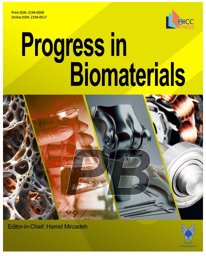

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Eco-Friendly Antibacterial Dyes from *Nerium oleander* and *Bougainvillea spectabilis* for Sustainable Wool Dyeing Applications

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Abstract:

This study evaluates plant-derived antibacterial dyes from *Nerium oleander* and *Bougainvillea spectabilis* for bio-based wool textile applications, with a focus on mordant effects. Wool yarns were pre-treated using various mordants-aluminum sulfate, stannous chloride, iron sulfate, and copper sulfate-and subsequently dyed with flower extracts. The dyeing was performed at different liquor ratios and temperatures, and outcomes were assessed for color stability, wash fastness, rubbing fastness, and antibacterial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Findings show that iron mordants enhanced light fastness for oleander-dyed fibers, while stannous mordant improved performance for bougainvillea-dyed samples. Copper mordants yielded strong antibacterial activity, particularly against *E. coli*, whereas iron mordants produced the largest inhibition zones overall, suggesting both Cu and Fe substantially contribute to antimicrobial efficacy in a dye- and plant-dependent manner. Wash and rubbing fastness remained satisfactory across samples, indicating stable dye-mordant-fiber complexes with minimal leaching and abrasion. Overall, the results support the potential of these natural dyes as sustainable alternatives in textile dyeing, offering functional benefits such as antibacterial properties and improved colorfastness. The study emphasizes the crucial role of mordant selection in optimizing dye performance and durability, contributing to eco-friendly textile processing.

Keywords: Natural dyes; Antibacterial textiles; Mordants; Colorfastness; Wool dyeing; Sustainable textiles

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1. Introduction

The application of natural dyes in textile coloration has a long-standing history, deeply embedded within the cultural, artistic, and economic traditions of many societies. In various cultures, particularly in handicrafts such as carpet weaving, plant-based dyes were valued not only for their aesthetic qualities but also as carriers of historical narratives, local identity, and representations of lifestyle and artistic heritage (Samanta and Agarwal, 2009; Shahid and Mohammad, 2013; Yusuf et al., 2017; Kasenova, 2020; Prabhu and Bhute, 2012; Fried et al., 2022). These dyes were typically extracted from flowers, leaves, roots, and barks, with each shade carrying symbolic meanings and specific uses.

With the advent of synthetic dyes in the twentieth century, natural dyes were largely replaced due to the synthetic counterparts' advantages in terms of shade variety, ease of availability, and low production cost. However, the widespread use of synthetic dyes has raised considerable concerns regarding their environmental and health impacts, as they are often non-biodegradable, toxic, and hazardous to both humans and ecosystems, contributing to soil and water contamination upon disposal (Shahid and Mohammad, 2013; Mani et al., 2018; Jabli et al., 2018). Such challenges have revived global scientific interest in eco-friendly, sustainable, and functional natural dyes for textile industries.

In recent years, researchers have increasingly investigated various plants as sustainable sources of natural dyes (García-Salinas and Ariza, 2019; Shrivastava, 2020). Bechtold et al. (2003) emphasized the integration of traditional knowledge with modern dyeing technologies, discussing issues such as color durability, wash and light fastness, production costs, and the ecological benefits of natural dyes compared to synthetic ones. Similarly, Ali et al. (2009) reported a laboratory-scale study on henna leaf extracts for cotton dyeing, demonstrating that optimized conditions of pH, temperature, and dyeing time can yield both high color strength and improved fastness. This study provided a practical example of substituting synthetic dyes with botanical alternatives.

Another line of research has focused on the chemical characterization of specific dye plants such as *Nerium oleander*. Khiari et al. (2011) investigated *Nerium oleander* and date palm as sources of lignocellulosic materials, showing that oleander fibers contain substantial cellulose and lignin, making them suitable substrates for papermaking and potential carriers of natural dyes. *Nerium oleander* has attracted particular attention not only due to its lignocellulosic composition but also because of its rich phenolic and flavonoid compounds, which provide both dyeing capability and antimicrobial potential. Jabli et al. (2017) compared the physicochemical properties of *Nerium oleander* fibers with *Posidonia oceanica*, analyzing parameters such as hemicellulose and lignin content, crystallinity, and specific surface area. Their results indicated that oleander fibers, with their high cellulose content, are appropriate for textile dyeing and environmental applications such as the adsorption of synthetic dyes from effluents. In a follow-up study, Jabli et al. (2018) analyzed the chemical composition of oleander stems, reporting high dye uptake but relatively poor wash and light fastness, which could be improved by the use of mordants.

More recent studies, including Lakshmi et al. (2023), explored the dyeing performance of oleander flower extracts on cotton using different chemical mordants. Their findings revealed that mordant type significantly influenced color strength, lightfastness, and durability, with metallic mordants such as alum and iron producing superior shade depth and stability against washing, perspiration, and rubbing. Nevertheless, most research on oleander-derived dyes has been limited to cellulosic or woody fibers, while their biological properties-particularly antimicrobial activity—have rarely been investigated.

Alongside oleander, *Bougainvillea spectabilis* has also emerged as a promising natural dye source. Ghoreishian demonstrated that Bougainvillea flower extracts can produce bright and moderately stable shades on textiles, though the influence of mordants and bio-functional properties was not assessed in that study (Ghoreishian, 2013). Subsequent investigations, such as those of Rasool et al. (2023) and Jaisri and Nandhini (2022), showed that bio-mordants like turmeric and henna enhanced color depth and shade variety, and that dyeing parameters including pH, temperature, and extraction time strongly affected dyeing performance. However, these studies primarily focused on process optimization rather than bioactivity.

In parallel, several works have highlighted the bio-functional aspects of these plants. More et al. (2022) evaluated methanolic and aqueous extracts of oleander flowers for antimicrobial and antifungal activity, finding notable effects against Gram-negative bacteria such as *Escherichia coli* and *Salmonella*, attributable to phenolic and flavonoid constituents. However, these assays were performed on crude extracts and did not assess dyed textiles or mordant effects. Likewise, Ravikumar and Thangaraj (2022) reported that *Bougainvillea glabra* flower extracts exhibit strong antibacterial, antioxidant, and anticancer activities when prepared with methanolic, ethanolic, aqueous, or hexane solvents, highlighting the potential of Bougainvillea as a dual-purpose natural dye with bioactive functionality.

Taken together, previous studies clearly indicate that while natural dyes from *Nerium oleander* and *Bougainvillea spectabilis* have been explored for dyeing cotton, silk, and other cellulosic substrates, there is still limited understanding of their application to protein-based fibers such as wool. Moreover, comprehensive studies simultaneously evaluating dyeing performance, fastness properties, and antimicrobial activity remain scarce. The role of mordant type in modulating both color durability and bio-functional performance also requires further exploration under more practical and industrially relevant conditions.

The present study aims to address these gaps by evaluating the dyeing potential of natural extracts from *Nerium oleander* and *Bougainvillea spectabilis* on wool yarns. Four mineral mordants-aluminum sulfate, tin chloride, ferrous sulfate, and copper sulfate-were employed to investigate their influence on color strength, wash and rub fastness, and antimicrobial performance of the dyed textiles. The novelty of this work lies in the integrated assessment of both chromatic and biological properties, offering new insights into the development of multifunctional natural dyes. The findings are expected to provide practical pathways for promoting sustainable textile dyeing, enhancing consumer safety, and preserving cultural heritage through the revival of eco-friendly dyeing practices.

2. Materials and methods

Materials

Woolen yarn (50 denier, 2-ply, twist = 180 turns/m) was sourced from a local wool spinning mill in Qom Province, Iran. Non-ionic surfactant and oxalic acid dihydrate (6153-56-6) were supplied by Merck. The mordants used in this study included aluminum sulfate (10043-01-3), stannous(II) chloride (7772-99-8), Iron(II) sulfate heptahydrate (7782-63-0), and copper(II) sulfate (7758-98-7), all of analytical grade and obtained from Merck. Fresh red flowers of *Nerium oleander* and pink flowers of *Bougainvillea spectabilis* were collected from Qom city, Qom Province, Iran (latitude: 34.6416° N, longitude: 50.8755° E). The collected flowers were air-dried under shade and pulverized into fine powder prior to extraction. For antibacterial testing, Mueller Hinton agar and Mueller Hinton broth were prepared from dehydrated culture media powder (Merck). Two bacterial strains were used as test organisms: *Escherichia* *coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Ciprofloxacin (10 µg/mL) served as the positive control. All chemicals and reagents used in this study were of analytical grade and applied without further purification.

Washing woolen yarn

To eliminate any natural or synthetic contaminants, 420 g of yarn were washed in a bath liquor ratio (L/R = 20). Initially, surfactant (non-ionic, 2 g/L) was introduced into the bath, after which the fibers were submerged for 15 minutes at a regulated temperature ranging from 40 to 50 °C. The fibers were subsequently washed with warm water and dried at room temperature.

Mordanting procedures

To evaluate the impact of mordanting, four distinct mordanting agents were utilized: Aluminum sulfate (10%), stannous(II) chloride (5%), iron(II) sulfate (5%), and copper(II) sulfate (5%). The mordanting process was performed prior to dyeing, and the outcomes were assessed after dyeing. For this experiment, 20 g of pre-washed wool yarns were mixed with 1 g of the mordanting agent (stannous chloride ($SnCl_2$) at 5% o.w.f.) and 0.2 g of oxalic acid at 1% o.w.f., resulting in a total volume of 600 mL (L/R = 30). The mixture was heated to the boiling point over a period of 40 minutes and maintained at that temperature for one hour. Throughout this duration, the yarns were agitated in the solution to enhance the effectiveness of the mordanting. Once the boiling period concluded and the solution cooled, the yarns were rinsed with lukewarm water to eliminate any excess mordanting agent, thereby preventing the formation of complexes between the surplus mordanting and the dye during the dyeing process.mordanting and dyeing steps were performed sequentially at specified condition for defined durations (Table 1).

Extraction of the dye

The collected flowers were air-dried in a shaded environment to preserve their bioactive compounds. To optimize the extraction efficiency, the dried flowers were pulverized into a fine powder. An extract was individually prepared by dispersing 20 g/L of the powdered flowers in water, followed by boiling for 60 minutes. The mixture was then passed through a filtration process, and the filtrate was diluted to reach a final volume of 250 mL.

Dyeing procedure

A total of one gram of wool yarns, including both mordanted and non-mordanted types, was dyed using a liquor ratio of 50:1. Dye concentrations were calculated based on the percentage of dye extract relative to the dry weight of the fiber (o.w.f.). For instance, a 50% o.w.f. concentration corresponds to 0.5 grams of dye extract per 1 gram of wool fiber. Accordingly, for each sample, 0.5 grams of dye extract was accurately weighed and dissolved in deionized water to prepare a final dye bath volume of 50 mL. This approach ensured consistent dye uptake across all samples and allowed for direct comparison of antibacterial performance at different concentration levels (e.g., 50% o.w.f. and 100% o.w.f.). It is important to note that natural dyes generally exhibit lower tinctorial strength compared to synthetic dyes. Therefore, 50% o.w.f. is commonly considered a medium shade concentration, while 100% o.w.f. is used to achieve deeper shades. These levels are standard in natural dyeing studies and provide sufficient color intensity for evaluating dyeing behavior and functional properties. Exceptions to this general trend include high-strength natural dyes such as indigo, madder, and weld, which can produce intense coloration even at lower concentrations. The wool fibers were fully immersed in the dye solution within designated dyeing containers, which were securely sealed to prevent evaporation and contamination. The dyeing process began at 40 °C and was gradually increased to boiling point (approximately 100 °C) over 40 minutes. The temperature was then maintained at boiling for 60 minutes to ensure thorough dye penetration and fixation. Finally, the temperature was reduced to 30 °C over a span of 30 minutes to complete the dyeing cycle. After dyeing, the samples were rinsed thoroughly and dried at room temperature prior to antibacterial testing.

Impact of L/R on the dyeing process (L/R = 30)

The liquor-to-material ratio of the dye bath was modified to allow for a larger amount of wool yarns and to investigate the impact of different L/R ratios on the dyeing process. Consequently, 5 g of wool yarns (including non-indented, iron-dented, and tin-dented samples) were combined with 125 mL of dye solution, and the total volume was adjusted to 150 mL (L/R = 30).

Antibacterial testing

Following the standard protocol established by Merck, 21 g/L of Mueller Hinton powder was dissolved in deionized water and sterilized using an autoclave at 121 °C for 20 minutes. Mueller Hinton agar was used as the culture medium for all antibacterial assays. Two bacterial strains were selected as representative models: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). For each test, two types of controls were included: Negative control: Wells containing sterile deionized water to confirm no inhibition in the absence of active agents. Positive control: Wells containing standard antibiotic (ciprofloxacin, 10 µg/mL) to validate the responsiveness of the bacterial strains.

The antibacterial activity of both dye extracts and dyed wool yarns was evaluated using the well diffusion method. Bacterial suspensions were prepared to match 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and uniformly spread across the agar surface using sterile cotton swabs. Wells of 6 mm diameter were created using sterile pipette tips. For extract testing, 100 µL of dye solution was introduced into each well. For yarn testing, 0.2 g of dyed wool yarn was placed directly into the wells. All plates were incubated at 37 °C for 24 hours. After incubation, the diameter of the inhibition zones (in mm) was measured using a digital caliper.

Statistical analysis

All experimental data were analyzed using standard statistical methods to ensure the reliability and significance of the

Table 1. Summary of experimental conditions for reproducibility.

Experimental section	Parameter	Conditions and specifications
Yarn Specifications	Material	Wool
	Yarn	Count 50 denier
	Ply	2-ply
	Twist	180 turns per meter
Pre-Treatment (Scouring)	Liquor Ratio (L/R)	20:1
	Detergent	Non-ionic surfactant (2 g/L)
	Temperature	40 – 50 °C
	Time	15 minutes
	Post-Wash	Rinsed with warm water and air-dried
Mordanting	Method	Pre-Mordanting
	Liquor Ratio (L/R)	30:1
	Mordant Type & Concentration (% o.w.f.)	Alum (10%), Stannous Chloride (5%), Iron Sulfate (5%), Copper Sulfate (5%)
	Additive	Oxalic Acid (1% o.w.f.)
	Temperature Program	Raised from room temperature to boil (100 °C) over 40 min
	Hold Time at Boil	60 minutes
	Final Step	Cooled naturally and rinsed with lukewarm water
Dye Extraction	Raw Material	Shade-dried flowers of *N. oleander* and *B. spectabilis*
	Form	Fine Powder
	Concentration	20 g/L
	Solvent	Water
	Time & Temperature	60 minutes at boiling
	Final Preparation	Filtered; the filtrate was used as the dye bath
Dyeing	Liquor Ratio (L/R)	50:1 (Standard) / 30:1 (for L/R impact study)
	Dye Concentration (% o.w.f.)	50% and 100%
	Starting Temperature	40 °C
	Temperature Program	Raised from 40 °C to boil (100 °C) over 40 minutes
	Fixation Time at Boil	60 minutes
	Cooling Phase	Cooled from boil to 30 °C over 30 minutes
	Post-Dyeing	Thoroughly rinsed and air-dried
Antibacterial Test	Test Method	Well Diffusion Assay
	Culture medium	Mueller Hinton Agar
	Bacterial Strains	*E. coli* (Gram-negative) and *S. aureus* (Gram-positive)
	Inoculum Density	0.5 McFarland Standard (approx. 1.5×10^8 CFU/mL)
	Sample	Dyed yarn (0.2 g)
	Incubation Conditions	24 hours at 37 °C
	Positive Control	Ciprofloxacin (10 µg/mL)
	Measurement	Inhibition Zone Diameter (mm)
Fastness Tests	Light Fastness	ISIRI 4084, Xenon arc lamp, 100 hours
	Wash Fastness	ISIRI 10076-Method A, 60 °C, 30 minutes
	Rubbing Fastness	ISIRI 204, 9 N force, Dry and Wet conditions

results. Quantitative values obtained from antibacterial assays (inhibition zone diameters) were expressed as mean ± standard deviation (SD) based on triplicate measurements. To compare the antibacterial effectiveness across different dye concentrations, mordant types, and plant sources, one-way analysis of variance (ANOVA) was performed. This allowed for the identification of statistically significant differences among treatment groups. Where ANOVA indicated significance ($p < 0.05$), Tukey's post-hoc test was applied to determine pairwise differences between specific groups. Additionally, independent sample t-tests were used to compare the antibacterial activity between Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) strains under identical dyeing conditions. All statistical analyses were conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA), and a significance level of $p < 0.05$ was considered statistically meaningful.

Testing for light fastness, wash fastness, and rubbing fastness

Assessment of light fastness

The evaluation of light fastness for samples dyed with paper flower and oleander flower (both with and without iron and tin mordants) was conducted in accordance with Iranian National Standard No. 4084, utilizing a xenon arc dyeing lamp. Six blue wool standards were placed alongside the samples within the color fastness testing apparatus, ensuring that one portion of the yarns was exposed to light while another portion remained shielded. The samples were exposed to light for 100 hours, and the resulting color difference between the irradiated and non-irradiated areas was measured using the blue wool standard.

Assessment of washing fastness

The color fastness to washing for each dyed item was evaluated using the Iranian National Standard Method No. 10076. A pressurized rotadyer, located at the Color Research Institute, served as the washing machine. Each dyed item underwent a washing process at 60 °C for 30 minutes, accompanied by 25 ceramic balls in a solution containing 1 g/L sodium perborate, 4 g/L detergent. A washing solution containing 1 g/L sodium carbonate was prepared with a liquor-to-fabric ratio of 50:1. The solution exhibited a pH close to 10.5. The yarns subjected to each washing cycle included acetate, cotton, nylon, polyester, acrylic, and wool. Following the washing process, the yarns were rinsed, dried, and assessed using the gray scale to determine color change and staining effects.

Measurement of rubbing fastness

The assessment of color fastness to abrasion for each dyed fiber was conducted by Iranian National Standard No. 204. In this procedure, the dyed yarns were positioned in a rubbing fastness testing apparatus and subjected to abrasion with a cotton cloth that was affixed to a weight of 9 N. The rubbing action was performed ten times (back and forth) over a distance of 10 cm. Rubbing fastness was evaluated in two conditions: Dry and wet, utilizing the gray scale for assessment.

All experimental conditions are detailed in Table 1.

3. Results and discussion

Effect of Mordant on dyeing process

The dyeing characteristics of textiles treated with natural dyes are influenced by the mordant used. The variation in color and enhancement of colorfastness for numerous natural dyes on textiles is attributed to the mordant. In several instances, the specific type of mordant, the chemicals employed during the mordanting process, and the technique of mordanting impact the hue and quality of the dyed textile. Furthermore, it has been noted that the mordant plays a crucial role in the context of natural dyes in the process of natural dyeing (Adeel et al., 2022). Wool yarns were mordanted with copper sulfate (5% o.w.f.), aluminum sulfate (10% o.w.f.), iron sulfate (5% o.w.f.), and stannous chloride (5% o.w.f.) using three distinct application methods: Pre-mordanting, meta-mordanting (simultaneous), and post-mordanting. The findings revealed that the iron mordant offered the greatest light stability for the dye derived from oleander flowers, whereas the stannous mordant exhibited superior performance for the dye from Bougainvillea flowers (Fig. 1). In general, the color fastness of natural dyes largely depends on the formation of chelate bonds within the dye–mordant–fiber system (Pervaiz et al., 2016; Gedik et al., 2013).

In earlier studies, lemon peel and cypress leaves have been utilized as herbal dyes in the dyeing process of Lawson cypress (Kilinc et al., 2015), while sorrel leaves have been employed in the dyeing of barberry (Haji, 2010). Furthermore, iron-based chemical dyes have been applied in the dyeing of daphne (Menekşe, 2020), and alum, along with potassium bichromate, have been used in the dyeing of henna and walnut shells (Mirjalili and Abbasipour, 2013). Although colorimetric analysis (including K/S values and CIELAB parameters) would provide deeper insight into dyeing performance and color quality, these measurements were not conducted due to lack of access to specialized equipment and dyed samples. This limitation has been acknowledged, and future studies are recommended to include exhaustive colorimetric evaluation.

Antibacterial activity

The antibacterial activity of wool yarns dyed with plant-derived dyes from *Nerium oleander* (oleander) and *Bougainvillea spectabilis* (Bougainvillea) was evaluated against two representative bacteria: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Inhibition zones were measured in millimeters (mm) and reported as mean ± standard deviation (SD) across replicates ($n = 3$) (Table 2). Across both plant sources, increasing the dye loading from 50% to 100% o.w.f. (weight of dye extract per weight of wool) generally enhanced antibacterial effects for the same mordant condition, indicating a dose-dependent contribution of plant phytochemicals to activity. This aligns with broader observations that higher concentrations of natural dyes increase bioactive compound delivery and antimicrobial outcome (Rasool et al., 2023; Saleh et al., 2018) *in vitro*. Oleander flowers are known to

Figure 1. Fiber colors with different mordants by L/R = 30. A: Oleander flower, B: Bougainvillea flower, 1: No mordant, 2: Aluminum mordant, 3: Iron mordant, 4: Copper mordant, 5: Tin mordant.

Table 2. The antibacterial activity of wool dyed with plant-derived dyes from *Nerium oleander* (oleander) and *Bougainvillea spectabilis* (Bougainvillea) extracts. Dyes were prepared at two concentrations, 50 and 100. Mordants used were None, Alum (10% o.w.f.), Iron (5% o.w.f.), Copper (5% o.w.f.), and Tin (5% o.w.f.). Inhibition zones were measured in millimeters (mm) against *Escherichia coli* and *Staphylococcus aureus*. Values are mean ± standard deviation (n = 3 replicates).

Dye (%o.w.f.)		Mordant type	Inhibition Zone (mm)	
Concentration	plant		*E. coli*	*S. aureus*
50	Oleander	None	2.1 ± 0.16	1.4 ± 0.13
		Alum (10% o.w.f.)	3.1 ± 0.18	2.8 ± 0.17
		Iron (5% o.w.f.)	16.6 ± 1.36	10.3 ± 1.03
		Copper (5% o.w.f.)	11.2 ± 1.36	9.8 ± 1.05
		Tin (5% o.w.f.)	2.5 ± 0.36	1.7 ± 0.63
	Bougainvillea	None	1.8 ± 0.25	1.5 ± 0.31
		Alum (10% o.w.f.)	2.7 ± 0.46	2.1 ± 0.25
		Iron (5% o.w.f.)	10.6 ± 1.25	8.9 ± 0.96
		Copper (5% o.w.f.)	9.6 ± 0.82	6.8 ± 0.59
		Tin (5% o.w.f.)	1.8 ± 0.26	1.2 ± 0.14
100	Oleander	None	3.8 ± 0.45	2.7 ± 0.16
		Alum (10% o.w.f.)	4.2 ± 0.39	3.6 ± 0.28
		Iron (5% o.w.f.)	17.5 ± 0.45	13.2 ± 0.86
		Copper (5% o.w.f.)	14.5 ± 0.95	11.2 ± 0.49
		Tin (5% o.w.f.)	4 ± 0.22	2.1 ± 0.38
	Bougainvillea	None	2.9 ± 0.11	1.8 ± 0.15
		Alum (10% o.w.f.)	3.5 ± 0.11	2.3 ± 0.18
		Iron (5% o.w.f.)	14.5 ± 0.82	13 ± 0.2
		Copper (5% o.w.f.)	12.3 ± 0.66	10.6 ± 0.16
		Tin (5% o.w.f.)	2.9 ± 0.06	1.7 ± 0.14

contain high levels of alkaloids, saponins, flavonoids, phenols, and tannins, which contribute to their antimicrobial activity by disrupting bacterial membranes and inducing oxidative stress (Saleh et al., 2018). Similarly, Bougainvillea flowers possess phenolic compounds and alkaloids with proven antibacterial potential (Sehrawat and Soni, 2019; Abarca-Vargas and Petricevich, 2018). The results show that increasing the dye concentration from 50% to 100% owf significantly enhances antibacterial efficacy, suggesting a dose-dependent mechanism. This is consistent with previous findings where higher concentrations of natural dyes led to increased bioactivity due to greater availability of active phytochemicals (Rasool et al., 2023). The differential inhibition observed between *Escherichia coli* and *Staphylococcus aureus* can be attributed to their structural differences. Gram-negative bacteria like *E. coli* possess an outer membrane rich in lipopolysaccharides, which can interact with metal ions and phenolic compounds, increasing membrane permeability and susceptibility to damage. In contrast, Gram-positive bacteria such as *S. aureus* have a thicker peptidoglycan layer but lack an outer membrane, making them relatively more resistant to certain dye-mordant complexes (Mirnezhad et al., 2016; Silhavy et al., 2010). Among the mordants tested, copper and iron significantly enhanced antibacterial activity. Copper ions are known to generate reactive oxygen species (ROS), disrupt membrane integrity, and interfere with essential enzymatic processes (Noyce et al., 2006; Lemire et al., 2013). Iron mordants may also catalyze oxidative reactions that damage bacterial DNA and proteins. In contrast, alum and tin showed limited enhancement, possibly due to weaker chelation with dye molecules or lower ion release under experimental conditions. These findings support the potential of oleander and Bougainvillea-based dyes, especially when combined with iron or copper mordants, as eco-friendly alternatives for antibacterial textile applications.

To evaluate the durability of antibacterial finishing, dyed samples were subjected to repeated laundering cycles. After 20 wash cycles, the antibacterial activity remained relatively stable, indicating acceptable durability of the treatment under practical conditions.

Assessment of the color fastness of dyed fibers

The extent to which the dye penetrates the fibers and its stability are closely linked to the fastness characteristics of the fibers, which often depend on the type of mordant employed and the complexes that form between the dye and the mordant. To assess the light fastness of the samples, a blue scale is utilized, which ranges from 1 to 8; with 1 indicating the lowest light fastness and 8 indicating the highest. Additionally, wash fastness is evaluated using a gray scale, where the numbers on this scale range from 1 to 5; with 1 denoting the weakest wash fastness and 5 denoting the best. These standardized grading methods facilitate an objective and precise comparison of the quality of color fastness in fibers.

Light fastness

The Light fastness of dyed fibers is influenced by various factors, including the type of fiber, the mordant used, the chemical structure of the dye, and the concentration of the dye. As indicated by the findings in Table 3, samples dyed with oleander exhibited greater Light fastness in comparison to those dyed with oleander. The fibers dyed with oleander and tin mordant demonstrated the highest Light stability,

Table 3. The fastness of dyed samples using Oleander and Bougainvillea flowers, and mordanted with Sn and Fe, in relation to light, washing, and rubbing.

Sample	Light fastness (Iranian National Standard No. 4084)	Washing fastness (Iranian National Standard No. 10076) Method A	Rubbing fastness (Iranian National Standard No. 304)	
Bogainvillea flower dye mordant by Sn	4	Color Change:3-4 Staining: Acetate(5) Cotton(5) Nylon(5) Polyester(5) Acrylic(5) Wool(5)	Dry: 5	Wet: 4-5
Bogainvillea flower dye mordant by Fe	3-4	Color Change: 4 Staining: Acetate(5) Cotton(5) Nylon(5) Polyester(5) Acrylic(5) Wool(5)	Dry: 4	Wet: 4
Bogainvillea flower dye without mordant	3-4	Color Change:3-4 Staining: Acetate(5) Cotton(5) Nylon(5) Polyester(5) Acrylic(5) Wool(5)	Dry: 4	Wet: 4-5
Oleander flower dye mordant by Sn	2	Color Change:3 Staining: Acetate(5) Cotton(5) Nylon(5) Polyester(5) Acrylic(5) Wool(5)	Dry: 4	Wet: 3-4
Oleander flower dye mordant by Fe	3	Color Change:2-3 Staining: Acetate(5) Cotton(5) Nylon(5) Polyester(5) Acrylic(5) Wool(5)	Dry: 4	Wet: 3-4
Oleander flower dye without mordant	3	Color Change:4 Stain: Acetate(5) Cotton(5) Nylon(5) Polyester(5) Acrylic(5) Wool(5)	Dry: 4	Wet: 4-5

Washing and rubbing fastnesses were evaluated according to the standard with a gray scale. (Number 5: Highest fastness, number 1: Lowest fastness)
Light fastnesses were evaluated according to the standard with a blue scale. (Number 8: Highest fastness, number 1: Lowest fastness)

whereas the lowest stability was observed in oleander dyed with the same mordant.

This variation can be attributed to the differing chemical structures of the two plants. Oleander is rich in tannin compounds that possess hydroxyl groups, enabling them to form stable complexes with metal mordants, such as tin. These complexes enhance Light stability by reinforcing the bond between the dye and the fiber. Conversely, oleander, although it contains tannins, has a lower concentration of these compounds, resulting in the formation of weaker complexes and consequently lower Light stability. Overall, polyphenols, including tannins, are crucial in enhancing light stability by establishing conjugated bonds and fortifying the dye structure. Additionally, the choice of an appropriate mordant, such as tin, serves as a supplementary factor in augmenting the dye's durability by enhancing the stability of the dye complexes.

Washing and staining fastness

Based on the data in Table 3, all mordant-dyed samples exhibited excellent stain fastness, with no color transfer to nearby fibers observed. However, fibers dyed with oleander and iron mordant showed a significantly greater color change compared to other samples. Overall, the wash fastness of all samples was satisfactory, likely due to the formation of stable complexes between the dye and mordant (or the fibers). These complexes help prevent dye leaching during washing by establishing strong chemical bonds (Eshaghloo-Galoogahi, 2015).

Rubbing fastness

According to Table 3, the dyed samples exhibited superior dry rubbing fastness compared to their performance in wet conditions. The reduction in rubbing fastness observed in wet environments can be attributed to the weak physical bonds formed by the deposited dyes on the fiber surface. These dyes tend to detach and transfer to the surface of the sample when they come into contact with other fibers, particularly in wet conditions. The fibers dyed with paper flower and tin dentin demonstrated the highest dry rubbing fastness, while the lowest was noted for those dyed with oleander flower and tin mordant. This variation can be explained by the fact that paper flower establishes a more robust bond between the dye and the fiber through the formation of a stable complex with tin mordant. In wet conditions, water acts as a lubricant, promoting the detachment of surface dyes that rely on non-covalent bonds. These observations align with broader research regarding the influence of dye solubility and the role of water in diminishing rubbing fastness.

4. Conclusion

This study explored the dyeing of wool yarns using natural dyes extracted from *Nerium oleander* and *Bougainvillea spectabilis* flowers, with emphasis on the role of different mordants-aluminum sulfate, stannous chloride, iron sulfate, and copper sulfate-in influencing dyeing performance, color fastness, and antibacterial properties. Quantitative analysis revealed that iron sulfate mordant yielded the highest light fastness for *Nerium oleander*-dyed samples (rating 5-6), while stannous chloride provided superior light fastness for *Bougainvillea spectabilis* (rating 6). Wash fastness across all mordanted samples was rated 4-5 (ISO 105-C06), indicating minimal staining and good durability. Dry rubbing fastness was consistently high (rating 4-5), whereas wet rubbing fastness showed moderate reduction (rating 3-4), likely due to partial dye solubility and weaker physical bonding. Both copper and iron mordants enhanced antibacterial activity, with iron generally producing larger inhibition zones than copper for most dye sources and concentrations, indicating that Fe (up to 17.5 mm) and Cu (up to 14.5 mm) contributions to antimicrobial effects are dye- and plant-dependent. The antibacterial effect remained relatively stable even after 20 laundering cycles, confirming the durability of the functional finish. Although colorimetric data (K/S, L, a, b, C, h^0) and MIC/MBC values were not assessed due to sample and equipment limitations, the study provides strong evidence that natural dyes from *Nerium oleander* and *Bougainvillea spectabilis*, in combination with environmentally acceptable mordants, offer promising potential for sustainable textile coloration and functional applications in hygienic or medical fabrics.

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Authors contributions

Mohsen Arsalaninia: Writing–original draft. Mahboubeh Sadat Sharif & Maryam Khoshsokhan-Mozaffar: Writing–original draft, visualization, supervision, software, project administration, methodology, investigation, formal analysis, data curation. Amir Mansour Arbabi: Methodology, investigation.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abarca-Vargas R., Petricevich V. L. (2018) *Bougainvillea genus*: A review on phytochemistry, pharmacology, and toxicology.

Adeel S., Kiran S., Shahid M., Habib S. R., Habib N., Hussaan M. (2022) Ecofriendly application of coconut coir (*Cocos nucifera*) extract for silk dyeing. *Environ Sci Pollut Res* 29:564–572. DOI: https://doi.org/10.1007/s11356-021-15669-6.

Ali S., Hussain T., Nawaz R. (2009) Optimization of alkaline extraction of natural dye from henna leaves and its dyeing on cotton by exhaust method. *Journal of Cleaner Production* 17 (1): 61–66.

Bechtold T., Turcanu A., Ganglberger E., Geissler S. (2003) Natural dyes in modern textile dyehouses – how to combine experiences of two centuries to meet the demands of the future? *Journal of Cleaner Production* 11 (5): 499–509.

Eshaghloo-Galoogahi S. (2015) Study on the dyeing and antimicrobial properties of wool fibers dyed with hawthorn fruit natural dye [Master's thesis]. Tabriz Islamic Art University

Fried R., Oprea I., Fleck K., Rudroff F. (2022) Biogenic colourants in the textile industry-a promising and sustainable alternative to synthetic dyes. *Green Chem* 24:13–35. DOI: https://doi.org/10.1039/d1gc02968a.

García-Salinas M. J., Ariza M. J. (2019) Optimizing a simple natural dye production method for dye-sensitized solar cells: Examples for betalain (Bougainvillea and beetroot extracts) and anthocyanin dyes. *Applied Sciences* 9 (12): 2515. DOI: https://doi.org/10.3390/app9122515.

Gedik G., Yavas A., Avinc O., Simsek O. (2013) Cationized natural dyeing of cotton fabrics with corn poppy (Papaver rhoeas) and investigation of antibacterial activity. *Asian J Chem* 25:8475–8481. DOI: https://doi.org/10.14233/ajchem.2013.14793.

Ghoreishian S. M. (2013) Preliminary study on dyeing of textiles using Bougainvillea spectabilis flower extract. *Journal of Natural Fibers* 10 (2): 135–144.

Haji A. A. D. (2010) Functional dyeing of wool with natural dye extracted from *Berberis vulgaris* wood and *Rumex hymenosepolus* root as biomordant. *Iran J Chem Chem Eng* 29 (3): 55–60.

Jabli M., Khiari R., Mauret E. (2017) Lignocellulosic fibers from *Nerium oleander* and *Posidonia oceanica*: Physicochemical characterization and potential applications. *Comptes Rendus Chimie* 20 (5): 485–493.

Jabli M., Tka N., Khiari R., Saleh T. A. (2018) Physicochemical characteristics and dyeing properties of lignin–cellulosic fibers derived from *Nerium oleander*. *Journal of Molecular Liquids* 249:1138–1144.

Jaisri R., Nandhini V. (2022) Optimization of natural dyeing of cotton using *Bougainvillea* flowers with henna as bio-mordant. *Journal of Natural Fibers* 19 (5): 845–857.

Kasenova K. B. (2020) Tracing cultural ties of carpet weaving in Kazakhstan and Central Asia. *Opción* 36 (26): 112–129.

Khiari R., Mhenni F., Belgacem M. N., Mauret E. (2011) Chemical composition and pulping of date palm rachis and *Nerium oleander*. *Industrial Crops and Products* 34 (3): 1573–1582.

Kilinc M., Canbolat S., Merdan N., Dayioglu H., Akin F. (2015) Investigation of the color, fastness and antimicrobial properties of wool fabrics dyed with the natural dye extracted from the cone of *Chamaecyparis lawsoniana*. *Procedia Soc Behav Sci* 195:2152–2159.

Lakshmi A. P., Murugan K., Vijayan C., Parvathy A. P. (2023) Natural dye from *Nerium oleander* L. and dyeing of cotton fabrics using different mordants with evaluation of colour fastness, strength and antifungal properties. *Int J Curr Sci Res Rev* 6 (5): 2748–2756. DOI: https://doi.org/10.47191/ijcsrr/V6-i5-06.

Lemire J. A., Harrison J. J., Turner R. J. (2013) Antimicrobial activity of metals: Mechanisms, molecular targets and applications. *Nature Reviews Microbiology* 11:371–384. DOI: https://doi.org/10.1038/nrmicro3028.

Mani S., Chowdhary P., Bharagava R. N. (2018) Textile wastewater dyes: Toxicity profile and treatment approaches. In: Emerging and eco-friendly approaches for waste management. Springer:219–244. DOI: https://doi.org/10.1007/978-981-10-8669-4"`11.

Menekşe S. T. (2020) Antibacterial activity of cotton, wool and silk fabrics dyed with *Daphne sericea* Vahl collected from Antalya. *NEScience* 5 (1): 30–36. DOI: https://doi.org/10.28978/nesciences.691689.

Mirjalili M., Abbasipour M. (2013) Comparison between antibacterial activity of some natural dyes and silver nanoparticles. *J Nanostruct Chem* 3:37. DOI: https://doi.org/10.1186/2193-8865-3-37.

Mirnezhad S., Safapour S., Sadeghi-Kiakhani M. (2016) Review on the properties and application of cochineal natural colorant in different industries. *J Stud Color World* 5 (4): 33–44.

More R., Patil S., Jadhav K. (2022) Evaluation of antimicrobial activity of natural dyes extracted from *Nerium oleander*. *Journal of Natural Products* 15 (3): 210–218.

Noyce J., Michels H. T., Keevil C. W. (2006) Potential use of copper surfaces to reduce survival of meticillin-resistant Staphylococcus aureus in the healthcare environment. *J Hosp Infect* 63:289–297. DOI: https://doi.org/10.1016/j.jhin.2005.12.008.

Pervaiz S., Mughal T. A., Najeebullah M., Khan F. Z. (2016) Extraction of natural dye from Rosa damascena Miller: A cost-effective approach for leather industry. *Int J Biosci* 8 (6): 83–92. DOI: https://doi.org/10.12692/ijb/8.6.83-92.

Prabhu K. H., Bhute A. S. (2012) Plant-based natural dyes and mordants: A review. *J Nat Prod Plant Resour* 2 (6): 649–664.

Rasool W., Adeel S., Batool F., Ahmad S. A., Mumtaz S., Hussaan M. (2023) Environmental friendly silk and cotton dyeing using natural colorant of *Bougainvillea glabra* flowers. *Environ Sci Pollut Res* 30 (8) DOI: https://doi.org/10.1007/s11356-022-23417-7.

Ravikumar P., Thangaraj P. (2022) Exploring the therapeutic properties of Bougainvillea glabra: A study of its antimicrobial and antioxidant effects in methanol extract. *Chin J Otorhinolaryngol Head Neck Surg* 26:399–406.

Saleh T. A., Jabli M., Tka N., Ramzi K. (2018) Physicochemical characteristics and dyeing properties of lignin-cellulosic fibers derived from *Nerium oleander*. *Journal of Molecular Liquids* 249:1138–1144.

Samanta A. K., Agarwal P. (2009) Application of natural dyes on textiles. *Indian J Fibre Textile Res* 34 (4): 384–399.

Sehrawat S., Soni H. (2019) Phytopharmacology profile of *Bougainvillea glabra*: An overview. *Br J Med Health Res* 6 (5)

Shahid M., Mohammad F. (2013) Perspectives for natural product based agents derived from industrial plants in textile applications – a review. *Journal of Cleaner Production* 57:2–18.

Shrivastava S. (2020) Sustainable approaches to rejuvenate the handloom and handicraft in India. *J Text Eng Fashion Technol* 6 (6): 240–248. DOI: https://doi.org/10.15406/jteft.2020.06.00257.

Silhavy T. J., Kahne D., Walker S. (2010) The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology* 2 (5): a000414. DOI: https://doi.org/10.1101/cshperspect.a000414.

Yusuf M., Shabbir M., Mohammad F. (2017) Natural colorants: Historical, processing and sustainable prospects. *Nat Prod Bioprospect* 7 (1): 123–145.