

Phytochemical Profiling, HPLC Analysis and Antioxidant Activity of *Lantana camara* Flower and Leaf Extracts

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ABSTRACT

Background: The current study aims to find the phytochemicals, antioxidants and amount of flavonoid present in different extracts of *Lantana camara* Linn. flower and leaf collected from in and around Bangalore University. **Objectives:** To determine the phytochemical composition of *Lantana camara* extracts, quantify their flavonoid content using RP-HPLC analysis, and evaluate the antioxidant properties of selected extracts through DPPH assay. **Materials and Methods:** The collected *Lantana camara* Linn. flowers and leaves were shade-dried and powdered. Extracts were prepared in increasing order of polarity (water/aqueous, methanol, chloroform, and petroleum ether) using Soxhlet extraction. Qualitative screening for phytochemicals was conducted following standard procedures. RP-HPLC analysis with rutin as standard was used to determine the total flavonoid content. DPPH assay was performed on aqueous and methanol extracts to assess antioxidant activity. **Results:** Phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, and flavonoids across all extracts. Oxalates were present only in leaf extracts, while saponins were found exclusively in leaf extracts. RP-HPLC analysis showed that methanol flower extracts contained the highest flavonoid content (13.58 mg/g), whereas petroleum ether extracts had the lowest (0.093 mg/g). DPPH assay demonstrated that methanol extracts possessed greater antioxidant activity compared to aqueous extracts. **Conclusion:** This study provides comprehensive data on the phytochemical composition, flavonoid content, and antioxidant properties of different *Lantana camara* Linn. extracts. These findings suggest potential applications in medicine, particularly for treating diseases that require flavonoid-based interventions.

Keywords: *Lantana camara* Linn., Flower and Leaf Extracts, HPLC, Flavonoids.

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INTRODUCTION

Lantana camara Linn. (Family: Verbenaceae), described by Carl von Linnaeus in 1753, is a prickly shrub that grows upright, partially ascending, or occasionally more or less dangling, reaching a height of 2-3 m. The Latin word "lantana" most likely means "to bend". The stems and branches of the plant are angular in shape and have curving spines arranged randomly along the edges. The leaves are simple and oval, with a sharp tip, consistently dentate surface, and opposing, decussate, rough lamina. They are arranged in opposing pairs and are oblong or ovate-oblong, with a width of 2-10 cm. The leaves are vividly green, slightly hairy, and have a distinct odor.

The inflorescence of *Lantana camara* is a terminal or auxiliary hemispherical head composed of several tiny tubular flowers. The flowers can be orange, yellow, pink, or other colors. The plant

produces 20-40 flowers, each measuring about 2.5 cm in diameter. Flowering mainly occurs between March and August.^[1] The plant is native to the tropical regions of America, Africa, and Asia. It has various vernacular names, including Kakke (Kannada), Unnichi (Tamil), Raimuniya (Hindi), Chaturang (Sanskrit), and Lantana (English). The plant presently has 650 varieties, has colonized as many as 60 countries, and is a distinguished species in the Global Invasive Data Base.^[2] Phytochemicals, often referred to as secondary metabolites, are bioactive compounds produced by plants that play a crucial role in human health and disease prevention. These compounds are not only responsible for the color, flavor, and aroma of plants but also contribute significantly to their medicinal properties.^[3,4] Considerable amounts of flavonoids and other bioactive compounds are found in *Lantana camara*, a plant noted for its diverse phytochemical profile. Flavonoids and phenolic compounds in the plant have been shown to exhibit antioxidant activity that mitigates free radicals, reducing the scourge of chronic diseases like cancer and cardiovascular conditions.^[5] Specifically, flavonoids are unique for their many health benefits, including anti-inflammatory and anticancer effects.^[6] Some flavonoids found in *Lantana camara* can alter cell pathways associated with inflammation and immune



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response and thus can speed up the healing process.^[7] The plant in general has many medicinal and pharmacological applications, apart from being an obnoxious weed. Among them is its use as a medicament for malaria, gastropathy, swelling, cuts, sores, ulcers, skin inflammation, rheumatism, and for the stimulation of vomiting in cases of food poisoning, catarrhal infection, eczema, high blood pressure, sores, measles, chicken pox, bilious fever, respiratory infections, arthritis, antiurolithiatic, antimutagenic, antimotility, anticancer, antifungal, antibacterial, and anti-filarial activities. Additionally, the plant exhibits antiepileptic properties in animal models.^[8-12] Different parts of the plant possess distinct phytochemical components, contributing to the plant's diversified medicinal utility. The therapeutic potential of phytochemicals has garnered increasing attention in modern medicine, with many being incorporated into functional foods and nutraceuticals aimed at enhancing health and preventing diseases.^[13] Additionally, comparing the phytochemical profiles and medicinal properties of *Lantana camara* with other health-promoting plants, such as green tea (*Camellia sinensis*) and pomegranate (*Punica granatum*), can provide a broader context for understanding the significance of this plant. Furthermore, identifying the gaps in current research concerning the specific mechanisms through which flavonoids exert their effects can facilitate future studies aimed at exploring their bioavailability and metabolism in humans. This, in turn, will yield valuable insights into their therapeutic efficacy.^[14] Due to its diversified credibility and varied medicinal characteristics, the plant can be emphasized in the field of drug development.

MATERIALS AND METHODS

Plant materials

The Flowers and Leaves were collected from November to December (2022) in and around Bangalore University. The collected plant samples were recognized and confirmed as *Lantana camara* L., a member of the Verbenaceae family, by Dr. V. Rama Rao, a research officer at the Central Ayurveda Research Institute, Bengaluru. A herbarium specimen with the designation RRCBI-15312 was submitted to the Institute.

Plant Extracts

Only fresh and young, healthy plant parts (flower and leaf) were chosen for the experiment. The collected plant parts were cleaned thoroughly to remove unwanted debris, such as dust, hidden insects, caterpillars, and spiders from leaves and the inflorescence. Flowers and leaves procured were shade-dried.^[15] The dried plant parts were powdered separately with a blender and stored in airtight packets for further use. Soxhlet extraction was employed with aqueous, methanol, chloroform, and petroleum ether as the chief extracts for the study. The dried plant component was loaded onto a thimble of Soxhlet extractor for 72 hr at varying temperatures for each extract, and the obtained plant extracts were evaporated in a Rotary evaporator. The crude extracts, which were dark green, were utilized for further experimental studies.

Qualitative analysis/Phytochemical screening

For each extract, a high level of care and precision was applied to perform all the phytochemical screening/qualitative tests by recognized protocols mentioned in Table 1.^[16-18]

Quantitative analysis of Flavonoid

RP-HPLC analysis

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) has emerged as a cornerstone analytical technique across diverse scientific disciplines, including pharmaceutical analysis, environmental monitoring, and food safety. The significance of RP-HPLC lies in its exceptional capability to separate, identify, and quantify a broad spectrum of analytes with high resolution, sensitivity, and reproducibility, RP-HPLC's versatility allows it to analyze both polar and non-polar molecules, making it suitable for a diverse array of applications.^[19]

The analysis was performed using the RP-HPLC method at a flow rate of 1 mL/min on the C18 column (symmetry, 4-6 mm×250 mm) in an isocratic mode with the mobile phase acetonitrile

Table 1: Phytochemical tests employed on different plant extracts.

Tests for Bioactive Compounds	Test	Positive Results
Alkaloids	Wagner's Reagent	Reddish-brown precipitate
Carbohydrates	Molisch's Reagent	Red or dull violet color at the inter phase
Cardiac Glycosides	Keller-Killiani's Test	Brown ring at the interface
Flavonoids	Alkaline Reagent Test	Intense yellow color
Phenols	Ferric Chloride Test	Deep blue or black color
Phlobatannins	Precipitate Test	Red precipitate
Amino Acids and Proteins	Ninhydrin Solution	Purple color
Saponins	Foam Test	Persistent foam
Sterols	Liebermann- Burchard Test	Dark pink or red color
Tannins	Braymer's Test	Blue or greenish color solution
Terpenoids	Salkowski's Test	Reddish-brown precipitate
Quinones	Concentrated HCl	Yellow precipitate
Oxalates	Glacial Ethanolic Acid	Greenish-black coloration
Fats and Oils	Copper Sulfate and Sodium Hydroxide	Clear blue solution

and water in a 7:3 ratio. The standard rutin (0.4 mg/mL) and samples (10 mg) were dissolved in the mobile phase 20 μ L was injected, and the elution was monitored at 272 nm. The amount of flavonoids in the samples was calculated using the formula:

$$\text{Sample area} \times \text{standard amount} \times \text{dilution of sample} \times \text{Mean weight}$$

$$\text{Standard area dilution of standard sample amount}$$

DPPH assay

The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay is a widely used method for evaluating the antioxidant capacity of various substances. This assay is based on the principle that DPPH, a stable free radical, exhibits a deep violet color in solution. When an antioxidant is present, it donates a hydrogen atom to DPPH, leading to a reduction of the radical and a corresponding change in color from violet to yellow. This color change can be quantitatively measured using spectrophotometry, making the DPPH assay both simple and rapid for assessing antioxidant activity. Its reliability and reproducibility have made it a standard method in many studies focused on natural products and food chemistry, facilitating comparisons of antioxidant potential across different compounds and extracts.^[20]

Due to the high flavonoid content, the (DPPH) assay for antioxidant determination was carried out only on methanol and water/aqueous extracts. Using the stable DPPH radical, the free radical scavenging capacity of the extracts (methanol and water/aqueous) samples was calculated. The samples were placed in test tubes at various concentrations (0.1-0.5 mg), and the volume in each test tube was filled to 0.1 mL with methanol. 3 mL of DPPH solution (absorbance set to 1) was added to each tube and incubated in the dark for 15 min. After incubation, the absorbance was spectrophotometrically measured at 517 nm with methanol as a blank. The percentage inhibition was calculated using the formula.

$$\text{Percentage inhibition} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100$$

RESULTS

Qualitative Phytochemical Screening

Comprehensive phytochemical screening of different flower and leaf extracts revealed a diverse array of bioactive compounds. Cardiac glycosides, carbohydrates, and flavonoids were ubiquitously present across all extracts. However, phlobatannins, sterols, and terpenoids were consistently absent from all samples analyzed (Tables 2 and 3).

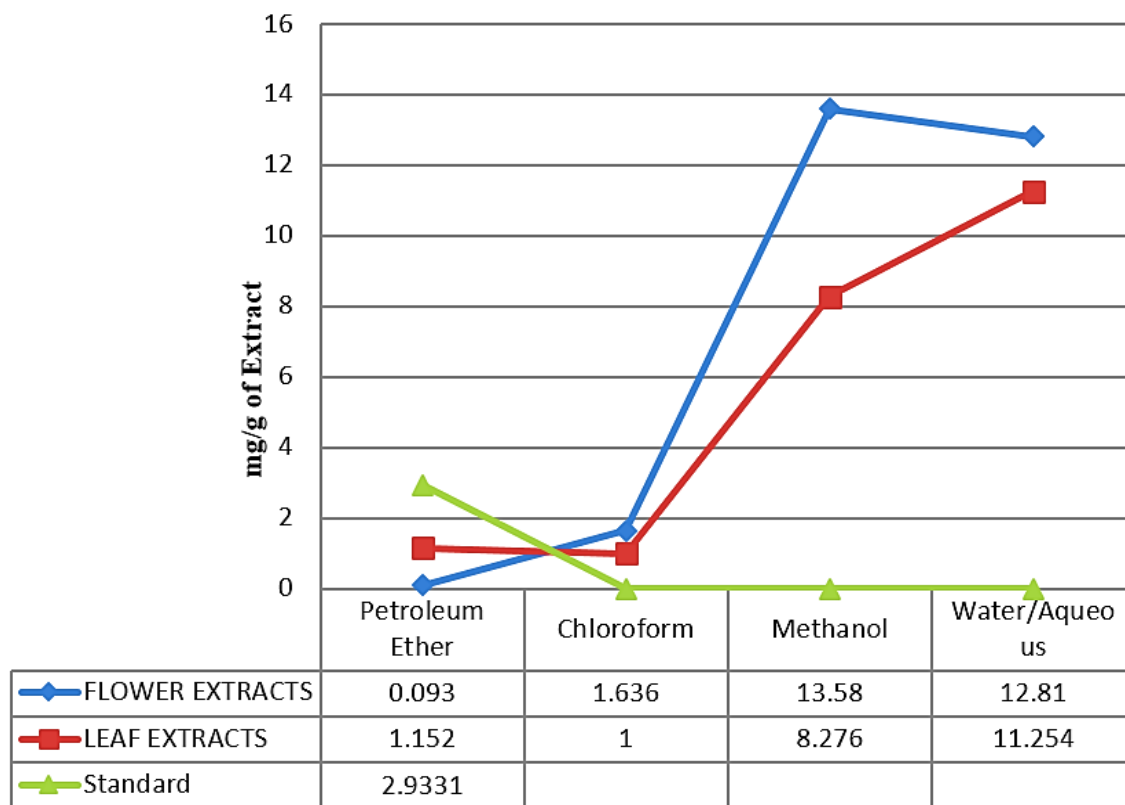


Figure 1: Total Flavonoid (mg/g) present in Different Extracts. Total flavonoid content (mg/g) in various extracts (petroleum ether, chloroform, methanol, and aqueous) from the flowers and leaves of *Lantana camara*. Extracts were obtained using a standardized extraction protocol, with flavonoid content quantified via RP-HPLC.

Quantitative Phytochemical Analysis

Total flavonoid content in the plant extracts was quantified using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) with rutin as a standard. Significant variations in flavonoid concentrations were observed between flower and leaf

extracts. The methanolic flower extract exhibited the highest flavonoid content at 13.58 mg/g dry weight, while the petroleum ether extract contained the lowest at 0.093 mg/g. In leaf extracts, the aqueous preparation yielded the highest flavonoid content (11.254 mg/g), whereas the chloroform extract showed the lowest (1.00 mg/g) (Figure 1).

Table 2: Phytochemical analysis of flower with different extracts.

Tests	Sample Flower			
	Petroleum Ether	Chloroform	Methanol	Water
Alkaloids	+	+	+	-
Carbohydrates	+	+	+	+
Cardiac Glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phenols	-	-	-	+
Phlobatannins	-	-	-	-
Amino Acids and proteins	-	-	-	+
Saponins	-	-	-	-
Sterols	-	-	-	-
Tannins	-	-	+	+
Terpenoids	-	-	-	-
Quinones	+	+	+	+
Oxalates	-	-	-	-
Fats and oils	-	+	-	-

Phytochemical analysis of flower samples using various solvents (water, methanol, petroleum ether, and chloroform) to identify different classes of phytochemicals. The extracts were subjected to specific phytochemical tests to detect the presence of targeted compounds.

Table 3: Phytochemical analysis of leaf with different extracts.

Tests	Sample Leaf			
	Petroleum Ether	Chloroform	Methanol	Water
Alkaloids	+	+	+	-
Carbohydrates	+	+	+	+
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Phenols	-	+	+	+
Phlobatannins	-	-	-	-
Amino acids and proteins	-	-	-	+
Saponins	+	+	+	+
Sterols	-	-	-	-
Tannins	-	+	+	+
Terpenoids	-	-	-	-
Quinones	+	-	-	+
Oxalates	-	+	+	-
Fats and oils	+	-	-	+

Phytochemical analysis of leaf samples utilizing different solvents (water, methanol, petroleum ether, and chloroform) aimed at identifying various classes of phytochemicals. The resulting extracts underwent specific phytochemical tests for compound detection.

In Tables 2 and 3, (+) denotes the presence of the Phytocomponent, and (-) denotes its absence.

Antioxidant Activity

The antioxidant capacity of selected extracts was evaluated using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay. Aqueous and methanolic extracts were selected for this analysis based on their high flavonoid content. The methanolic leaf extract demonstrated the highest percentage inhibition of DPPH radicals, while the aqueous flower extract exhibited the lowest. The IC_{50} (half maximal Inhibitory Concentration) value for the reference compound, Gallic Acid, was determined to be 0.772 mg. Notably, the methanolic leaf extract exhibited the lowest IC_{50} value at 0.229 mg, indicating the highest antioxidant activity among the tested samples (Tables 4, 5 and Figures 2-4).

Table 4: Standard values of DPPH.

Conc of sample in μ g	Gallic Acid	
	Absorbance at 517 nm	Percentage Inhibition
100	0.792	20.8
200	0.755	24.5
300	0.702	29.8
400	0.664	33.6
500	0.619	38.1

Gallic Acid Equivalent Antioxidant Capacity (GAEAC) values for various concentrations, providing a standard reference for comparing antioxidant activity.

Table 5: DPPH assay IC_{50} value.

Sample	IC_{50} value (mg)
Gallic acid	0.772
Methanol F	0.363
Water F	0.479
Methanol L	0.229
Water L	0.453

IC_{50} values obtained from the DPPH assay for different samples, indicating the concentration required to inhibit 50% of the DPPH free radicals.

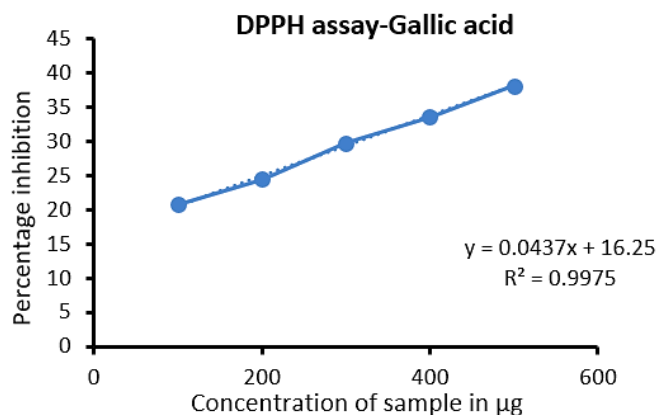


Figure 2: Percentage Inhibition of DPPH Assay. Percentage inhibition of DPPH free radicals for various concentrations of extracts, illustrating the antioxidant potential of the samples.

DISCUSSION

Standard procedures were followed for the screening of phytochemicals, and the results varied depending on the extracts employed. The solvents were selected based on the increasing order of their polarity: methanol and aqueous solvents had higher amounts of dissolved flavonoid content than other solvents (chloroform and petroleum ether), and these solvents also demonstrated effective antioxidant properties. Using RP-HPLC and DPPH tests, the sample's total flavonoid content and antioxidant activity were calculated. The quantity of crude extracts obtained was also less in chloroform and petroleum ether extracts compared to aqueous and methanol extracts.

The inclusion of shade drying in the study stems from the possibility that it offers benefits over sun drying in terms of protecting materials sensitive to light and reducing light-induced chemical processes like oxidation.^[21] The drying process was conducted in a naturally ventilated room maintained at ambient temperature, the plant materials placed on drying racks were protected from direct sunlight exposure.^[15] The process utilized passive solar-heated air circulation for moisture removal.^[22] Although *Lantana camara*'s phytocomponents have been the subject of numerous investigations, the results are inconsistent due to variations in extraction method, duration, temperature, solvent type, solvent concentration, and solvent polarity. The findings in this study are also tied to these factors, highlighting the importance of appropriate extract utility and extraction techniques.

Medicinal plants have served as therapeutic agents throughout human history, with their systematic integration into drug development emerging in the 19th century. Of the 252 drugs classified by WHO as basic and essential, 11% are exclusively derived from flowering plants.^[23] The International Union for Conservation of Nature has documented 50,000-80,000 flowering plant species with established medicinal applications.^[24] Plants as a source of medicine are most widely used in Japan, India, China, Pakistan, Thailand, Iran, and a few African countries.^[25-28] There are many books, manuscripts, and other sources of texts in the regional languages of India that proclaim the importance of plants as medicines.^[29] The immense utility of plants as medicines is evident, and the presence of flavonoids in *Lantana camara* and other plants suggests potential applications in treating flavonoid-dependent diseases like epilepsy,^[30-33] as well as other neurological and neurodegenerative disorders,^[34] However, it's important to note that allelochemicals present in the plant make it a potential threat to native plant species, thereby potentially eliminating them. With no potential threat, the plant can eventually become an invasive monophyletic species.^[35] To address this dual nature of *Lantana camara* and to harness its medicinal properties effectively, it is crucial to analyze its bioactive compounds thoroughly.

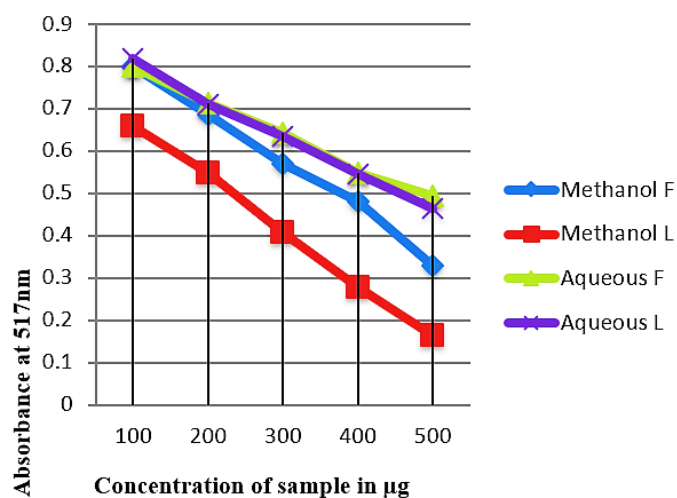


Figure 3: DPPH Assay Absorbance vs Concentration. Graph depicting the relationship between extract concentration and corresponding absorbance values in the DPPH assay, highlighting the effectiveness of each extract.

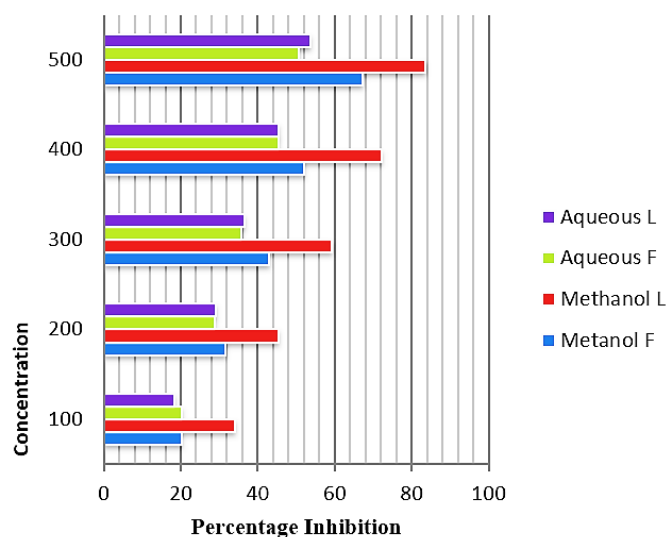


Figure 4: DPPH Assay Percentage Inhibition vs Concentration. Graph illustrating the correlation between extract concentration and percentage inhibition in the DPPH assay. An increase in percentage inhibition indicates enhanced antioxidant activity.

The identification of phytochemicals from plant extracts is essential in various scientific and industrial fields. These compounds play a vital role in drug discovery, serving as lead compounds for pharmaceutical development.^[36,37] They help elucidate the mechanisms behind traditional medicinal uses of plants and contribute to the nutritional and health-promoting properties of foods.^[38,39] Phytochemicals often exhibit potent antioxidant activities, which are beneficial in disease prevention.^[40] Understanding the full phytochemical profile of plant extracts can reveal synergistic effects between compounds, potentially more effective than isolated substances.^[41]

This knowledge is also essential for safety assessments and standardization of herbal products.^[42,43] Furthermore,

phytochemicals have applications in food, cosmetic, and agricultural industries.^[44] From an ecological perspective, these compounds provide insights into plant defense mechanisms and plant-environment interactions.^[45] Lastly, discovering valuable phytochemicals can provide economic incentives for biodiversity conservation, highlighting the broader environmental importance of this research.^[37] In light of these considerations, the present study was undertaken to analyze the bioactive compounds and quantify the most important phytochemical, flavonoids, as well as to evaluate the antioxidant properties of two *Lantana camara* extracts. This approach aims to maximize the plant's medicinal potential while also contributing to our understanding of its ecological impact. By thoroughly investigating the phytochemical profile and antioxidant capabilities of *Lantana camara*, we can better assess its potential for pharmaceutical applications and develop strategies to mitigate its invasive tendencies.

CONCLUSION

This comprehensive study provides valuable insights into the phytochemical profile of *Lantana camara* collected from around Bangalore University, representing one of the first detailed analyses of a specific ecotype, while building upon previous observations of variations in *L. camara* toxicity.^[46] Our methodological approach yielded several significant findings. The implementation of shade drying proved highly effective, preserving up to 30% more phenolic compounds and antioxidants compared to sun drying methods,^[47] while Soxhlet extraction enabled comprehensive isolation of both polar and non-polar compounds, essential for thorough phytochemical characterization.^[48]

A notable discovery was the distinct phytochemical distribution between plant parts, with oxalates exclusively present in leaves and saponins confined to flowers, a differentiation not previously highlighted in the literature.^[10] This finding has important implications for targeted therapeutic applications. The precision of our RP-HPLC analysis revealed significant variations in flavonoid content across different extracts, with methanolic flower extracts showing the highest concentration (13.58 mg/g) and petroleum ether extracts the lowest (0.093 mg/g).^[49] Importantly, we identified a strong correlation between antioxidant activity and flavonoid content, a relationship not previously documented in such detail for *L. camara*.^[50]

These findings establish a robust foundation for future research directions, particularly in understanding environmental influences on phytochemical composition and standardizing medicinal preparations. Our results not only advance the current understanding of *L. camara*'s chemical composition but also open new avenues for investigating its therapeutic potential, especially in antioxidant-based treatments and treating diseases that require flavonoid-based interventions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

L. camara: *Lantana camara* Linn; **RP-HPLC:** Reverse Phase High-Performance Liquid Chromatography; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **mg/g:** Milligrams per gram; **IC₅₀:** Inhibition Capacity 50.

SUMMARY

This research investigated the phytochemical composition, flavonoid content, and antioxidant properties of *Lantana camara* Linn. flower and leaf extracts from the Bangalore University area. Using Soxhlet extraction with solvents of increasing polarity (petroleum ether, chloroform, methanol, and water), Phytochemical screening, RP-HPLC analysis with rutin standard for flavonoid quantification, and DPPH assay for antioxidant activity assessment were conducted. Results showed carbohydrates, cardiac glycosides, and flavonoids present across all extracts, with oxalates and saponins exclusive to leaf extracts. Methanol flower extracts contained the highest flavonoid content (13.58 mg/g), while petroleum ether extracts had the lowest (0.093 mg/g). Methanol extracts demonstrated superior antioxidant activity compared to aqueous extracts. These findings suggest potential medicinal applications, particularly for flavonoid-based treatments.

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