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Preliminary phytochemical characterization and lc–ms/ms-based metabolite profiling of *Bauhinia Tomentosa* L. from Northern Maharashtra, India

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Abstract
Bauhinia tomentosa L. is a medicinally important member of Fabaceae with reported traditional use and phytochemical diversity. The present study investigated the preliminary phytochemical composition of leaf and stem extracts collected from the Patnadevi forest region near Chalisgaon, Jalgaon District, Maharashtra, India, and profiled the ethanol leaf extract by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using electrospray ionization (ESI) in positive and negative modes. Dried leaf and stem powders were extracted separately with ethanol and petroleum ether by Soxhlet extraction. Qualitative screening indicated alkaloids, glycosides, phenolics, terpenoids, lipids, proteins and carbohydrates, with differences according to plant part and solvent. Ethanol extracts showed broader detection of polar and semi-polar constituents, whereas petroleum ether extracts were particularly positive for lipids and terpenoids. LC–MS/MS profiling produced a diverse chemical fingerprint. Positive-mode data were characterized mainly by putative flavonoid, flavonoid glycoside, phenolic and coumarin annotations, whereas negative-mode data showed prominent putative phenolic acids, organic acids, flavonoids and fatty acids. Reported annotations included apigenin, quercetin, isoquercitrin, genistein, ellagic acid, rosmarinic acid, umbelliferone, p-coumaric acid, ferulic acid, caffeic acid, linoleic acid and oleic acid. The dual-polarity approach broadened metabolite coverage. Because the instrumental data were based on spectral-library matching without authentic-standard confirmation or validated calibration curves, the compounds are interpreted as putative annotations rather than definitively identified or absolutely quantified constituents. The study provides a preliminary chemical fingerprint of B. tomentosa from northern Maharashtra and establishes a basis for targeted phytochemical and pharmacological investigations.

Keywords: *Bauhinia Tomentosa*, phytochemical screening, LC–MS/MS, electrospray ionization, Khandesh, Maharashtra

Introduction

Medicinal plants are important sources of structurally diverse natural products and have long contributed to traditional healthcare. Phytochemicals including alkaloids, phenolics, flavonoids, terpenoids and glycosides are commonly investigated because they may contribute to biological properties of plant-derived preparations (Harborne, 1998; Sofowora, 2008; Trease & Evans, 2002; Dhale, 2013; 2022; 2023; 2024; 2026; Dhale & Chamle, 2018) [1, 2, 3, 4, 5, 6, 9, 17, 18]. Preliminary phytochemical screening provides a rapid indication of major chemical classes, whereas chromatographic and mass-spectrometric techniques provide more detailed chemical fingerprints.

Bauhinia tomentosa L., commonly known as yellow bauhinia, belongs to Fabaceae and occurs in tropical and subtropical regions. Traditional and pharmacognostic literature has described medicinal relevance for different parts of the plant, and earlier studies have reported diverse phytochemical classes in its leaves and roots (Kirtikar & Basu, 2006; Kumar *et al*., 2011; Gautam *et al*., 2012) [8, 10, 12]. Pharmacognostic investigations of leaves have also reported alkaloids, flavonoids, glycosides, phenolics and related constituents (Rhama & Madhavan, 2012) [16].

Recent analytical investigations further support the chemical diversity of the species. Mini *et al*. (2021) [13] reported phytochemical screening and GC–MS characterization of *B. tomentosa* leaves, with phytol, n-hexadecanoic acid and squalene among prominent constituents. A recent flower study also demonstrated chemical diversity using GC–MS profiling (Trisha & Balaji, 2026) [19]. These findings indicate that plant part, geographical origin, extraction solvent and analytical platform can influence the observed chemical profile.

The present investigation was undertaken to characterize leaf and stem extracts of *B. tomentosa* collected from the Khandesh region of Maharashtra. Conventional qualitative screening was combined with LC–MS/MS profiling of the ethanol leaf extract in ESI positive and negative modes. The study emphasizes comparative phytochemical screening and exploratory metabolite annotation rather than validated absolute quantification.

Materials and Methods

1. Plant collection, authentication and voucher specimen

Fresh, healthy leaf and stem samples of *B. tomentosa* were collected from the Patnadevi forest region near Chalisgaon tehsil, Jalgaon District, Maharashtra, India (Fig. 1). The plant was authenticated using diagnostic morphological characters and the regional reference Flora of Jalgaon District by Kshirsagar and Patil (2008) [11]. A voucher specimen was prepared, labelled and deposited in the departmental herbarium of SSVPS's L. K. Dr. P. R. Ghogrey Science College, Dhule.

Fig. 1: Habit of selected plant *Bauhinia tomentosa* L.

2. Sample preparation

The collected materials were washed with tap water followed by distilled water, shade-dried at approximately 25–30 °C for 10–14 days, pulverized, sieved and stored in airtight containers at 4 °C until extraction.

3. Soxhlet extraction

Five grams of dried powdered leaf and stem material were extracted separately using ethanol and petroleum ether in a Soxhlet apparatus. Extraction was continued for approximately 6–8 h. Extracts were filtered through Whatman No. 1 filter paper and concentrated at approximately 40–45 °C. Crude extracts were stored at −18 °C until analysis. Ethanol was used as a polar solvent and petroleum ether as a non-polar solvent.

4. Preliminary phytochemical screening

Qualitative screening was performed in triplicate following standard procedures described by Harborne (1998), Sofowora (2008) and Trease and Evans (2002) [9, 17, 18]. Mayer's and Dragendorff's tests were used for alkaloids; Keller–Killiani and Borntrager's tests for glycosides; ferric chloride and lead acetate tests for phenolics; Salkowski reaction for terpenoids; Sudan III and grease-spot tests for lipids; Biuret and Xanthoproteic tests for proteins; and Molisch's and Benedict's tests for carbohydrates. Results were recorded semi-qualitatively as +, ++, +++ or ±; these scores represent test responses and are not concentration measurements.

5. LC–MS/MS analysis

The ethanol leaf extract was analysed using an LC–MS/MS system equipped with a triple-quadrupole/QTRAP mass analyser and electrospray ionization.

Untargeted screening with library matching was performed using the NIST 2023 [2] spectral library in ESI positive and negative modes. Chromatographic separation was carried out on a reverse-phase C18 column (250 × 4.6 mm, 5 µm) at 40 °C. Mobile phase A was water containing 0.1% formic acid and mobile phase B was acetonitrile: methanol (50:50, v/v). Gradient elution was performed at 0.6 mL/min with a 10 µL injection volume. Extracts were reconstituted in HPLC-grade methanol at 1 mg/mL and filtered through a 0.22 µm membrane before injection. Data processing was performed using SCIEX OS.

6. Data interpretation

The source project states that experiments were conducted in triplicate. However, the reported LC–MS/MS results are primarily library matches, retention times and peak areas rather than concentrations derived from authentic standards and calibration curves. Therefore, the LC–MS/MS results are treated here as exploratory/relative profiling and not validated absolute quantification.

Results

1. Preliminary phytochemical screening

The screening results demonstrated differences between plant parts and solvents. Ethanol extracts showed broader positive responses for alkaloids, glycosides, phenolics, proteins and carbohydrates, while petroleum ether extracts showed strong responses for lipids and detectable terpenoids. The leaf ethanol extract showed the strongest phenolic response (+++).

Table 1: Qualitative phytochemical screening of leaf and stem extracts of *B. tomentosa*.

Group	Test	Leaf EtOH	Leaf PE	Stem EtOH	Stem PE
Alkaloids	Mayer's	++	−	+	−
Alkaloids	Dragendorff's	++	−	+	−
Glycosides	Keller–Killiani	+	−	+	−
Glycosides	Borntrager's	+	−	±	−
Phenolics	Ferric chloride	+++	−	++	−
Phenolics	Lead acetate	+++	−	++	−
Terpenoids	Salkowski	+	++	++	++
Lipids	Sudan III	+	+++	+	+++
Lipids	Grease spot	+	+++	+	+++
Proteins	Biuret	++	−	±	−
Proteins	Xanthoproteic	++	−	±	−
Carbohydrates	Molisch's	++	−	+	−
Carbohydrates	Benedict's	++	−	±	−

Note: +++ = strongly present; ++ = moderately present; + = weakly present; ± = trace; − = absent.

2. LC–MS/MS profiling in ESI positive mode

The ethanol leaf extract produced a broad LC–MS/MS profile in positive ionization mode. Reported retention times extended from approximately 3.67 to 35.66 min, with precursor ions in the approximate range m/z 153–595. The project reported NIST 2023 [2] library match scores of 83.3–100%. Flavonoids, flavonoid glycosides, phenolic

derivatives and coumarins were prominent among the putative annotations.

Table 2: Summary of LC–MS/MS parameters reported for the ethanol leaf extract in ESI positive mode.

Sr. No.	Parameter	Observation
1	Ionization	ESI positive
2	Retention time	3.67–35.66 min
3	Precursor-ion range	m/z 153–595
4	Library	NIST 2023
5	Reported library scores	83.3–100%
6	Collision energy	30 eV

Table 3: Selected putative compound annotations reported in ESI positive mode.

Putative annotation	Class	Approx. RT (min)
Apigenin	Flavonoid	26.53
Quercetin	Flavonoid	~25
Isoquercitrin	Flavonoid glycoside	~24
Genistein	Isoflavone	~23
Ellagic acid	Polyphenol	~20
Rosmarinic acid	Phenolic acid	~18
Umbelliferone	Coumarin	~15
Kaempferol	Flavonol	Not specified
Daidzein	Isoflavone	Not specified

3. LC–MS/MS profiling in ESI negative mode

Negative-mode analysis showed a complementary profile with greater representation of acidic and deprotonatable metabolites. Reported retention times ranged from approximately 3.63 to 35.64 min and precursor ions were approximately m/z 115–447. The project reported library confidence values mostly ≥99–100%. Phenolic acids, organic acids, flavonoids and fatty acids were prominent.

Table 4: Selected putative compound annotations reported in ESI negative mode.

Putative annotation	Class	Approx. RT (min)
p-Hydroxybenzoic acid	Phenolic acid	~10
p-Coumaric acid	Phenolic acid	~12
Umbelliferone	Coumarin	~15
Esculetin	Coumarin	~16
Vanillin	Phenolic aldehyde	~14
Linoleic acid	Fatty acid	~30
Oleic acid	Fatty acid	~32
Isorhamnetin	Flavonoid	~25
Emodin	Anthraquinone	~28
Baicalein	Flavonoid	~26

4. Comparative interpretation of ionization modes

Table 5: Comparative distribution of phytochemical classes across ESI positive and negative modes.

Chemical class	ESI+	ESI−	Observation
Flavonoids	High	High	Detected in both modes
Flavonoid glycosides	High	Moderate	More evident in positive mode
Phenolic acids	Moderate	High	More evident in negative mode
Coumarins	Moderate	Moderate	Detected in both modes
Fatty acids	Low	High	Favoured in negative mode
Organic acids	Low	High	Favoured in negative mode
Simple phenolics	Moderate	High	More evident in negative mode

Discussion

The preliminary screening indicates that *B. tomentosa* contains diverse extractable phytochemical classes and that solvent choice influences the observed profile. Stronger responses for alkaloids, glycosides, phenolics, proteins and carbohydrates in ethanol extracts and the strong lipid response in petroleum ether extracts are consistent with solvent-selectivity principles (Harborne, 1998; Sofowora, 2008) [9, 17]. Similar qualitative diversity has been reported for B. tomentosa leaves and roots (Rhama & Madhavan, 2012; Gautam *et al*., 2012; Kumar *et al*., 2011) [12, 16].

The LC–MS/MS data provided a more detailed chemical fingerprint. Positive-mode analysis was richer in putative flavonoid and glycoside annotations, whereas negative-mode analysis emphasized phenolic acids, organic acids and fatty acids. Such complementary behaviour is expected because ESI positive mode favours protonation of suitable analytes, while negative mode favours deprotonation of acidic compounds (Niessen, 2006; Wolfender *et al*., 2015) [14, 20].

The findings are broadly consistent with previous studies. Mini *et al*. (2021) [13] reported phytol, n-hexadecanoic acid and squalene among major constituents in methanolic leaf extract using GC–MS. Trisha and Balaji (2026) [19] likewise demonstrated chemical diversity in B. tomentosa flowers using GC–MS. Differences among studies are expected because plant part, extraction solvent and analytical platform affect the observed metabolite profile.

Flavonoids and phenolic acids are widely studied for antioxidant and anti-inflammatory properties (Panche *et al*., 2016) [15]. However, the present experiment did not directly evaluate antioxidant, antibacterial, anti-inflammatory or anticancer activity; such activities are therefore not presented as experimental findings.

A methodological distinction is important for publication. In the absence of authentic standards, analyte-specific calibration curves, recovery, precision, matrix-effect and limit-of-quantification validation, the LC–MS/MS results are more appropriately described as putative annotations and relative peak-area observations rather than definitive identification or absolute quantification.

Conclusion

Bauhinia tomentosa L. collected from the Khandesh region of Maharashtra showed diverse phytochemical responses in leaf and stem tissues. Ethanol extracts gave broader qualitative responses for polar and semi-polar classes, whereas petroleum ether extracts showed strong lipid and terpenoid responses. LC–MS/MS profiling of the ethanol leaf extract revealed complementary chemical fingerprints in ESI positive and negative modes. The findings provide a preliminary LC–MS/MS chemical profile for regional B. tomentosa and support further targeted phytochemical investigation. Authentic-standard confirmation, validated quantitative analysis, compound isolation and appropriate biological and toxicological studies are recommended before therapeutic applications are proposed.

Declarations

1. Ethics statement

No human or animal subjects were involved in the present study.

2. Conflict of interest

The authors declare that no conflict of interest is associated with the present work.

3. Funding

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