

Green Dyeing: Harnessing Flower Pigments from *Lagerstroemia speciosa* (L.) Pers., *Nerium oleander* L., and *Tagetes erecta* L. for Sustainable Textile Applications

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Abstract

This study explores the potential of *Lagerstroemia speciosa*, *Tagetes erecta*, and *Nerium oleander* as sustainable natural dyes with dual functionality—providing vibrant coloration and antimicrobial properties. Through phytochemical analysis and antimicrobial testing, *L. speciosa* demonstrated superior antibacterial activity (particularly against *Escherichia coli* and *Enterococcus faecalis*) and excellent textile dyeing performance, attributed to its high flavonoid and phenolic content. *T. erecta* exhibited strong potential for candle applications due to thermally stable carotenoid pigments, while *N. oleander* showed limited efficacy. Environmental assessments revealed that these plant-based dyes significantly reduce chemical oxygen demand (89–92%) compared to synthetic alternatives while remaining fully biodegradable. The antimicrobial properties of *L. speciosa* suggest promising applications in medical textiles and infection-resistant fabrics. This research validates the use of these floral species as eco-friendly alternatives to synthetic dyes, supporting sustainable manufacturing and circular bioeconomy principles. Future studies should focus on optimizing extraction techniques and scaling production for industrial applications.

Keywords: Natural Dyes; Antimicrobial Textiles; Plant Pigments; Eco-Friendly Coloration; Circular Bioeconomy

1. Introduction

India is recognized as one of the world's most biodiverse nations, ranking 11th globally in plant biodiversity, with approximately 490,000 plant species [1]. This vast botanical wealth serves as a treasure trove of natural products, including dyes that have been used since ancient times. The art of dyeing predates written records, with early techniques involving the rubbing of crushed plant pigments into fabrics [2]. Over time, dye extraction methods evolved, leading to sophisticated applications in textiles, art, and crafts. However, the advent of synthetic dyes in the 19th century led to a decline in natural dye usage due to their cost-effectiveness and vibrant color range [3].

Recently, there has been a global resurgence in the demand for natural dyes, driven by increasing environmental awareness and health concerns associated with synthetic dyes. Synthetic dyes often contain carcinogenic and allergenic compounds, posing risks to human health and contributing to environmental pollution through toxic wastewater discharge [4]. In contrast, natural dyes derived from plants are biodegradable, non-toxic, and eco-friendly, making them a sustainable alternative [5]. Natural dyes may have a wide range of shades and can be obtained from various parts of the plant, including roots, bark, leaves, flowers, and fruits [6]. Among these, floral dyes are particularly valued for their vibrant hues and potential medicinal properties. In India, over 500 plant species are known to produce natural dyes, yet many remain underutilized [7]. The revival of natural dye applications extends beyond textiles to industries such as cosmetics, food, pharmaceuticals, and leather, emphasizing their economic and ecological benefits [8].

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The present study focuses on extracting natural dyes from three flowering species—*Lagerstroemia speciosa* (Pride of India), *Nerium oleander* (Oleander), and *Tagetes erecta* (Marigold)—selected for their attractive pigmentation, medicinal properties, and eco-friendly dyeing potential. The primary objectives of this research are: (1) to evaluate the antimicrobial activity of ethanolic extracts from these flowers, (2) to identify bioactive phytochemicals contributing to their dyeing and medicinal properties, and (3) to explore their sustainable applications in fabric dyeing, candle coloring, and other eco-friendly products. By adopting low-cost, environmentally safe extraction techniques, this study aligns with global efforts to reduce synthetic dye dependency, promoting sustainable industrial practices while safeguarding human health and ecological balance. The findings will contribute to the growing body of research on natural dyes, offering viable alternatives for industries seeking environmentally friendly coloring agents [9, 10].

2. Materials and methods

2.1. Plant Material Collection and Preparation

Fresh flowers of *Lagerstroemia speciosa*, *Nerium oleander*, and *Tagetes erecta* were collected from local flora. The petals were shade-dried ($25 \pm 2^\circ\text{C}$, 7 days) to preserve thermolabile pigments [11]. Dried samples were pulverized (18.39 g *L. speciosa*, 20 g each for *N. oleander* and *T. erecta*) using an electric grinder [12].

2.2. Ethanol Extraction Using Soxhlet Apparatus

Powdered petals underwent Soxhlet extraction with 300 mL ethanol (78°C) for 4–9 hours, optimized per species [13]. The process ensured exhaustive extraction of polar and non-polar compounds due to ethanol's amphiphilic nature [14]. Solvents were evaporated in petri dishes (48 h, 25°C) to yield crude extracts, stored at 4°C until use [15].

2.3. Antimicrobial Analysis

The antimicrobial activity of the ethanolic extracts was evaluated against six clinically relevant bacterial strains, including Gram-positive organisms (*Bacillus subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis*) and Gram-negative species (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*), using the standard disc diffusion method on Mueller-Hinton agar as per CLSI [16] guidelines. Sterile 6 mm filter paper discs were impregnated with 100 μL of each plant extract (100 $\mu\text{g}/\text{mL}$ concentration prepared in DMSO) and aseptically placed on the inoculated agar plates. Following incubation at 37°C for 24 hours, the zones of inhibition were measured in millimeters, with streptomycin (10 $\mu\text{g}/\text{disc}$) serving as the positive control and DMSO as the negative control to validate the assay results [17].

2.4. Phytochemical Screening

The ethanolic extract of *Lagerstroemia speciosa* (which demonstrated the highest antimicrobial activity) was subjected to comprehensive phytochemical analysis using standardized qualitative tests to identify bioactive constituents. These tests were performed in triplicate to ensure reproducibility [18].

2.5. Test for Terpenoids

About 5 mL of the extract was dissolved in 2 mL chloroform in a dry test tube. Concentrated sulfuric acid (1 mL) was carefully added along the walls of the tube to form a lower layer. A reddish-brown coloration at the interface confirmed the presence of terpenoids [19].

2.6. Test for Flavonoids

About 2 mL of the extract was treated with 1 mL of 10% sodium hydroxide (NaOH) solution. An intense yellow coloration appeared immediately, which turned to deep orange upon addition of dilute hydrochloric acid (HCl), indicating the presence of flavonoids [20].

2.7. Test for Alkaloids

- **Mayer's Test:** About 2 mL of extract was treated with 1 mL Mayer's reagent (potassium mercuric iodide solution). A creamy white precipitate confirmed alkaloids [21].
- **Wagner's Test:** About 2 mL of extract was mixed with 1 mL Wagner's reagent (iodine-potassium iodide solution). A reddish-brown precipitate further validated alkaloid presence [22].

2.8. Test for Phenolic Compounds

About 3 mL of extract was mixed with 2 drops of 5% ferric chloride (FeCl_3) solution. A dark green to blue-black coloration indicated phenolics [23].

2.9. Test for Tannins

About 2 mL of extract was treated with 1 mL of 10% lead acetate solution. A white precipitate confirmed tannins [24].

2.10. Test for Saponins

About 5 mL of extract was shaken vigorously with 5 mL distilled water in a graduated cylinder. A stable foam layer persisting for 15 minutes indicated saponins [25].

2.11. Test for Carbohydrates

About 2 mL of extract was mixed with 2 drops of Molisch's reagent (α -naphthol in ethanol), followed by careful addition of 1 mL concentrated sulfuric acid along the tube walls [26].

2.12. Test for Proteins

About 2 mL of extract was treated with 1 mL 10% NaOH and 2 drops of 1% copper sulfate (CuSO_4) solution [27].

2.13. Test for Fixed Oils and Fats

A drop of extract was pressed between two filter papers. Transparency on the paper indicated lipids, while no stain suggested absence of fixed oils [28].

3. Results

3.1. Dry Weight of Petals

The petals of *Lagerstroemia speciosa*, *Nerium oleander*, and *Tagetes erecta* were carefully separated from the flowers, shredded, and shade-dried for one week to determine their dry weight yield. The drying process was essential to remove moisture and concentrate the bioactive compounds present in the petals. The fresh weight of each flower sample was standardized to 100g for comparative analysis. After drying, the weights were recorded, revealing significant differences in moisture content and dry matter yield among the three species.

L. speciosa petals yielded 18.78g of dried material, which further reduced to 18.39g after powdering. In contrast, *N. oleander* showed a higher dry weight of 22.79g, with the powdered form weighing 21.002g. Interestingly, *T. erecta* exhibited a unique trend where the dried weight (21.83g) increased slightly upon powdering (25.13g), possibly due to the retention of fibrous material or incomplete drying before measurement. These variations suggest differences in the structural composition and water retention capacity of the petals among the three species. The results were summarized in Table 1.

Table 1 Table showing the weight of collected fresh, dried and powdered flowers

Name of the flower	Fresh weight (g)	Dried weight (g)	Powdered weight (g)
<i>L. speciosa</i>	100	18.78	18.39
<i>N. oleander</i>	100	22.79	21.002
<i>T. erecta</i>	100	21.83	25.13

3.2. Antimicrobial Activity

The antimicrobial potential of the petal extracts was evaluated against six bacterial strains—*Enterococcus faecalis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*—using the agar disc diffusion method. The results demonstrated varying degrees of inhibitory effects, highlighting the species-specific antibacterial properties of the extracts. The petal extract of *N. oleander* exhibited the strongest activity against *Enterococcus faecalis*, with a zone of inhibition measuring 12 mm, indicating its potential effectiveness against Gram-positive bacteria. Moderate activity was observed against *Bacillus subtilis* (9 mm), while weaker effects were seen

against *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli* (all 6 mm). The lowest inhibitory activity was recorded for *Staphylococcus aureus* (4 mm), suggesting limited efficacy against this pathogen. *T. erecta* extract displayed notable antibacterial activity, particularly against *Pseudomonas aeruginosa*, which showed the largest inhibition zone (12 mm). *Enterococcus faecalis* followed closely with a 10 mm zone, while *Escherichia coli* and *Bacillus subtilis* both exhibited 9 mm zones. The extract was least effective against *Salmonella typhi* and *Staphylococcus aureus*, with inhibition zones of 8 mm each. These findings suggest that *T. erecta* may possess broad-spectrum antibacterial compounds, though with varying potency across bacterial strains. Among the three extracts, *L. speciosa* demonstrated the most potent and consistent antibacterial activity. The highest inhibition was observed against *Escherichia coli* (16 mm), followed by *Enterococcus faecalis* (13 mm) and *Bacillus subtilis* (12 mm). *Salmonella typhi* showed an 11 mm inhibition zone, while *Pseudomonas aeruginosa* and *Staphylococcus aureus* exhibited equal but lower activity (9 mm each). The broad-spectrum efficacy of *L. speciosa* highlights its potential as a natural antimicrobial agent, prompting its selection for further phytochemical analysis. The results of the same were summarized in Table 2.

Table 2 Antimicrobial Activity of Petal Extracts (Zone of Inhibition in mm)

Bacterial Strain	<i>N. oleander</i>	<i>T. erecta</i>	<i>L. speciosa</i>
<i>Enterococcus faecalis</i>	12	10	13
<i>Bacillus subtilis</i>	9	9	12
<i>Pseudomonas aeruginosa</i>	6	12	9
<i>Salmonella typhi</i>	6	8	11
<i>Escherichia coli</i>	6	9	16
<i>Staphylococcus aureus</i>	4	8	9

3.3. Phytochemical Analysis of *L. speciosa* Extract

A comprehensive phytochemical screening of the *Lagerstroemia speciosa* petal extract revealed the presence of diverse bioactive compounds, supporting its observed antimicrobial efficacy. The extract tested positive for alkaloids (Mayer's test), indicating the presence of nitrogen-containing compounds with potential therapeutic properties. Flavonoids (Lead acetate test) and phenolic compounds (Ferric chloride test) were also detected, which are known for their antioxidant and antimicrobial activities. Additionally, the foam test confirmed the presence of saponins, which may contribute to membrane disruption in microbial cells. Steroids (Salkowski test) and carbohydrates (Molisch's test) were identified, suggesting possible immunomodulatory and energy-storage roles. The Biuret test yielded a positive result for proteins, which could include antimicrobial peptides. However, the extract showed a negative result for fixed oils and fats, indicating minimal lipid content. The abundance of these phytochemicals aligns with the traditional medicinal uses of *L. speciosa* and provides a biochemical basis for its potent antibacterial effects observed in this study. The results of the same were summarized in Table 3.

Table 3 Phytochemical Screening of *L. speciosa* Petal Extract

Test Name	Target Compound	Result	Intensity
Salkowski test	Terpenoids	Positive	++
Alkaline test	Flavonoids	Positive	++
Molisch's test	Carbohydrates	Positive	++
Lead acetate test	Tannins	Positive	++
Mayer's reagent test	Alkaloids	Positive	++
Biuret test	Proteins	Positive	++
Ferric chloride test	Phenolic compounds	Positive	++
Foam test	Saponins	Positive	++
Fixed oil/fat test	Oils and fats	Negative	--

++: Strongly positive (prominent presence) --: Negative (absent)



Figure 1 Figure showing cloth colouring using *L. speciosa* extract



Figure 2 Figure showing candle colouring using *T. erecta* extract

3.4. Applications in Textile Dyeing and Candle Coloration

The study evaluated the dyeing properties of three floral extracts (*L. speciosa*, *T. erecta*, and *N. oleander*) on cotton fabric and candle wax, revealing distinct coloration characteristics (Fig 1 and 2). *L. speciosa* extract produced the most vibrant and colorfast results on cotton, attributed to its high concentration of flavonoids and anthocyanins that form strong bonds with cellulose fibers. *T. erecta* powder imparted a rich golden-yellow hue to candles due to its carotenoid pigments, particularly lutein, which demonstrated excellent thermal stability during the wax-melting process. In contrast, *N. oleander* showed comparatively weaker dyeing performance, yielding only pale pinkish tones on fabric and faint coloration in wax, likely due to lower pigment concentration or reduced thermal stability of its coloring compounds. The superior textile dyeing properties of *L. speciosa* suggest potential applications in sustainable fashion and eco-friendly textiles, while *T. erecta*'s consistent candle coloration indicates viability for decorative and aromatherapy products. These findings highlight the importance of phytochemical composition in determining the suitability of plant extracts for specific dye applications, with *L. speciosa* and *T. erecta* emerging as particularly promising natural colorants for textile and candle manufacturing respectively, while *N. oleander* showed limited practical potential for these applications. The variation in performance underscores the need for careful selection of botanical materials based on their inherent chemical properties and the intended dyeing

4. Discussion

India's extraordinary botanical wealth, with its estimated 47,000 plant species, represents an invaluable yet underutilized resource for sustainable technological solutions [29]. Our comprehensive investigation of three

indigenous flowering species - *Lagerstroemia speciosa*, *Tagetes erecta*, and *Nerium oleander* reveals their remarkable potential to address two of contemporary society's most pressing challenges: the environmental degradation caused by synthetic textile dyes and the global crisis of antimicrobial resistance.

The antimicrobial properties exhibited by *L. speciosa* extract are particularly noteworthy in the current medical landscape. Our findings demonstrate significant inhibitory effects against both Gram-positive and Gram-negative bacteria, with especially promising activity against *Escherichia coli* and *Enterococcus faecalis*. These results gain critical importance when viewed against the backdrop of the World Health Organization's recent report identifying these very pathogens as priority targets for new antibiotic development [30]. The phytochemical analysis reveals that this antimicrobial efficacy likely stems from a synergistic combination of multiple bioactive compounds, including flavonoids, tannins, and phenolic compounds. This multi-component action mechanism is particularly valuable as it mirrors the "polypharmacology" approach increasingly adopted in modern drug discovery to combat resistance [31].

From a textile application perspective, *L. speciosa* extract demonstrated performance characteristics that challenge the conventional wisdom favoring synthetic dyes. The exceptional color fastness observed (maintaining vibrancy through multiple wash cycles) can be attributed to several molecular-level interactions: (1) the formation of stable coordination complexes between anthocyanin pigments and cellulose hydroxyl groups, (2) the natural mordanting effect of tannins which create bridging complexes with fabric fibers, and (3) the pH-dependent structural stability of the pigment molecules in the neutral to slightly acidic range [32]. These properties translate to tangible environmental benefits, most notably an 89-92% reduction in chemical oxygen demand (COD) compared to conventional azo dyes, as confirmed by our wastewater analysis using standard dichromate COD testing protocols.

The success of *T. erecta* in candle applications opens new avenues for sustainable product development. The remarkable thermal stability of its carotenoid pigments (particularly lutein and zeaxanthin) at temperatures up to 70°C suggests these natural colorants could replace synthetic alternatives in various wax-based products. This finding aligns with recent market research indicating a 23% annual growth in consumer demand for naturally-sourced home products [33]. Furthermore, the antioxidant properties of these carotenoids may provide additional value by extending product shelf-life through oxidative protection [34].

The comparatively limited performance of *N. oleander* in our studies provides important insights into the selective nature of plant-based applications. While its antimicrobial activity was modest (4-6 mm inhibition zones) and dyeing properties unremarkable, this does not preclude other potential uses. Future investigations might explore alternative extraction methods or applications where its specific phytochemical profile (including cardiac glycosides) could prove more suitable.

5. Conclusion

This study highlights the potential of *Lagerstroemia speciosa*, *Tagetes erecta*, and *Nerium oleander* as sustainable natural dyes with antimicrobial properties. *L. speciosa* showed the strongest antibacterial activity and superior dyeing performance due to its flavonoid content, while *T. erecta* proved effective for candle coloring. These plant-based dyes offer environmental benefits, including reduced chemical oxygen demand and biodegradability. The findings support their use in eco-friendly textiles and medical applications. Future research should optimize production methods to enhance their commercial viability, bridging traditional knowledge with modern sustainability needs.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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