ORIGINAL ARTICLE

# Phytochemical Screening and Thin-layer Chromatography Fingerprint of *Lagerstroemia Parviflora*

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## Abstract

**Background:** The plant *Lagerstroemia parviflora* has been traditionally used for various medicinal applications, yet its phytochemical profile remains insufficiently explored. Investigating the secondary metabolites of this species could reveal bioactive compounds with potential pharmacological benefits. **Aims:** The study aims to conduct phytochemical screening and establish a thin-layer chromatography (TLC) fingerprint of *L. parviflora* to identify key secondary metabolites. **Materials and Methods:** Leaves of *L. parviflora* were collected, dried, and subjected to maceration extraction using chloroform, methanol, and water. Extracts were analyzed for presence of phytoconstituents using standard qualitative assays. TLC was performed on silica gel 60F254 plates, with a mobile phase of toluene: ethyl acetate: Formic acid (5:4:1). The chromatograms were visualized under normal light, short ultraviolet (UV) (254 nm), and long UV (365 nm), and retention factor (Rf) values were recorded. **Results:** Phytochemical screening of *L. parviflora* revealed flavonoids in chloroform and aqueous extracts; diterpenes, phenols, proteins, and carbohydrates in the methanolic extract; saponins and tannins in the aqueous extract; and sterols in the chloroform extract, while alkaloids were absent. TLC analysis showed multiple flavonoid-related bands, with the chloroform extract exhibiting six spots under UV light (Rf = 0.4–1.0), the methanolic extract showing up to 7 spots (Rf = 0.12–1.0), and the aqueous extract displaying fewer bands. Notably, several spots near Rf = 0.64 align with quercetin, indicating flavonoid-rich profiles with potential antioxidant and therapeutic relevance. **Conclusion:** The findings suggest that *L. parviflora* possesses significant bioactive compounds, particularly flavonoids and phenolics, which could be explored for antioxidant and therapeutic applications. Further investigation into isolation, purification, and bioactivity assays of identified compounds is needed to establish their pharmacological potential.

**Key words:** *Lagerstroemia parviflora*, phytochemical screening, secondary metabolites, thin-layer chromatography

## INTRODUCTION

The plant kingdom represents a significant biological resource for the isolation and characterization of pharmacologically active compounds. Phytomedicine, encompassing botanical and herbal medicine, refers to therapeutic agents derived from whole plants, plant components, or plant-derived substances subjected to minimal chemical modification.[1,2] This broad category includes raw plant materials (e.g., leaves, flowers, fruits, seeds, stems, wood, bark, roots, rhizomes, and isolated active constituents), herbal preparations, and formulated products where plant-derived materials constitute the primary components. While plants synthesize primary metabolites essential for ontogenetic processes, they also elaborate a diverse array of secondary metabolites, also termed natural products.[3] These compounds arise through enzymatic biotransformation of primary metabolic intermediates and are broadly classified based on their biosynthetic origins into phenols, terpenes, and alkaloids.[1,3] Terpenoids, phenolics, flavonoids, alkaloids, and glycosides constitute major classes of secondary metabolites that are important sources of isolated bioactive compounds for both nutraceutical and medicinal applications. Possessing notable antioxidant properties, they can be effectively utilized as

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natural antioxidants in nutraceuticals. Their diverse therapeutic efficacy stems from direct interactions at the molecular level with receptors, cellular membranes, and nucleic acids.[4,5]

Leveraging the structural diversity and biochemical specificity of these secondary metabolites, herbal therapy has emerged as a cornerstone of traditional medicine, utilizing whole plants or their components for therapeutic intervention and health maintenance.[6] Numerous phytomedicinal products possess a broad spectrum of pharmacological properties, including antimicrobial, antifertility, antidiabetic, antiarthritic, anti-aging, antidepressant, sedative, antispasmodic, anxiolytic, anti-inflammatory, analgesic, vasodilatory, anti-human immunodeficiency virus, and hepatoprotective actions and are employed in the management of conditions such as hepatic cirrhosis, acne, asthma, menopause, erectile dysfunction, cholelithiasis, migraine, Alzheimer's disease, chronic fatigue syndrome, and memory enhancement.[6,7]

The historical prevalence of herbal remedy utilization in Asia reflects a protracted history of human interaction with the natural world. Several crude drugs are derived from plant taxa yet to be scientifically determined.[8] As the leading global producer of plant species with therapeutic properties, India is appropriately referred to as the "Botanical Garden of the world." The ethnomedicinal use of plants for the treatment of various pathological states has a deep historical basis in the region.[9] *Lagerstroemia parviflora* (of the family Lythraceae), commonly designated as Landia throughout India and Seja in the Bundelkhand region, is a species exhibiting extensive distribution across virtually all moist and dry deciduous zones of India. The primary economic value of this tree lies in its timber; however, the bark of *L. parviflora* contains a notable percentage of tannins (7–10%) and is utilized at a local level for the tanning of hides and the dyeing of cotton yarns.[9-11] Ethnobotanical evidence suggests the widespread utilization of this plant species by indigenous female populations for the management of lactational insufficiency. Traditional knowledge posits the pan-plant application of this organism in the treatment of gastrointestinal obstruction and syphilis. Furthermore, this herbaceous species demonstrates potential efficacy in the symptomatic management of cough, pyrexia, asthma, and bronchitis.[10]

This investigation employs phytochemical screening and thin-layer chromatography to elucidate the phytochemical profile of *L. parviflora* and identify potential bioactive constituents for therapeutic applications.

## MATERIALS AND METHODS

### Preparation of plant material

Specimens of *L. parviflora* foliage [Figure 1] were sourced from the Bhimbetka region of Bhopal. On collection, the plant material was meticulously purified through a multi-step process. Initially, senescent or compromised components

**Figure 1:** Procurement of leaves of *Lagerstroemia parviflora*

were eliminated. This was followed by serial rinsing with tap water and subsequently with distilled water. Residual water was absorbed by encasing the washed material in blotting paper. The plant material was subsequently desiccated through indirect sun drying under shade.

### Preparation of plant extracts

The efficient isolation of bioactive secondary metabolites from plant materials represents a critical step in phytochemical processing. Fifty grams of *L. parviflora* leaf tissue, previously dehydrated under shade and mechanically processed to a coarse powder, were sequentially extracted with chloroform, methanol, and water using the maceration technique.[12] Each extraction was conducted for a 24-h interval. The resulting extracts (filtrates) were separated from the solid plant material by filtration through Whatman paper. Subsequent solvent removal from each filtrate was performed through controlled evaporation at 50°C using a water bath until a concentrated extract was obtained. The yield of each anhydrous extract was then quantified as a percentage of the initial plant material mass.

### Determination of percentage yield

The percentage yield serves as a quantitative metric for evaluating the overall efficiency of the extraction procedure. This value is determined through the following formula:

$$Percentage\ Yield = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} \times 100$$

### Qualitative phytochemical screening

The extract was subjected to qualitative phytochemical screening following standard procedures to determine the presence of different natural compound groups. The results of this screening indicated the presence of phenolics, flavonoids, tannins, saponins, alkaloids, and proteins, as inferred from diagnostic color changes and precipitation phenomena in extracts.[13]

### Detection of alkaloids

Alkaloid detection in plant extracts was performed on acidic filtrates through established qualitative assays. (a)

Hager's test, a precipitation reaction with saturated picric acid resulting in a yellow precipitate, and (b) Wagner's test, a colorimetric reaction with Wagner's reagent producing a reddish hue, were employed as positive indicators for the presence of these secondary metabolites.

### Detection of glycoside

An aqueous extract is subjected to a screening utilizing concentrated sulfuric acid. The generation of a red color is indicative of glycoside presence.

### Detection of flavonoids

The qualitative chemical characterization of the extract for the estimation of flavonoids involved two distinct color-based assays.

a. Alkaline Reagent Test: Flavonoid presence in the extracts is indicated by the formation of an intense yellow coloration upon exposure to sodium hydroxide, with concomitant decolorization on the addition of dilute acid.
b. Lead acetate Test: The addition of lead acetate solution to the extracts resulted in the formation of a yellow precipitate, indicative of flavonoid presence.

### Detection of diterpenes

The quantification of diterpenes was performed using the copper(II) acetate test, a colorimetric method. The appearance of an emerald green chromophore in aqueous extracts treated with the reagent provided evidence for the presence of diterpenoid compounds.

### Detection of phenols

Qualitative assessment of phenolic constituents in the extract was performed using two chromogenic assays: (a) The ferric chloride test, based on the formation of a bluish-black complex indicative of phenolic hydroxyl groups, and (b) the Folin–Ciocalteu assay, a colorimetric method yielding a blue-green chromophore in the presence of potential phenolic antioxidants.

### Detection of proteins

Extracts were subjected to the xanthoproteic test to determine protein content, where the observation of a yellow coloration after the introduction of concentrated nitric acid constituted a positive detection.

### Detection of carbohydrates

Individual extracts were subjected to aqueous dissolution (5 mL), filtration, and the resulting filtrates were analyzed for carbohydrate content.

a. Fehling's Test: The filtrates underwent acid-catalyzed hydrolysis and were subsequently neutralized with a base. On thermal incubation with Fehling's solution (a mixture of solutions A and B), a red precipitate formed, indicating the presence of reducing sugars.
b. Benedict's Test: The filtrates were analyzed for reducing sugars using Benedict's reagent and subsequent controlled heating. A positive result, indicated by the precipitation of an orange-red solid, confirmed the presence of these analytes.

### Detection of saponins

A foam test was employed to ascertain the presence of saponins. Extract solutions (20 mL in distilled water) were mechanically agitated for 15 min. The sustained generation of a foam column ≥1 cm in a graduated cylinder constituted a positive saponin indication.

### Detection of tannins

The gelatin test is a qualitative assay employed for the detection of tannins, based on their capacity to precipitate gelatin. The methodology involves combining a sample extract with a 1% gelatin-sodium chloride solution. The observation of white precipitate signifies the presence of tannins.

### Detection of sterols

For the qualitative identification of sterols, the Salkowski test was performed by introducing 3–4 drops of concentrated sulfuric acid into a chloroform extract. A positive reaction is visually confirmed by the development of a reddish hue at the interphase of the two liquid phases.

### Separation and identification

TLC profiling serves as a preliminary step toward the isolation and characterization of these phytoconstituents. Silica gel 60F254 plates (7 cm × 6 cm, Merck), prepared by cutting, served as the stationary phase. A 1 µL volume of each sample was applied 1 cm from the plate origin at five distinct locations using glass capillaries. Chromatographic development was performed in a pre-saturated twin-trough chamber using a mobile phase system of toluene: Ethyl acetate: Formic acid (5:4:1), optimized for flavonoid separation as per reference.[3] The mobility of separated analytes was quantified by their retention factor (Rf). Visualization of the developed chromatograms was achieved under normal light, short-wavelength ultraviolet (UV) (254 nm), and long-wavelength UV (365 nm) using a TLC documentation system (Electronic India). The Rf-value for each resolved spot was determined by the ratio of the distance traveled by the solute to the distance traveled by the solvent front:[14]

$$Rf = \frac{Distancentraveled\ by\ solute}{Distancentraveled\ by\ solvent}$$

## RESULTS

### % yield of leaves extract of *L. parviflora*

The percentage yield of *L. parviflora* leaf extracts obtained using different solvents is presented in Table 1. Among the three solvents tested, the aqueous extract exhibited the highest yield at 7.82% (w/w), followed by the methanolic extract with 4.36% (w/w), and the chloroform extract with the lowest yield at 2.50% (w/w). The variation in extraction yield reflects the differential solubility of phytoconstituents in solvents of varying polarity, with water demonstrating superior extraction efficiency for polar compounds compared to methanol and chloroform.

### Result of phytochemical screening

Phytochemical screening of medicinal plants is crucial for assessing their potential therapeutic applications and identifying the bioactive compounds responsible for their established pharmacological effects. Table 2 and Figure 3a-c present the results of a qualitative phytochemical analysis conducted on chloroform, methanolic, and aqueous extracts of *L. parviflora* leaves to determine the presence of various secondary metabolites.

**Table 1:** % yield of leaves extract of *Lagerstroemia parviflora*

| S. No. | Extracts | % Yield (W/W) |
|---|---|---|
| 1. | Chloroform | 2.50 |
| 2. | Methanolic | 4.36 |
| 3. | Aqueous | 7.82 |


**Figure 2:** Extraction of plant leaves by the maceration process

The phytochemical composition of *L. parviflora* leaf extracts was investigated using solvents of varying polarity. Methanol extraction yielded alkaloids and glycosides. Flavonoids and phenolic compounds were present in both methanol and water extracts, exhibiting differential reactivity to specific reagents. Diterpenes and sterols were not observed. Carbohydrates were detected in both polar extracts, with positive results in distinct reducing sugar assays. Saponins and tannins were uniquely extracted by water, while proteins were absent across all solvent systems [Figure 2].

### Separation and identification by TLC

TLC profiling was carried out using the mobile phase toluene: Ethyl acetate: Formic acid (5:4:1) to analyze flavonoid content in different extracts of *L. parviflora*. Quercetin was used as a standard reference compound. As shown in Table 3, the standard quercetin exhibited a single spot under normal light, short-wave UV (254 nm), and long-wave UV (366 nm) with an Rf = 0.64, corresponding to a solute travel distance of 3.2 cm (total solvent front: 5.0 cm). This confirms the suitability of the selected mobile phase for flavonoid separation.

The chloroform extract exhibited no visible spots under normal light but revealed six distinct spots under both short- and long-wave UV light. The Rf values under short UV ranged from 0.48 to 0.90, and under long UV from 0.40 to 1.00, indicating a broad range of flavonoid-like compounds with varying polarity.

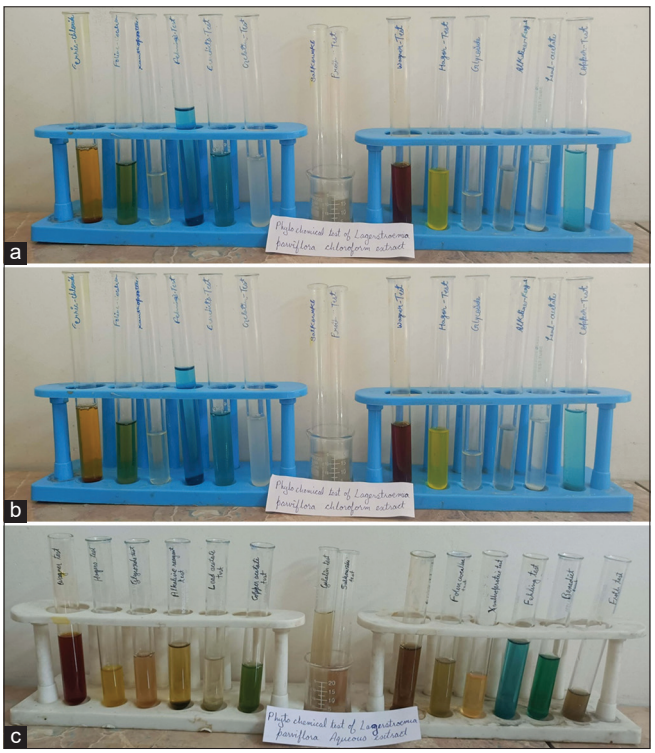


**Figure 3:** Phytochemical screening of the extract of (a) chloroform extract, (b) methanolic extract, and (c) aqueous extract of *Lagerstroemia parviflora*

The methanolic extract demonstrated the highest complexity, revealing two spots under visible light, seven under short-wave UV, and five under long-wave UV. The Rf values under normal light were 0.76 and 1.00, while UV detection showed multiple bands with Rf values ranging from 0.12 to 1.00, suggesting the presence of a diverse spectrum of flavonoids with varying degrees of polarity and fluorescence characteristics. In contrast, the aqueous extract showed no spots under visible light, a single spot under short UV (Rf = 0.72), and two spots under long UV (Rf = 0.60 and 0.76), indicating a lower flavonoid content compared to organic solvent extracts.

The presence of a spot at Rf = 0.64 in both the methanolic and chloroform extracts under short and long UV wavelengths suggests the possible presence of quercetin or structurally related flavonoids [Figure 4].

**Table 2:** Result of phytochemical screening of the leaf extract of *Lagerstroemia parviflora*

| S. No. | Constituents | Chloroform extract | Methanolic extract | Aqueous extract |
|---|---|---|---|---|
| 1. | Alkaloids | | | |
| | Wagner's test | –ve | +ve | –ve |
| | Hager's test | –ve | –ve | –ve |
| 2. | Glycosides | | | |
| | Conc. $H_2SO_4$ test | –ve | +ve | –ve |
| 3. | Flavonoids | | | |
| | Lead acetate test | –ve | +ve | –ve |
| | Alkaline test | –ve | +ve | +ve |
| 4. | Diterpenes | | | |
| | Copper acetate test | –ve | –ve | –ve |
| 5. | Phenol | | | |
| | Ferric chloride test | –ve | –ve | +ve |
| | Folin–Ciocalteu test | –ve | +ve | –ve |
| 6. | Proteins | | | |
| | Xanthoproteic test | –ve | –ve | –ve |
| 7. | Carbohydrate | | | |
| | Fehling's test | –ve | +ve | +ve |
| | Benedict's test | –ve | –ve | –ve |
| 8. | Saponins | | | |
| | Froth test | –ve | –ve | +ve |
| 9. | Tannins | | | |
| | Gelatin test | –ve | –ve | +ve |
| 10. | Sterols | | | |
| | Salkowski test | –ve | –ve | –ve |

+Ve: Positive, –Ve: Negative

## DISCUSSION

The study on *L. parviflora* was conducted to explore its phytochemical composition and chromatographic profile, given its traditional medicinal applications in treating respiratory ailments, gastrointestinal disorders, and lactational insufficiency. Despite its widespread use, scientific validation of its bioactive compounds remains limited. This study aimed to screen secondary metabolites and establish a TLC fingerprint to identify potential therapeutic constituents.

Phytochemical screening is crucial for identifying bioactive compounds responsible for medicinal properties. The phytochemical screening of *L. parviflora* was conducted to identify its secondary metabolites, which contribute to its medicinal properties. The study examined extracts obtained using chloroform, methanol, and water, revealing the presence of flavonoids, phenolics, alkaloids, tannins, saponins, glycosides, diterpenes, sterols, proteins, and carbohydrates in varying concentrations. By employing qualitative assays and TLC profiling, this study provides insights into the chemical diversity of *L. parviflora*, supporting its pharmacological relevance.

The present study showed that the aqueous extract had the highest yield, indicating greater extraction efficiency for polar compounds, while the chloroform extract had the lowest yield, suggesting limited solubility of non-polar compounds. This finding is consistent with Bharadwaj and Chaturvedi, who also reported efficient extraction of phenolic and flavonoid compounds using aqueous solvents in *L. parviflora*.[8]

Qualitative phytochemical screening demonstrated a differential distribution of secondary metabolites across solvent extracts. Lipophilic compounds, specifically flavonoids and diterpenes, were identified in both chloroform

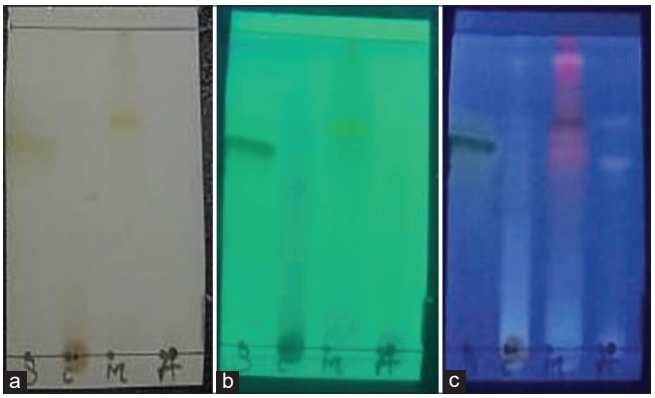


**Figure 4:** Thin-layer chromatography of flavonoids (a) normal light, (b) short ultraviolet (UV), and (c) long UV. 1st spot: Standard quercetin, 2nd spot: Chloroform extract, 3rd spot: Methanolic extract, 4th spot: Aqueous extract

**Table 3:** TLC of the extract of *Lagerstroemia parviflora* (Flavonoids)

| S. No. | Mobile phase Toluene: Ethyl acetate formic acid (5:4:1) | Distance of solute | *Rf*-value |
|---|---|---|---|
| 1. | (Quercetin) | | |
| | Dis. travel by mobile phase=5.0 cm | Normal Light-3.2 | Normal-0.64 |
| | No. of spot at normal light=1 | Short-3.2 | Short-0.64 |
| | No. of spot at short UV=1 | Long-3.2 | Long-0.64 |
| | No. of spot at long UV=1 | | |
| 2. | (Chloroform extract) | | |
| | No. of spot at normal light=0 | Normal Light-0 | Normal-0 |
| | No. of spot at short UV=6 | Short-2.4, 2.7, 3, 3.5, 4, 4.5 | Short-0.48, 0.54, 0.6, 0.7, 0.8, 0.9 |
| | No. of spot at long UV=6 | Long-2, 2.5, 3, 3.7, 4.8, 5 | Long-0.4, 0.5, 0.6, 0.74, 0.96, 1.0 |
| 3. | (Methanolic extract) | | |
| | No. of spot at normal light=2 | Normal Light-3.8, 5 | Normal-0.76, 1.0 |
| | No. of spot at short UV=7 | Short-0.6, 3.2, 3.7, 4, 4.2, 4.6, 5 | Short-0.12, 0.64, 0.74, 0.8, 0.84, 0.92, 1.0 |
| | No. of spot at long UV=5 | Long-3.2, 3.8, 4.6, 4.8, 5 | Long-0.64, 0.76, 0.8, 0.96, 1.0–0.6, 0.7, 0.92, 1.0 |
| 4. | (Aqueous extract) | | |
| | No. of spot at normal light=0 | Normal Light-0 | Normal-0 |
| | No. of spot at short UV=1 | Short-3.6 | Short-0.72 |
| | No. of spot at long UV=2 | Long-3, 3.8 | Long-0.6, 0.76 |

and methanolic fractions. Phenols and proteins exhibited preferential solubility in methanol, indicative of intermediate polarity. Highly polar compounds, namely, saponins and tannins, were exclusively detected in the aqueous extract. These results are aligning with previously reported study.[8,9]

TLC was employed to profile the phytochemical constituents of *L. parviflora* extracts. Utilizing silica gel 60F254 plates and a mobile phase system of toluene: Ethyl acetate: Formic acid (5:4:1), chromatographic separation was achieved. Visualization under white light, short-wavelength (UV 254 nm), and long-wavelength (UV 365 nm) irradiation revealed distinct metabolite fingerprints for each extract. A quercetin standard exhibited an Rf = 0.64 under all visualization conditions. The chloroform extract displayed six resolved compounds under both short and long UV wavelengths (Rf = 0.48–1.0). The methanolic extract presented two visible spots under normal light and seven and five spots under short (Rf = 0.12–1.0) and long UV (Rf = 0.64–1.0), respectively. The aqueous extract showed one spot under short UV (Rf = 0.72) and two spots under long UV (Rf = 0.6–0.76). These data suggest a diverse array of flavonoids and phenolic compounds, with the methanolic extract exhibiting the greatest metabolite complexity. The TLC methodology effectively facilitated the separation and presumptive identification of bioactive constituents within *L. parviflora*. The findings align with prior research on phytochemical profiling of medicinal plants. A study on *Maytenus emarginata* reported the presence of flavonoids, phenolics, tannins, and alkaloids, similar to the results obtained for *L. parviflora*. In addition, research on *Oroxylum indicum* and *Pongamia pinnata* demonstrated TLC-based flavonoid separation, reinforcing the methodology used in this study. The presence of quercetin-like compounds in *L. parviflora* corresponds with findings from polyherbal formulations, where flavonoids exhibited antioxidant and anti-diabetic properties.[14]

## Limitations

Despite successfully identifying secondary metabolites and establishing the TLC fingerprint of *L. parviflora*, certain limitations must be acknowledged. First, the study relied on qualitative phytochemical screening, which does not provide precise quantification of the detected compounds, necessitating more advanced techniques such as high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS) for accurate measurement. Second, the TLC method offers preliminary separation of compounds but does not confirm their chemical identity or purity, requiring further spectroscopic analyses such as UV-Vis, Fourier transform infrared, and nuclear magnetic resonance [NMR] to validate findings. In addition, bioactivity testing was not performed, meaning the therapeutic relevance of the identified flavonoids and phenolics remains speculative and needs *in vitro* and *in vivo* pharmacological studies.

## Future directions

Future research should focus on quantitative phytochemical profiling (HPLC, LC-MS, and NMR) for compound confirmation in *L. parviflora*. Preparative chromatography for bioactive compound isolation and structural elucidation is crucial. Bioactivity assays (antioxidant, anti-inflammatory, and antimicrobial) and pharmacokinetic/toxicological evaluations are needed to assess therapeutic potential and safety. Extraction optimization and geographical/seasonal

variation analyses should also be conducted to fully characterize the species' therapeutic value.

## CONCLUSION

This study provides a preliminary phytochemical characterization and TLC fingerprint of *L. parviflora*, revealing a diverse array of secondary metabolites, including flavonoids, phenolics, diterpenes, and sterols, thereby substantiating its traditional ethnomedicinal applications. The observed phytochemical richness, particularly the potential presence of quercetin-like compounds in methanolic and chloroform extracts, suggests a promising source of bioactive molecules with potential antioxidant and therapeutic properties. However, the qualitative nature of these findings necessitates further quantitative investigation utilizing advanced analytical techniques, coupled with bioactivity validation and exploration of environmental influences, to fully elucidate and harness the therapeutic potential of *L. parviflora*.

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