

## Extraction and Characterization of Natural Dyes from Transvaal Daisy and Lavender Petals: A Sustainable Approach

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### ABSTRACT

This study explores the use of Transvaal daisy and Lavender flowers as sustainable sources for natural pigments. The project aims to address environmental and health concerns associated with synthetic dyes by developing an eco-friendly extraction process. Two methods, aqueous-ethanol extraction and Soxhlet extraction, were employed and compared for efficiency. Thin-Layer Chromatography (TLC) and biochemical tests confirmed the presence of Xanthophyll pigment and other compounds in the extracted dyes. The Soxhlet extraction method demonstrated higher efficiency and purity than aqueous-ethanol extraction. The extracted dyes were successfully applied to cotton fabrics and evaluated for their wash and light fastness properties. While the dyes proved to be biodegradable and non-toxic, limitations such as reduced durability under prolonged sunlight and washing were identified. This research highlights the potential of natural dyes as sustainable alternatives to synthetic ones. It underscores the need for further improvements in fastness properties through advanced mordanting techniques and the exploration of alternative natural dye sources. The findings contribute to the development of environmentally friendly dyes for applications in art, textiles, and beyond.

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## 1. Introduction:

The growing environmental and health concerns associated with synthetic dyes have driven significant interest in natural dyes as sustainable alternatives. Synthetic dyes are widely used across various industries, including textiles, food, cosmetics, and leather. However, their production and usage are linked to severe environmental pollution due to the presence of toxic, non-biodegradable chemicals that persist in ecosystems. Effluents from synthetic dye manufacturing are known for their high chemical oxygen demand (COD), complex molecular structures, and resistance to degradation, making them a major contributor to water pollution and ecological imbalance (Verma et al., 2012). Furthermore, synthetic dyes often contain harmful substances, some of which have been identified as carcinogens, posing long-term health risks to humans (Samanta & Agarwal, 2009). These issues have necessitated the exploration of eco-friendly alternatives that can meet industrial demands without compromising environmental and public health.

Natural dyes, derived from renewable sources such as plants, animals, and minerals, are increasingly being recognized as viable substitutes for synthetic dyes. Unlike synthetic counterparts, natural dyes are biodegradable, non-toxic, and often have antimicrobial properties, making them safer for both the environment and human use (Rather et al., 2023). Historically, natural dyes have been used for textile dyeing, food coloring, and medicinal applications. However, their widespread adoption was hindered during the industrial revolution, as synthetic dyes gained prominence due to their cost-effectiveness, vibrant shades, and superior fastness properties. Despite these advantages, the detrimental effects of synthetic dyes have reignited interest in natural dyes, leading to extensive research focused on improving their extraction processes, fastness properties, and scalability (Geetha & Sumathy, 2013).

Flowers, in particular, represent a rich and renewable source of natural dyes. The vibrant pigments present in flowers, such as anthocyanins, flavonoids, carotenoids, and tannins, make them ideal for producing a wide range of colors (Samanta & Agarwal, 2009). For example, the petals of Transvaal daisy and Lavender are known for their high pigment content and potential applications in fabric dyeing and art paints. The use of flower-based dyes aligns with sustainability goals, as these raw materials are biodegradable and can often be sourced from agricultural byproducts or floral waste, reducing the environmental footprint of dye production. However, challenges remain, including the limited light and wash fastness of natural dyes, variability in shades due to differences in raw material quality, and the lack of standardized extraction and application methods (Reddy et al., 2023).

Recent advancements in extraction techniques have significantly improved the efficiency and yield of natural dyes. Traditional methods, such as maceration and boiling, are often time-consuming and energy-intensive, with lower dye recovery rates. In contrast, modern approaches like Soxhlet extraction, ultrasonic-assisted extraction, and the use of green solvents have demonstrated superior performance in terms of yield, efficiency, and environmental sustainability (Sivakumar et al., 2009). Soxhlet extraction, for instance, offers a controlled environment for dye extraction by continuously recirculating the solvent, ensuring maximum pigment recovery. Ultrasonic-assisted extraction further enhances the process by breaking down plant cell walls through acoustic cavitation, facilitating the release of pigments with minimal solvent usage and energy input. These methods not only improve the economic viability of natural dye production but also align with green chemistry principles, minimizing environmental impact.

The use of mordants plays a crucial role in improving the application and durability of natural dyes. Mordants are substances that fix dyes onto fabrics, enhancing their affinity and fastness properties. While traditional mordants such as copper, chromium, and tin are effective, they often pose environmental and health hazards due to their heavy metal content (Pizzicato et al., 2023). Natural mordants like alum, myrobalan, and tannins offer a safer alternative, enabling sustainable dyeing practices. Research has shown that combining natural mordants with advanced dyeing techniques, such as ultrasonic dyeing, can significantly improve the color fastness and vibrancy of natural dyes, making them competitive with synthetic dyes in industrial applications (Repon et al., 2024).

This study focuses on the extraction and application of natural dyes from the petals of Transvaal daisy and Lavender flowers. The primary objective is to evaluate the efficiency of two extraction methods: Soxhlet extraction (Inbarajan et al., 2022) and aqueous-ethanol extraction (Ramprasath et al., 2017). These methods are analyzed for their yield, efficiency, and environmental impact. The extracted dyes are characterized using Thin-Layer Chromatography (TLC) and subjected to various biochemical tests to identify their chemical composition, including pigments like Xanthophyll, alkaloids, phenolic compounds, and carbohydrates (Khan et al., 2017; Porwal et al., 2023). The application of these dyes to cotton fabrics is also explored, with assessments of their wash and light fastness properties to determine their suitability for practical use.

By addressing key challenges associated with natural dye use, such as extraction efficiency, fastness properties, and environmental sustainability, this research contributes to the growing efforts to replace

synthetic dyes with eco-friendly alternatives. The findings aim to provide insights into the development of cost-effective and environmentally sustainable dyes suitable for diverse applications, including textiles, art paints, and cosmetics. This work also highlights the importance of integrating modern technologies and natural resources to promote sustainability in industries reliant on coloration processes (Burrows, 2004; Shahid et al., 2013).

In conclusion, the shift towards natural dyes is not only a response to the environmental and health issues posed by synthetic dyes but also a step toward harnessing the potential of renewable resources in a sustainable manner. This study seeks to bridge the gap between traditional knowledge and modern science, offering practical solutions for scaling up the use of natural dyes in industrial applications. By leveraging advanced extraction techniques and natural mordants, this research aims to demonstrate the feasibility of replacing synthetic dyes with natural alternatives, ultimately contributing to a greener and more sustainable future.

## 2. Methodology:

**Experimental materials:** The culture is isolated from soil samples and the banana peels are collected from local areas and used as experimental materials. A dark yellow variety of Transvaal daisy, Lavender was purchased from flower market. Substrates and Chemicals (Ethanol, Methanol, Acetone, Diethyl Ether, Mayer's Reagent, Wagner's Reagent, Ferric Chloride, Benedict's Reagent) and the Equipments (Weighing balance, Water Bath, Soxhlet apparatus, Hot Air Oven, Colorimeter, Spectrophotometer, etc.) were used during the experiment.

After obtaining all the required materials for the study, the next step was the Extraction of coloured pigments from the flowers of Transvaal Daisy and Lavender, aqueous-ethanol extraction and soxhlet extraction methods were used.

**The aqueous-ethanol extraction:** The aqueous-ethanol extraction method is a sustainable approach to isolating natural dyes from flower petals. Flowers of Transvaal Daisy and Lavender were cleaned, dried, and crushed into powder. This powder was mixed with ethanol-water solutions (60%, 70%, or 100% ethanol) in conical flasks, using approximately 10–15 grams of material per 100 mL of solvent. The flasks are incubated in a water bath at 68°C for four hours to dissolve the pigments efficiently. After extraction, the solution is filtered to remove residues and evaporated in a hot air oven overnight to obtain a concentrated dye extract.

**The Soxhlet extraction:** The Soxhlet extraction process is a widely used technique for efficiently extracting natural dyes from plant materials such as flowers. The process begins with the preparation of the raw material, where flowers are cleaned, dried, and ground into a fine powder. This powdered material is then placed in a thimble made of filter paper or cellulose, which is inserted into the Soxhlet extractor. The extractor is connected to a round-bottom flask containing the chosen solvent, such as ethanol or methanol, and a condenser is attached to the top of the apparatus to recycle the solvent vapor.

During the extraction, the solvent is heated in the round-bottom flask until it vaporizes and rises into the condenser, where it cools and condenses back into liquid form. The liquid solvent drips onto the material in the thimble, dissolving the pigments. Once the solvent in the extractor reaches a specific level, it siphons back into the round-bottom flask, leaving the extracted dye behind. This cycle is repeated continuously for 4–5 hours to ensure complete pigment extraction. After the extraction, the solvent is removed from the dye solution using a rotary evaporator or by evaporation in a hot air oven, leaving behind the concentrated dye extract, which is then collected and stored for further use.

Thin-Layer Chromatography (TLC) was used to analyze the composition of the extracted natural dyes. A TLC plate coated with silica gel served as the stationary phase. Pencil marks were made near the bottom of the plate to indicate the spots where the dye samples would be applied. Small amounts of the dye extracts were carefully spotted onto these marked points using a capillary tube. The plates were then placed in a TLC chambers, one containing a mobile phase solvent mixture, acetone and hexane, and other with ethanol as solvent, ensuring that the solvent level remained below the spots.

As the solvent ascended the plate by capillary action, the components of the dye mixture separated based on their interaction with the stationary and mobile phases. After the solvent front reached a predetermined height, the plate was removed and air-dried. The separated spots were visualized under UV light and their positions were recorded. The retention factor ( $R_f$ ) values for each spot were calculated to identify the specific pigments present in the dye extracts and assess the purity of the samples.

Several biochemical tests were performed to characterize the natural dye extracts. For alkaloids, two tests were conducted: Mayer's test and Wagner's test. In Mayer's test, the dye extract was treated with Mayer's reagent (a solution of potassium mercuric iodide), and the mixture was observed for any interaction indicating the presence of alkaloids. Similarly, Wagner's test involved adding Wagner's reagent (iodine in potassium iodide) to the dye extract to confirm the presence of alkaloids. For

carbohydrates, Benedict's test was used, where the extract was mixed with Benedict's reagent and heated in a boiling water bath to assess the presence of reducing sugars.

Phenolic compounds were tested using the Ferric Chloride test, where the extract was dissolved in distilled water and a few drops of 5% ferric chloride solution were added to observe the reaction. Proteins were analyzed using Millon's test, in which the extract was treated with Millon's reagent and heated to identify protein content. These tests provided essential insights into the chemical composition of the dye extracts, helping to confirm the presence of key bioactive compounds for further application and analysis.

### 3. Results:

The results of the Soxhlet extraction method showed superior performance in terms of yield, concentration, and purity of the natural dyes compared to the aqueous-ethanol extraction method. The Soxhlet process, which involves continuous solvent circulation, allowed for maximum pigment recovery from the flower materials. The extracted dye appeared more concentrated, with vibrant coloration and better solubility, indicating higher quality. Thin-Layer Chromatography (TLC) analysis revealed distinct and well-defined spots with consistent retention factor (Rf) values, confirming the presence of Xanthophyll pigments and fewer impurities. The Soxhlet extraction was also highly efficient, completing the process in 4–5 hours, with ethanol and methanol proving effective solvents for enhancing pigment solubility and extraction.

In contrast, the aqueous-ethanol extraction method produced a moderate yield of dye, which was lower than that obtained through the Soxhlet method. The extracted dyes appeared less concentrated, with slightly diluted coloration, likely due to limited solvent interaction with the flower materials. TLC analysis indicated the presence of multiple spots, suggesting that some impurities were co-extracted along with the pigments. Although the Rf values confirmed the presence of Xanthophyll pigments, the separation was less defined compared to the Soxhlet-extracted dyes. This method, while simpler and more environmentally friendly, required longer incubation times in a water bath, and the yield was influenced by the ethanol-water ratio used during the process.

**Table 1.**By using Acetone and Hexane as solvent systems the Rf value of the Dye

Sr. No	Rf value
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1	0.78
2	0.85
3	0.95

**Table 2.** By using ethanol as solvent system the Rf value of the dye

Sr. No	Rf value
1	0
2	0.86
3	0.60

The biochemical analysis revealed the following detailed results for the natural dye extracts:

i. **Alkaloids:**

- **Mayer's Test:** When the dye extracts were treated with Mayer's reagent (potassium mercuric iodide solution), a white creamy precipitate formed along the sides of the test tube. This result confirmed the presence of alkaloids in the extracts, indicating that the flowers used in the extraction contained these nitrogenous compounds, which are often associated with biological activity.
- **Wagner's Test:** Adding Wagner's reagent (iodine in potassium iodide solution) to the dye extracts resulted in a reddish-brown precipitate, further validating the presence of alkaloids in the samples.

ii. **Carbohydrates:**

- **Benedict's Test:** The addition of Benedict's reagent to the dye extracts, followed by heating in a boiling water bath, led to the formation of a brick-red precipitate. This confirmed the presence of reducing sugars, suggesting that the flower extracts contained carbohydrate compounds that could contribute to their properties as natural dyes.

iii. **Phenolic Compounds:**

- **Ferric Chloride Test:** Upon adding a few drops of 5% ferric chloride solution to the dye extracts dissolved in distilled water, a dark green coloration developed. This indicated the presence of phenolic compounds, which are known for their antioxidant properties and their role in producing stable pigments for dyeing applications.

iv. **Proteins:**

**Millon’s Test:** The dye extracts reacted with Millon’s reagent to form a white precipitate that turned red upon heating. This result confirmed the presence of proteins in the extracts, which might contribute to the binding properties of the dyes when applied to fabrics.



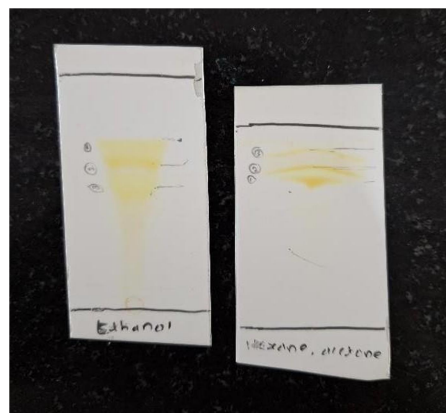
*Fig. 1. Crushing of Flowers1*



*Fig. 3. Dye Extract*



*Fig. 4. Thin Layer Chromatography of Dye*



*Fig. 5. Millon's Test 1*





Fig. 4. Thin Layer Chromatography of Dye



Fig. 8. Mayer's test



Fig. 7. Benedict's test

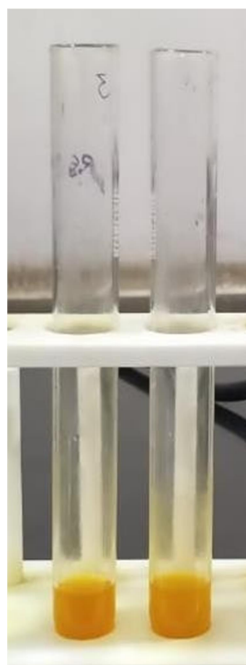


Fig. 8. Wegner's test



Fig. 8. Ferric Chloride test

## 5. Conclusion:

**Summary and Conclusion** This experiment explored the extraction of natural dyes from Transvaal Daisy and Lavender petals using Soxhlet and aqueous-ethanol extraction methods. The Soxhlet method demonstrated superior performance, yielding more concentrated and purer dyes due to continuous solvent circulation and effective pigment recovery. In contrast, the aqueous-ethanol method provided moderate yields and slightly diluted extracts but offered a simpler and environmentally friendly approach. Biochemical tests confirmed the presence of alkaloids, phenolic compounds, carbohydrates, and proteins in the dyes, while Thin-Layer Chromatography (TLC) analysis revealed the presence of Xanthophyll pigments, with the Soxhlet-extracted dyes displaying greater purity.

The findings indicate that natural dyes are a viable and sustainable alternative to synthetic dyes, offering non-toxic and eco-friendly solutions for various applications, including textiles and art paints. While the Soxhlet method is more suitable for large-scale production, the aqueous-ethanol method remains a practical option for smaller-scale uses. These results highlight the potential of natural dyes to contribute to sustainable industrial practices, and future research should focus on improving their fastness properties and identifying additional natural sources to enhance their applicability.

## References:

1. Burrows, V. A. (2004). Experiments and Other Learning Activities Using Natural Dye Materials. *Chemical Engineering Education*, 8(2), 132–141.
2. Geetha, B., & Sumathy, V. J. H. (2013). Extraction of Natural Dyes from Plants. *International Journal of Chemistry and Pharmaceutical Sciences*, 1(8), 502–509.
3. Inbarajan, K., Sowmya, S., & Janarthanan, B. (2022). Direct and soxhlet extraction of dyes from the peels of *Allium cepa* and its effective application in dye – Sensitized solar cells as sensitizer. *Optical Materials*, 129, 112487.
4. Khan, H., Raza, M. L., Sheikh, D., Usmani, S., & Sana, A. (2017). Extraction and Preliminary Phytochemical Analysis of Different Extracts of *Cassia fistula* Linn. Pods. *RADS J. Pharm. Pharm. Sci.*, 5(3), 17–23.
5. Pizzicato, B., Pacifico, S., Cayuela, D., Mijas, G., & Riba-Moliner, M. (2023). Advancements in Sustainable Natural Dyes for Textile Applications: A review. *Molecules*, 28(16), 5954.

6. Porwal, N., Gupta, B., Gakkhar, A. K., Tiwari, R. C., & Mittal, B. (2023). An Evaluation of Physicochemical Parameters and Quantitative Phytochemical Analysis of Datura Metel- A Research Article. *Journal of Ayurvedic Herbal and Integrative Medicine*, 3(2), 1–13.
7. Ramprasath, R., Kavi, G. G., & Rathi, T. (2017). Isolation of Natural Dyes from Hibiscus Rosa Sinensis and Marigold Flower and Dyeing Properties of the Dyes On Cotton Cloth. *IOSR Journal of Applied Chemistry*, 10(05), 74–79.
8. Rather, L. J., Shabbir, M., Ganie, S. A., Zhou, Q., Singh, K. P., & Li, Q. (2023). Natural dyes: Green and sustainable alternative for textile colouration. In *CRC Press eBooks* (pp. 41–69).
9. Reddy, A. R., Reddy, N. M., Kalyani, P., Tejasri, G., & Rao, T. R. (2023). A Review On Utilization Of Floral Waste To Develop Value Added Products. *Gradiva Review Journal*, 9(12), 116–131.
10. Repon, M. R., Dev, B., Rahman, M. A., Jurkonienė, S., Haji, A., Alim, M. A., & Kumpikaitė, E. (2024). Textile dyeing using natural mordants and dyes: a review. *Environmental Chemistry Letters*, 22(3), 1473–1520.
11. Samanta, A. K., Agarwal, P., & Institute of Jute Technology. (2009). Application of natural dyes on textiles. In *Indian Journal of Fibre & Textile Research: Vol. Vol. 34* (pp. 384–399).
12. Shahid, M., Shahid-Ul-Islam, N., & Mohammad, F. (2013). Recent advancements in natural dye applications: a review. *Journal of Cleaner Production*, 53, 310–331.
13. Sivakumar, V., Anna, J. L., Vijayeeswarri, J., & Swaminathan, G. (2009). Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather. *Ultrasonics Sonochemistry*, 16(6), 782–789.
14. Verma, A. K., Dash, R. R., & Bhunia, P. (2011). A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. *Journal of Environmental Management*, 93(1), 154–168.