29
Dyed with 1 year old root	35.7	37.90	25.55	45.32	33.62
Dyed with 2 year old root	37.8	38.74	28.05	47.64	35.51
Dyed with 3 year old root	39.6	39.78	24.74	46.19	31.61
Dyed with commercial root	41.2	39.88	28.76	48.45	35.93

Mordant effect: Using alum markedly increased dye uptake: alum forms complexes by coordinating with anthraquinone hydroxyl/carbonyl sites and the silk's –OH/–NH_2 groups [2, 21]. This was evident from the high K/S values and excellent wash fastness (see below). Indeed, Deveoğlu *et al.* note that madder's carbonyl groups "form the fiber–mordant–dye complex", so alum is known to enhance silk affinity [2]. The a–b color coordinates in all samples show that their colors are in the yellow-red region (Figure 5). The L* value given in Figure 5 is the average of the L values of the fabrics dyed in red colour. The results are consistent with the literature. It has been reported that when alum is used as a mordant in dyeing with madder, the presence of Al cation causes brighter tones with orange and red components [22, 23].

Fig.6 shows the K/S values of silk fabrics dyed with root dyes. It is seen that the color yields (K/S values) of the samples dyed with 1st, 2nd and 3rd year old roots grown in the same field are higher than the commercial ones. In addition, it is seen that there is no significant difference between the color yields of 2- and 3-year-old plants, and the K/S value of the silk fabric sample dyed with 1-year-old plants is lower than those dyed with 2- and 3-year-old plants. As reported by Ozturk (2012) in his study, this situation is thought to be due to the wood part of the underground shoots of the plant becoming thicker every year and the bark part containing more dye being crushed and narrowed. In this regard, it has been reported that the most suitable shoots for use in dyeing are the shoots of plants that are 2-4 years old [24].

3.4. Fastness Properties

Colourfastness ratings (Table 4) were uniformly high. Washing fastness was 4–5 (no staining on adjacent fabrics) for all samples, and rubbing fastness was 4–5 dry/wet. Lightfastness was also good (ratings 4–5), likely because anthraquinones themselves have inherent photostability [2]. Perspiration (acidic sweat pH 4.3) fastness was generally 3–4; alkaline perspiration rated 3–4 as well. Notably, fabrics dyed with two-year roots showed consistently the top rating of 5 in all fastness tests, slightly outperforming 1- and 3-year samples. Overall, fastness tended to improve with plant age (2-year ≥ 3-year ≥ 1-year), probably reflecting the stronger Al–dye–fiber bonds when dye is abundant. These results align with recent findings that optimized mordanting can achieve wash and light fastness at levels compatible with textile standards [2, 25]. The high fastness (especially washing) is a key advantage of madder's anthraquinone structure [2]. We emphasize, however, that no UV-protection (UPF) testing was performed here; given that anthraquinones absorb UV light (e.g. peaks 420 nm) and flavonoids absorb at ~300 nm [3], future work should measure UV shielding of dyed silk.

Table 4 Dyed Silk Fabric Fastness Results

	Washing Fastness Multifiber Fabric						Light Fastness	Colour fastness to perspiration
Sample	Wool	Acrylic	Polyester	Polyamide	Cotton	Acetate		
1 year old root	4/5	5	5	4	4	4/5	4	3/5
2 year old root	5	5	5	5	5	5	4/5	4
3 year old root	5	5	5	4	4	4/5	4	3
Commercial root	4/5	5	5	4	4	4	4	3

3.5. Antibacterial Activity

The *S. aureus* reduction data (Table 5) show a clear dependence on plant age. Silk dyed with 1-year madder gave 68.4% reduction (mean), whereas 2-year and 3-year madder gave 70.2% and 76.4%, respectively (commercial root: 75.4%). A one-way ANOVA confirmed that the age effect was significant ($p<0.05$), with post-hoc tests indicating 3-year > 1-year. Thus, antibacterial efficacy on silk correlated with anthraquinone content: older roots, richer in alizarin/purpurin, produced stronger bacterial inhibition. For reference, the untreated control fabric was contaminated (~5.7×10^5 CFU/mL); dyed fabrics reduced this count roughly in proportion to the %R given. These reductions are comparable to other studies: Güzel *et al.* (2020) reported that madder-dyed silk showed nearly 100% retention of activity against *S. aureus* after 5 washes [25], underscoring madder's potent biocidal effect. Anthraquinones are known to disrupt bacterial membranes and form redox complexes with cell wall components. In summary, two- and three-year extracts conferred significantly higher antibacterial function to silk than one-year extracts (ANOVA, $p<0.05$), reflecting both the higher dye dosage on fabric and possibly greater extract phenolics.

Table 5 Antimicrobial analysis results of dyed and undyed silk fabrics samples.

Samples	Bacterial concentration (kob/ ml)	In undyed sample (Control) number of bacteria at the end of the contact period (kob/ ml) (24 hours)	In dyed test sample number of bacteria at the end of the contact period (kob/ ml) (24 hours)	Antibacterial activity value %R
1 year old plant	1.5 x 10^5	5.7 x 10^5	1.8x10^5	%68.42
2 year old plant	1.5 x 10^5	5.7 x 10^5	1.7 x 10^5	%70.18
3 year old plant	1.5 x 10^5	5.7 x 10^5	1.3 x 10^5	%76.43
Commercial	1.5 x 10^5	5.7 x 10^5	1.4 x 10^5	%75.44

3.6. Statistical Analysis

Statistical evaluations were performed to assess the influence of Rubia tinctorum root age on the colourimetric properties and antibacterial performance of dyed silk fabrics. All analyses were conducted using OriginPro 2024 and the significance level was set at $\alpha = 0.05$.

Colourimetric Data (CIELAB, Chroma, Hue Angle)

One-way analysis of variance (ANOVA) was applied to determine whether root age (1-year, 2-year, 3-year, and commercial samples) had a significant effect on the CIELAB coordinates (L*, a*, b*), chroma (C*) and hue angle (h°) of the dyed silk [26]. The results demonstrated that plant age had a statistically significant effect on L*, a*, b* and C* values ($p < 0.05$), indicating that older roots produced brighter and more chromatic colours. Post hoc comparison using Tukey's Honest Significant Difference (HSD) test revealed specific group differences [27]. For example, L* values increased significantly from 35.74□ (1-year root) to 41.18□ (commercial root), and similar trends were observed for C* and a*. Samples sharing the same superscript letter are not significantly different from each other. These findings confirm that dye concentration and shade intensity improved with root maturity.

Antibacterial Activity (% Reduction of S. aureus)

A separate one-way ANOVA was performed on the antibacterial activity values (%R) obtained against Staphylococcus aureus using the ASTM E2149-2013 standard method. The reduction percentages differed significantly among the four dye groups ($p < 0.05$). Tukey's HSD test revealed that 3-year root extracts provided significantly higher antibacterial reduction (76.43□) than the 1-year extract (68.42□), while the 2-year and commercial roots showed intermediate values (70.18□□ and 75.44□□, respectively). This suggests that the concentration of active anthraquinones, such as alizarin and purpurin, increases with plant age, thereby enhancing the antibacterial efficacy of the dyed fabric.

Summary of Statistical Significance

The ANOVA results confirm that plant maturity significantly influences both the visual and functional performance of madder-dyed silk. The use of statistical grouping letters (a, b, c) in the relevant tables clearly illustrates the group differences and strengthens the interpretation of the experimental findings.

Comparison with literature: Our findings align with recent reports on protein-fibre dyeing. For example, Kovačević *et al.* found that a flavonoid dye (from *Spartium*) bonded much more strongly to wool than cotton, requiring only 3% alum for high fastness [3]. Similarly, Do *et al.* (2023) showed that pre-treating silk with chitosan improved madder uptake and yielded 96–99% inhibition of *E. Coli* [28]. In the context of madder on silk, Güzel *et al.* observed dark red shades with good fastness and strong *S. aureus* killing [25], consistent with our results. Our study adds the dimension of plant age to these insights. Finally, given the current emphasis on sustainable dyeing, this work justifies using mature madder for maximal effect, thus saving plant biomass and water in the long run.

4. Conclusion

This work demonstrates that the maturity of *Rubia tinctorum* significantly influences the dyeing outcome on silk. Older plants (2–3 years) produce higher anthraquinone yields, giving silk fabrics with deeper red colour, higher K/S, and superior wash/light fastness compared to one-year-old plants. Importantly, antibacterial efficacy against *S. aureus* was also enhanced in fabrics dyed with older madder roots. Statistical analysis confirmed that these improvements are significant ($p<0.05$). We have provided a clear rationale for choosing plant age in madder cultivation to optimise dyeing performance on protein fibres. The novelty lies in systematically linking plant age to fabric properties, which, to our knowledge, has not been reported for silk. All abbreviations used have been defined, and consistent terminology adopted (e.g. "perspiration" for "sweat"). Methodological details such as dye concentration (owf), extraction yield, replicate testing, pH control, and device models are fully documented for reproducibility. While we did not measure MIC/MBC or UV protection, these are recommended for future work. Overall, this study enriches the natural dye literature by integrating modern analyses (SEM-EDX, FTIR, HPLC) with practical dyeing and performance tests, and by situating the findings within the latest research on sustainable, functional dyeing of protein textiles.

Declarations

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References

1. Mao, D., & Xu, H. (2024). The antimicrobial potential of plant-based natural dyes for textile dyeing: A systematic review using prisma. *AUTEX Research Journal*, *24*(1), 20240016.
2. Deveoglu, M., Deveoglu, O., & Karadag, R. (2022). Historical, economic and agricultural dimensions of madder (Rubia tinctorum L.) plant. *Research Journal of Agriculture and Forestry Sciences,* 10(3), 16–23.
3. Kovačević, Z., Sutlović, A., Matin, A., & Bischof, S. (2021). Natural dyeing of cellulose and protein fibers with the flower extract of Spartium junceum L. plant. *Materials*, *14*(15), 4091.
4. Muruganandham, M., Tamilselvi, Y., Sivasubramanian, K., Velmurugan, P., Oleyan Al-Otibi, F., Sivakumar, S. (2025). Sustainable dyeing of cotton, silk and leather using natural dye from Bixa orellana seeds: Extraction, optimization and assessment of antibacterial activity. *Frontiers in Chemistry*, 13, 1474160.
5. Mao, D., & Xu, H. (2024). The antimicrobial potential of plant-based natural dyes for textile dyeing: A systematic review using prisma. *AUTEX Research Journal*, *24*(1), 20240016.
6. Santiago, D., Cunha, J., & Cabral, I. (2023). Chromatic and medicinal properties of six natural textile dyes: A review of eucalyptus, weld, madder, annatto, indigo and woad. *Heliyon*, *9*(11).
7. A. K. Samanta and A. Konar, "Dyeing of Textiles with Natural Dyes," in *Dyeing of Textiles with Natural Dyes*, A. K. Samanta and A. Konar, Eds., IntechOpen, 2011.
8. G. Cuoco, C. Mathe, P. Archier, F. Chemat, and C. Vieillescazes, "A Multivariate Study of Ultrasound- Assisted Extraction of Madder Dyes and Characterization by Liquid Chromatography-Photodiode Array Detection," Ultrason Sonochem, vol. 16, no. 3, pp. 75–82, 2009.
9. C. Akyürek, "Anadolu'da Bulunan Rubia tinctorum L.'lardaki Antrakinon Türevi Boyarmaddelerin HPLC ile Kantitatif Olarak Araştırılması," M.S. thesis, Selçuk University, Turkey, 2001.
10. Ozturk, A., (2012). Dyeing of cotton fabric with natural dyes extracted from *Rubia tinctorum* L.: effect of mordant and dye concentration, International Journal of the Physical Sciences, 7(13), 2068–2075.
11. S. Ghandehari, M. H. Tabrizi, P. Ardalan, A. Neamati, and R. Shali, "Green Synthesis of Silver Nanoparticles Using Rubia tinctorum Extract and Evaluation of Anti-Cancer Properties In Vitro," IET Nanobiotechnol., vol. 13, no. 3, pp. 269–74, 2019. doi: 10.1049/iet-nbt.2018.5190.
12. H. Veisi, L. Mohammadi, S. Hemmati, T. Tamoradi, and P. Mohammadi, "In situ immobilized silver nanoparticles on Rubia tinctorum extract coated ultrasmall iron oxide nanoparticles: an efficient nanocatalyst with magnetic recyclability for synthesis of propargylamines by A3 coupling reaction," ACS Omega, vol. 4, no. 9, pp. 13991-14003, 2019, doi: 10.1021/acsomega.9b01720.
13. "Organic Chemistry I," Kwantlen Polytechnic University, 2024. [Online]. Available: https://kpu.pressbooks.pub/organicchemistry/chapter/6-3-ir-spectrum-and-characteristic-absorption-bands/. [Accessed: Mar. 1, 2024].
14. L. Morsch and S. Farmer, "Alcohols and Phenols," in Organic Chemistry, L. Morsch and S. Farmer, Eds., Libre Texts, 2024, pp. 17.11.1-7.
15. R. Yang, Y. Zhang, S. Ranjitkar, M. Li, Y. Guo, X. Yan, et al., "Reusing Waste Root of Rubia wallichiana Dyeing from Monpa of Tibet in China," Sci. Rep., vol. 11, no. 1, pp. 14331, 2021. doi: 10.1038/s41598-021-93839- 1.

16. X. Liu, *Structural Identification of Organic Compounds- IR and NMR Spectroscopy*, in *Kwantlen Polytechnic University Organic Chemistry I*, Libre Texts, 2024, pp. 6.3.1-3.
17. O. Deveoglu, R. Karadag, A. Spinella, E. T. Guzel, "Examination of Dyeing Properties on Silk of Some Flavonoids by Spectroscopic Techniques," *J. Nat. Fibers*, vol. 18, no. 2, pp. 238–49, 2021.
18. A. Pars, *Use of Non-Destructive and Micro Methods and Method Development in the Restoration of Textile Cultural Heritage*, Ph.D. thesis, Marmara Univ., Istanbul, Turkey, 2021.
19. X. Zhang and P. Wyeth, "Using FTIR Spectroscopy to Detect Sericin on Historic Silk," *Sci. China Chem.*, vol. 53, pp. 626–31, 2010.
20. Guo, Y., Zhou, X., Zhang, L., Luo, X., Wu, M., & Zhang, W. (2024). Experimental and theoretical study of madder-associated dyestuffs on silk: Adsorption of kinetics, thermodynamics and molecular docking. *Journal of Natural Fibers*, *21*(1), 2324302.
21. Septhum, C., Rattanaphani, S., Bremner, J. B., & Rattanaphani, V. (2009). An adsorption study of alum-morin dyeing onto silk yarn. *Fibers and Polymers*, *10*, 481-487.
22. Madder Root. Dyeing With Madder Root (Rubia Tinctorum) — Shepherd Textiles. Accessed 10 May 2025.
23. Madder Rubia Cordifolia & RUBIA TINCTORUM https://naturaldyes.ca/madder. Accessed 10 May 2025.
24. M. Ozturk, "Comparative Investigation of the Morphology and Dyeing Properties of Roots of *Rubia peregrina* L. and *Rubia tinctorum* L.", M.S. thesis, Bursa Uludag University, Turkey, 2012.
25. Güzel, E. T., Karadag, R., & Alkan, R. (2020). Durability, antimicrobial activity and HPLC analysis of dyed silk fabrics using madder and gall oak. *Journal of Natural Fibers.*
26. Montgomery, D. C. (2017). Design and Analysis of Experiments (9th ed.). Wiley.
27. Tukey, J. W. (1949). Comparing individual means in the analysis of variance. Biometrics, 5(2), 99–114.
28. Do, K. L., Su, M., Mushtaq, A., Ahsan, T., & Zhao, F. (2023). Functionalization of silk with chitosan and Rubia cordifolia L. dye extract for enhanced antimicrobial and ultraviolet protective properties. *Textile Research Journal*, *93*(15-16), 3777-3789.

Figures

Figure 1

The process of obtaining madder involves three main stages: first, harvesting the plant, followed by washing and drying process and finally, grinding.

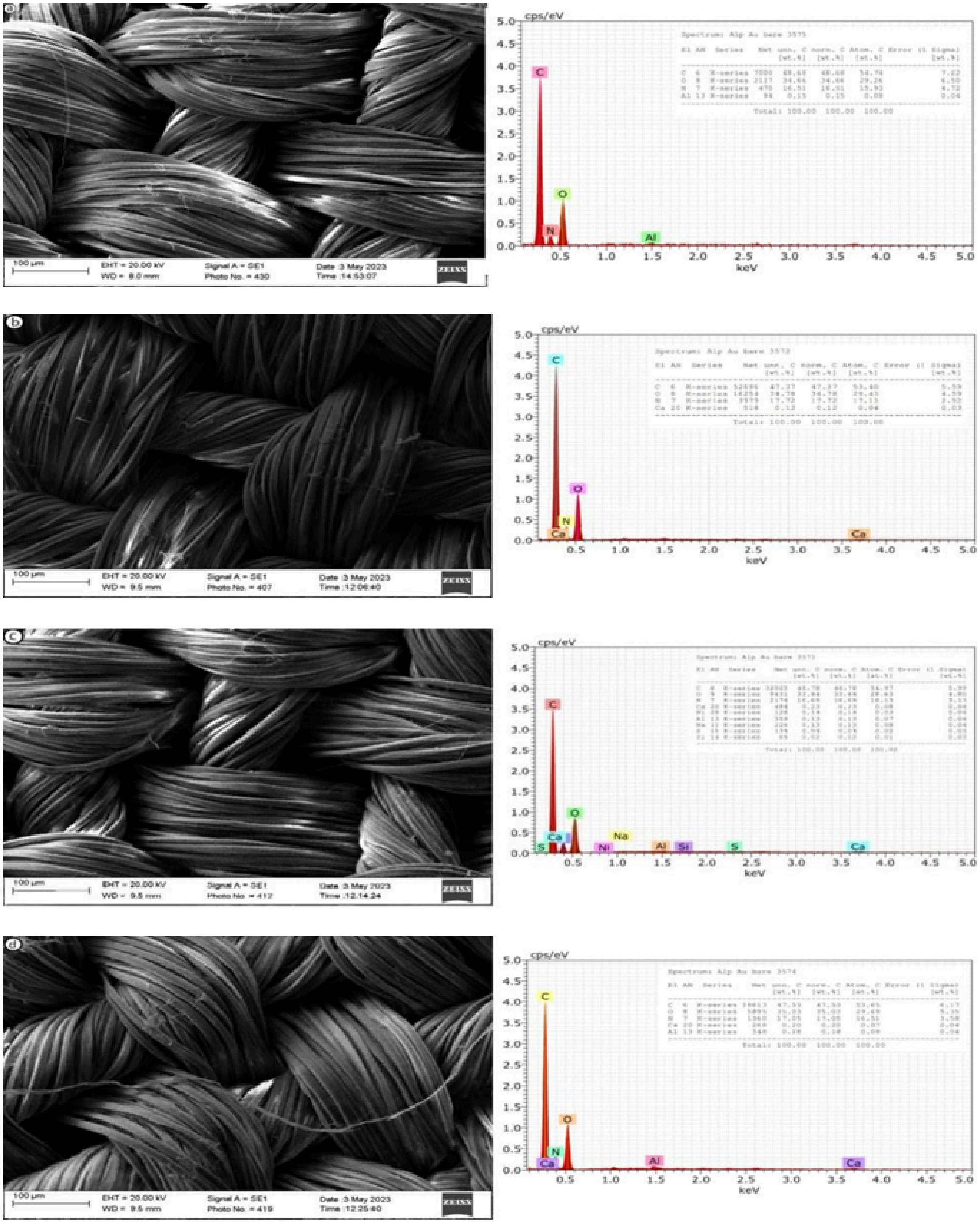

Figure 2

The SEM-EDX Images of fabrics dyed with mader dyes (a: 1 year, b: 2 years c: 3 years, d: commercial).

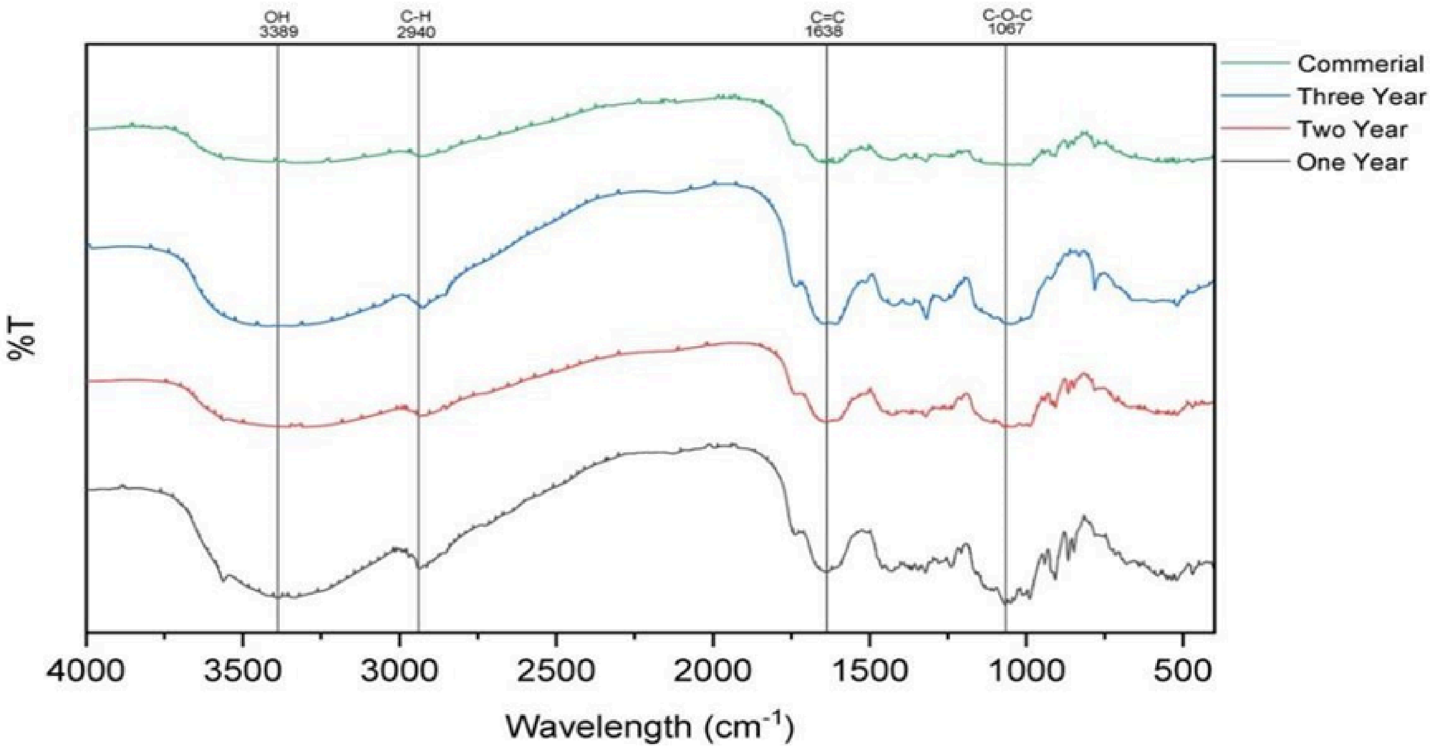

Figure 3

FTIR Analysis of Madder

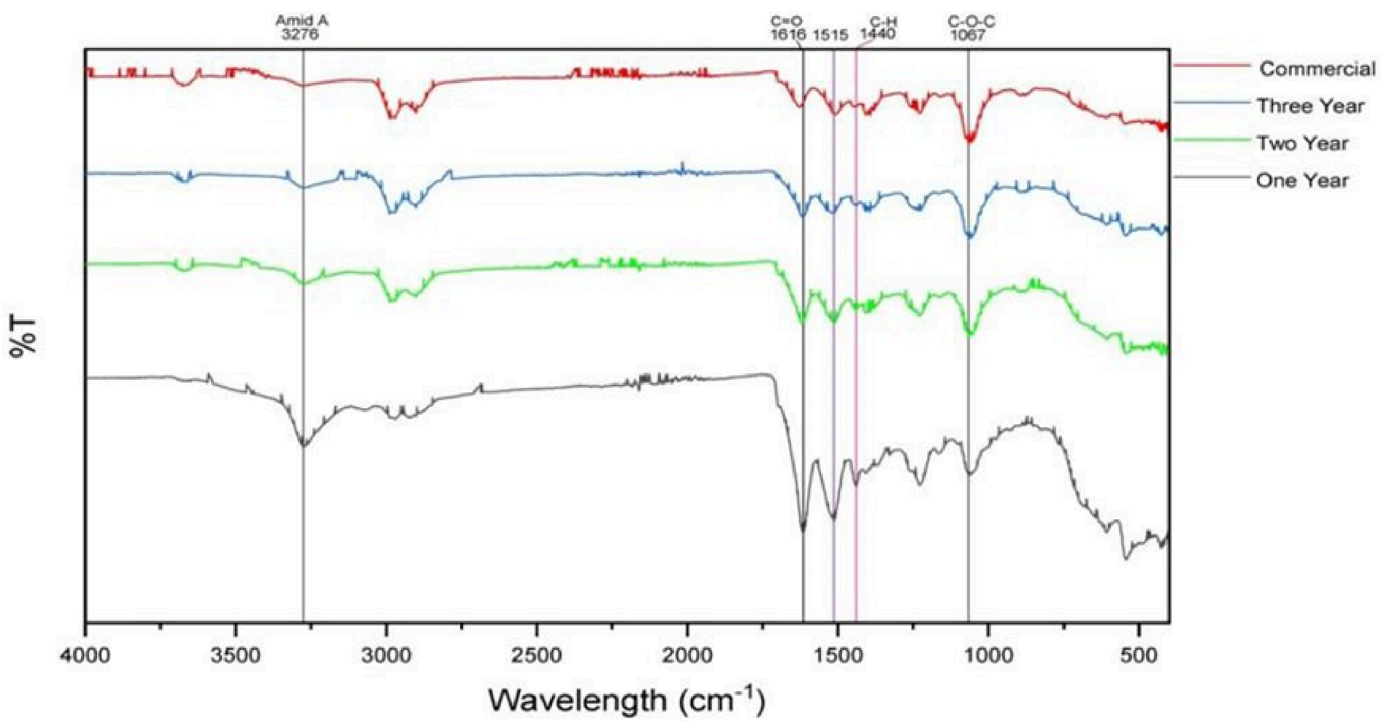

Figure 4

FTIR Analysis of dyed samples

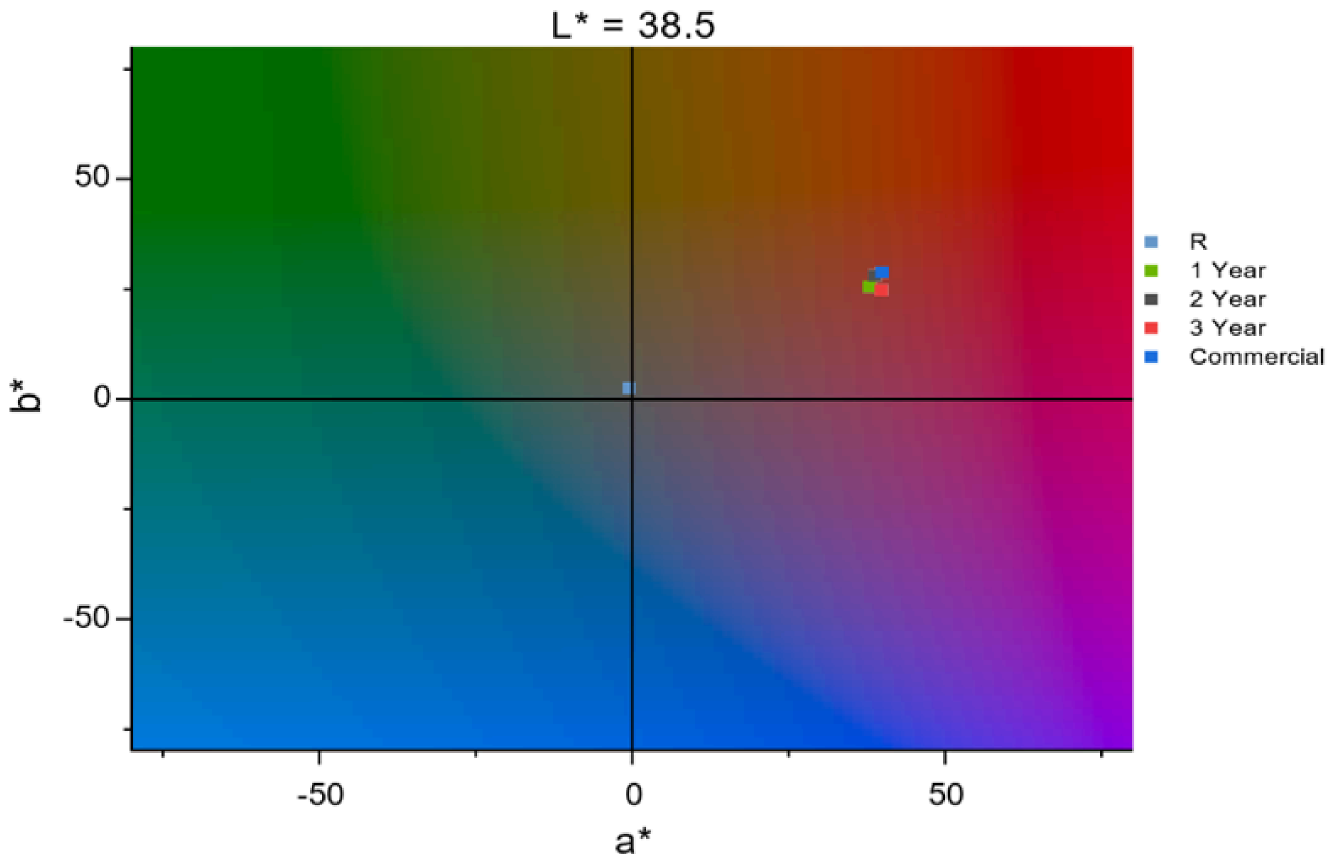

Figure 5

The CIE-LAB color graph was drawn based on the a* and b* color values of the samples.

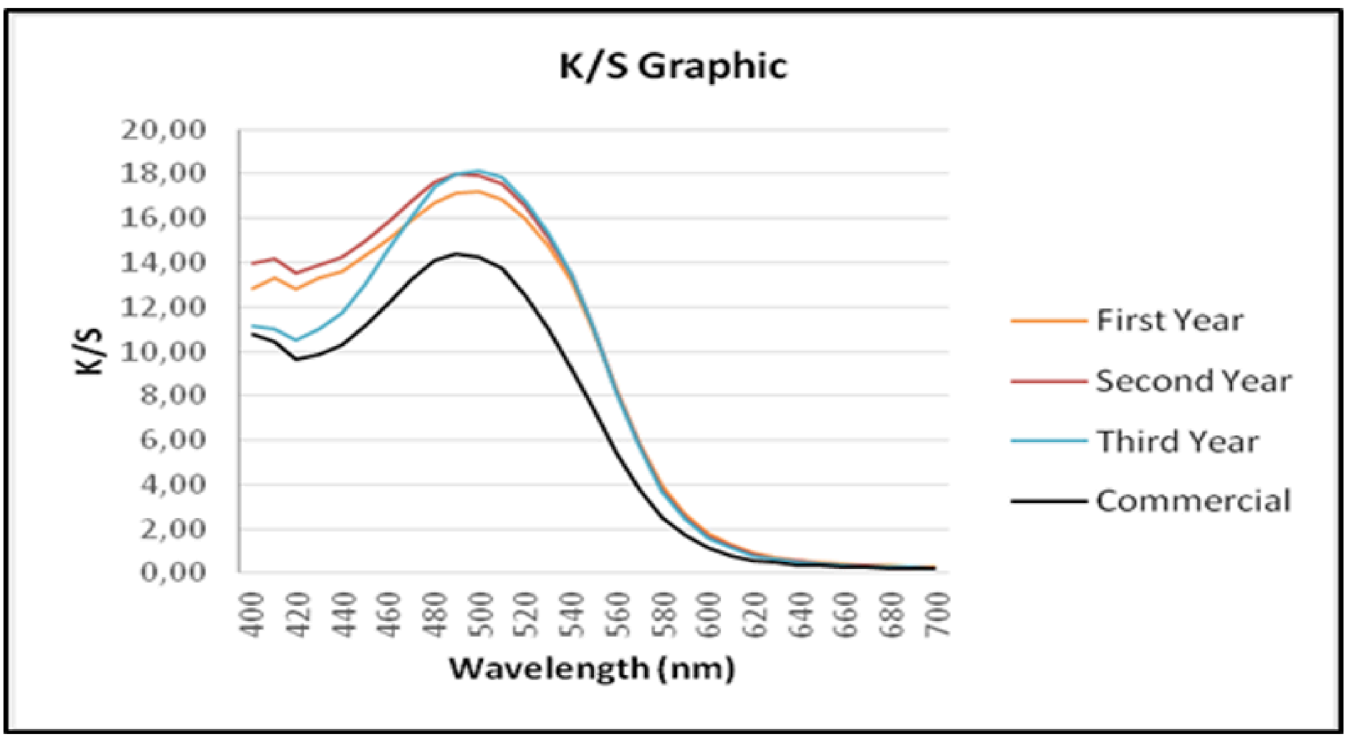

Figure 6

The K/S graph