

LC–MS/MS-Based Putative Metabolite Profiling of the Stem Ethanol Extract of *Bauhinia tomentosa* L.

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ABSTRACT

Bauhinia tomentosa L. is a medicinally important member of Fabaceae with a documented history of phytochemical investigation. The present study profiled the ethanol extract of *B. tomentosa* stem by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using electrospray ionization in positive and negative modes and NIST23 spectral-library matching. Dried powdered stem was extracted by Soxhlet extraction and analyzed on a triple-quadrupole/QTRAP platform coupled to reversed-phase C18 chromatography. The analytical reports contained 22 positive-mode and 28 negative-mode component annotations (50 total). Positive-mode library scores ranged from 25.6 to 100.0, while negative-mode scores ranged from 32.7 to 100.0. The profiles included flavonoids/flavan-3-ols, phenolic acids, coumarins, fatty acids, organic acids, anthraquinone-related compounds and terpenoid/volatile-type annotations. The study provides a stem-specific chemical fingerprint and identifies candidates for future targeted confirmation, isolation, quantitative analysis and comparative phytochemical investigation.

Keywords: *Bauhinia tomentosa*; LC–MS/MS; electrospray ionization; NIST23; metabolite profiling; putative annotation.

INTRODUCTION

The genus *Bauhinia* comprises medicinally important plants investigated for diverse phytochemical and pharmacological properties. Reported constituents across the genus include flavonoids, phenolic compounds, saponins, terpenoids and other specialized metabolites, while biological studies have described antioxidant, antimicrobial, anti-inflammatory, antidiabetic and related activities (Singh et al., 2023; Gudavalli et al., 2024; Verma et al., 2024; Dhale, 2026). This chemical breadth makes *Bauhinia* suitable for chromatographic and mass-spectrometric profiling.

Previous work on *B. tomentosa* has included phytochemical screening, biological assays, GC–MS profiling and isolation of individual constituents. Radha et al. (2016) isolated chemically characterized constituents from flowering buds, demonstrating that targeted isolation can establish structures that cannot be inferred from library matching alone. Janaki et al. (2018) investigated leaf extracts for phytochemicals and biological activities, while Mini et al. (2021) used GC–MS to characterize volatile and lipid-associated constituents in leaves. Reviews by Shanthini Nachiar and Sameemabegum (2023) and Kumar et al. (2025) consolidate the broader ethnopharmacological and phytochemical evidence for the species. These studies establish a strong background but are not equivalent to an untargeted, dual-polarity LC–MS/MS survey of the stem ethanol extract.

LC–MS/MS complements GC–MS by permitting chromatographic and tandem-mass investigation of compounds that are not necessarily volatile or sufficiently thermally stable for GC analysis. At the same time, LC–MS/MS library matching must be interpreted conservatively: spectral similarity alone does not necessarily distinguish positional isomers, stereoisomers or structurally related metabolites. The Metabolomics Standards Initiative and subsequent confidence frameworks therefore recommend explicit reporting of the evidence supporting metabolite annotation (Fiehn et al., 2007; Sumner et al., 2007; Schymanski et al., 2014; Blaženović et al., 2018).

The specific gap addressed here is the limited published characterization of the stem ethanol extract of *B. tomentosa* by LC–MS/MS using both ESI+ and ESI– acquisition. The objective was therefore to organize the available LC–MS/MS annotations into polarity-specific datasets, distinguish annotation confidence from definitive identification, compare the detected chemical space with earlier *B. tomentosa* reports, and identify suitable for future targeted validation.

MATERIALS AND METHODS

Plant material

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

Sample preparation and extraction

Collected stem material was cleaned to remove adhering soil and extraneous matter, shade-dried under ambient conditions, powdered using a mechanical grinder and stored in a clean, dry container until extraction. Five grams of dried powdered stem were extracted in a Soxhlet apparatus with ethanol and petroleum ether for approximately 6–8 h. The extracts were filtered through Whatman No. 1 filter paper and concentrated at approximately 40–45 °C. Crude extracts were stored at –18 °C. Only the ethanol extract was subjected to LC–MS/MS profiling. Ethanol was selected to recover a broad range of polar and moderately polar phytoconstituents.

LC–MS/MS instrumentation

LC–MS/MS analysis was performed using liquid chromatography coupled to an electrospray ionization (ESI) source and a triple-quadrupole/QTRAP mass analyzer. Data were acquired independently in positive (ESI+) and negative (ESI–) ionization modes and processed using SCIEX OS software. The available record specifies an approximate ion-spray/capillary voltage of 3–5 kV and a source temperature of approximately 450 °C, with nitrogen used as nebulizing gas.

Chromatographic conditions

Chromatographic separation was achieved using a reversed-phase C18 analytical column (250 × 4.6 mm, 5 µm). Solvent A was water containing 0.1% formic acid, while solvent B was reported as acetonitrile: methanol (50:50, v/v). The flow rate was 0.6 mL min^{–1}, injection volume 10 µL and column temperature 40 °C.

Data acquisition and processing

Raw LC–MS/MS data were processed using SCIEX OS. The workflow comprised chromatographic peak detection, spectral evaluation, precursor-mass assessment, fragmentation-spectrum assessment and comparison with NIST23 library spectra. Retention time, precursor m/z, detector response (peak area) and library similarity score were recorded for detected components. Positive- and negative-mode datasets were evaluated independently and subsequently compared for recurrent annotations.

Spectral-library matching and annotation confidence

Annotations were assigned from the combined evidence of precursor m/z, available MS/MS fragmentation information and NIST23 spectral similarity. All reported compounds are designated as putative library-based annotations. The library score is treated as an indicator of spectral similarity and not as a measure of concentration, purity, biological activity or structural certainty.

False-positive risk should be minimized by applying the following criteria in the final validated workflow: (i) retention-time consistency across replicate injections where available; (ii) precursor-mass agreement within the validated mass tolerance of the instrument method; (iii) agreement of diagnostic product ions with the library spectrum; (iv) appropriate polarity/adduct assignment; (v) exclusion of chemically implausible matches; (vi) blank/control comparison where available; (vii) duplicate or replicate confirmation where available; and (viii) authentic-standard confirmation for high-priority compounds.

RESULTS

Overview of LC–MS/MS profiling

The analytical report of stem contained 22 explicitly listed positive-mode component rows and 28 explicitly listed negative-mode component rows, giving 50 polarity-specific annotations in total. The positive-mode library scores ranged from 25.6 to 100.0 and the negative-mode scores from 32.7 to 100.0. The results are interpreted as library-supported putative annotations.

Positive ESI-mode annotations

Table 1. Putative metabolite annotations detected in positive ESI mode.

No.	Putative annotation	Retention time (min)	Precursor m/z	Product ions / fragment m/z	Peak area	Library score	Annotation confidence
1	Carvacrol	24.21	151	NR	1.610×10^6	100.0	Putative (NIST23 library)
2	Acetophenone	35.55	121	NR	1.453×10^4	100.0	Putative (NIST23 library)
3	Carnitine	4.35	162	NR	1.500×10^5	99.8	Putative (NIST23 library)
4	Eugenol	29.64	165	NR	1.046×10^5	99.6	Putative (NIST23 library)
5	Isoquercitrin	4.60	465	NR	1.314×10^5	99.1	Putative (NIST23 library)
6	Naringenin	25.33	273	NR	1.906×10^5	97.8	Putative (NIST23 library)
7	Geraniol	35.17	155	NR	1.146×10^5	97.6	Putative (NIST23 library)
8	Umbelliferone	27.57	163	NR	6.635×10^5	97.5	Putative (NIST23 library)
9	Apigenin	25.34	271	NR	3.063×10^5	95.7	Putative (NIST23 library)
10	Farnesol	28.94	223	NR	2.919×10^5	95.4	Putative (NIST23 library)



11	Coumarin	29.70	147	NR	4.717×10^4	94.9	Putative (NIST23 library)
12	Catechin	28.78	291	NR	3.922×10^5	86.4	Putative (NIST23 library)
13	Epicatechin	28.79	291	NR	3.789×10^5	86.4	Putative (NIST23 library)
14	Menthol	27.22	157	NR	2.207×10^4	83.7	Putative (NIST23 library)
15	Scopoletin	29.41	193	NR	2.545×10^4	75.2	Putative (NIST23 library)
16	Betaine	35.88	118	NR	1.252×10^4	73.9	Putative (NIST23 library)
17	Linalool	35.75	155	NR	7.266×10^3	73.3	Putative (NIST23 library)
18	Epigallocatechin	31.07	307	NR	3.024×10^4	42.4	Putative (NIST23 library)
19	Magnoflorine	26.31	342	NR	1.559×10^5	38.1	Putative (NIST23 library)
20	Quercetin	27.90	303	NR	1.309×10^4	28.4	Putative (NIST23 library)
21	Isorhamnetin	25.65	317	NR	8.083×10^4	26.3	Putative (NIST23 library)
22	Esculetin	28.47	179	NR	2.120×10^6	25.6	Putative (NIST23 library)

Annotation confidence is reported conservatively as putative (NIST23 library-based). Product-ion information was not available in the supplied result report and is therefore reported as NR (not reported). Library score is the reported NIST23 similarity score.

Negative ESI-mode annotations

Table 2. Putative metabolite annotations detected in negative ESI mode.

No.	Putative annotation	Retention time (min)	Precursor m/z	Product ions / fragment m/z	Peak area	Library score	Annotation confidence
1	p-Hydroxybenzoic acid	21.20	137	NR	4.548×10^5	100.0	Putative (NIST23 library)
2	p-Coumaric acid	22.97	163	NR	4.211×10^4	100.0	Putative (NIST23 library)
3	Esculetin	29.10	177	NR	6.761×10^5	100.0	Putative (NIST23 library)



4	Lawsone	32.74	173	NR	4.709×10^5	100.0	Putative (NIST23 library)
5	Oleic acid	29.62	281	NR	1.290×10^7	100.0	Putative (NIST23 library)
6	Linoleic acid	30.88	279	NR	8.191×10^6	100.0	Putative (NIST23 library)
7	Arachidic acid	31.54	311	NR	8.154×10^7	100.0	Putative (NIST23 library)
8	Maleic acid	34.98	115	NR	4.028×10^5	100.0	Putative (NIST23 library)
9	Palmitic acid	0.04	255	NR	1.218×10^6	99.0	Putative (NIST23 library)
10	Baicalein	32.15	269	NR	2.304×10^5	98.2	Putative (NIST23 library)
11	Juglone	32.56	173	NR	1.151×10^4	98.1	Putative (NIST23 library)
12	Catechin	32.80	289	NR	3.049×10^5	97.1	Putative (NIST23 library)
13	Epicatechin	32.80	289	NR	3.070×10^5	97.1	Putative (NIST23 library)
14	Behenic acid	25.73	339	NR	2.647×10^5	96.9	Putative (NIST23 library)
15	Isorhamnetin	25.69	315	NR	9.916×10^4	96.8	Putative (NIST23 library)
16	Succinic acid	8.30	117	NR	4.168×10^5	96.3	Putative (NIST23 library)
17	Malic acid	4.95	133	NR	5.519×10^5	95.6	Putative (NIST23 library)
18	Azelaic acid	22.90	187	NR	5.505×10^6	94.3	Putative (NIST23 library)
19	Emodin	32.15	269	NR	2.176×10^5	92.4	Putative (NIST23 library)
20	Aloe-emodin	29.19	269	NR	1.269×10^4	92.3	Putative (NIST23 library)
21	Protocatechuic acid	14.22	153	NR	7.947×10^4	89.4	Putative (NIST23 library)
22	Linolenic acid	29.70	277	NR	1.192×10^4	87.4	Putative



					10^6		(NIST23 library)
23	Gallic acid	26.56	169	NR	1.350×10^5	81.8	Putative (NIST23 library)
24	Coumarin	0.10	147	NR	6.838×10^6	81.5	Putative (NIST23 library)
25	Caffeic acid	33.49	179	NR	4.727×10^5	79.7	Putative (NIST23 library)
26	Gentisic acid	14.21	153	NR	8.724×10^4	72.7	Putative (NIST23 library)
27	Vanillic acid	17.85	167	NR	3.266×10^4	72.5	Putative (NIST23 library)
28	Quercetin	29.41	301	NR	1.806×10^5	32.7	Putative (NIST23 library)

Annotation confidence is reported conservatively as putative (NIST23 library-based). Product-ion information was not available in the supplied result report and is therefore reported as NR (not reported). Library score is the reported NIST23 similarity score.

Compounds observed in both ionization modes

Observation	ESI+	ESI−	Interpretation
Total annotated components	22	28	50 polarity-specific component records
Library-score range	25.6–100.0	32.7–100.0	Similarity indicator, not structural certainty
Highest peak area	Esculetin: 2.120×10^6	Arachidic acid: 8.154×10^7	Detector response, not concentration
Detected in both modes	6	6	Catechin, epicatechin, quercetin, isorhamnetin, esculetin, coumarin
Product-ion information	NR	NR	-

Six annotations occurred in both polarity-specific datasets: catechin, epicatechin, quercetin, isorhamnetin, esculetin and coumarin. Repeated observation in both modes is supportive of their analytical recurrence in the sample, but it does not independently establish structural identity because ionization, adduct formation and fragmentation behaviour can differ between ESI+ and ESI−. The overlap should therefore be regarded as a prioritization criterion for targeted confirmation rather than as confirmation itself (Fiehn et al., 2007; Schymanski et al., 2014).

Comparison with previous reports of *B. tomentosa*

The comparison is deliberately conservative. A compound is marked as previously reported only where the supplied *B. tomentosa* literature supports its occurrence or where the literature provides only a broader class-level indication. Accordingly, 'detected as putative annotation' does not mean that the compound is newly discovered in *B. tomentosa*. Earlier studies have focused on leaves, flowering buds and broader phytochemical/GC–MS characterization, leaving the present stem-specific dual-polarity LC–MS/MS dataset as



a complementary analytical record (Janaki et al., 2018; Mini et al., 2021; Radha et al., 2016; Kumar et al., 2025).

Table 3. Comparison with previous reports of *B. tomentosa*

Compound	Evidence in supplied <i>B. tomentosa</i> literature	Status in present study
Isoquercitrin	-	Detected as putative annotation
Naringenin	-	Detected as putative annotation
Apigenin	-	Detected as putative annotation
Catechin	-	Detected as putative annotation
Epicatechin	-	Detected as putative annotation
Epigallocatechin	-	Detected as putative annotation
Quercetin	-	Detected as putative annotation
Isorhamnetin	-	Detected as putative annotation
p-Hydroxybenzoic acid	Reported phenolic constituents; exact compound not established in supplied references	Detected as putative annotation
p-Coumaric acid	Reported phenolic constituents; exact compound not established in supplied references	Detected as putative annotation
Protocatechuic acid	Reported phenolic constituents; exact compound not established in supplied references	Detected as putative annotation
Gallic acid	Reported phenolic constituents; exact compound not established in supplied references	Detected as putative annotation
Caffeic acid	Reported phenolic constituents; exact compound not established in supplied references	Detected as putative annotation
Gentisic acid	-	Detected as putative annotation
Vanillic acid	-	Detected as putative annotation
Coumarin	-	Detected as putative annotation
Umbelliferone	-	Detected as putative annotation
Scopoletin	-	Detected as putative annotation
Esculetin	-	Detected as putative annotation
Carvacrol	-	Detected as putative annotation
Eugenol	-	Detected as putative annotation
Geraniol	-	Detected as putative annotation



Farnesol	-	Detected as putative annotation
Menthol	-	Detected as putative annotation
Linalool	-	Detected as putative annotation
Palmitic acid	GC–MS literature indicates lipid-associated compounds, but exact match not established from supplied references	Detected as putative annotation
Oleic acid	GC–MS literature indicates lipid-associated compounds, but exact match not established from supplied references	Detected as putative annotation
Linoleic acid	GC–MS literature indicates lipid-associated compounds, but exact match not established from supplied references	Detected as putative annotation
Linolenic acid	-	Detected as putative annotation
Arachidic acid	-	Detected as putative annotation
Behenic acid	-	Detected as putative annotation
Emodin	-	Detected as putative annotation
Aloe-emodin	-	Detected as putative annotation
Juglone	-	Detected as putative annotation
Baicalein	-	Detected as putative annotation
Lawsone	-	Detected as putative annotation
Maleic acid	-	Detected as putative annotation
Succinic acid	-	Detected as putative annotation
Malic acid	-	Detected as putative annotation
Azelaic acid	-	Detected as putative annotation
Acetophenone	-	Detected as putative annotation
Carnitine	-	Detected as putative annotation
Betaine	-	Detected as putative annotation
Magnoflorine	-	Detected as putative annotation

DISCUSSION

Phytochemical breadth and complementarity with earlier studies

The dual-polarity LC–MS/MS profile demonstrates substantial chemical heterogeneity in the ethanol extract of *B. tomentosa* stem. The annotated chemical space includes flavonoids and flavan-3-ols, phenolic acids,

coumarin-type compounds, fatty acids, organic acids and several terpenoid/volatile-type constituents. This broad profile is consistent with the wider phytochemical diversity reported for *Bauhinia* and with earlier investigations of *B. tomentosa* (Gudavalli et al., 2024; Kumar et al., 2025; Shanthini Nachiar & Sameemabegum, 2023). The present LC–MS/MS dataset complements, rather than duplicates, previous GC–MS and isolation-based studies: GC–MS has been used to characterize volatile and lipid-associated constituents in *B. tomentosa*, whereas isolation work provides stronger structural evidence through purified compounds and orthogonal characterization (Mini et al., 2021; Radha et al., 2016).

The positive-mode dataset included isoquercitrin, naringenin, apigenin, catechin, epicatechin, epigallocatechin, quercetin and isorhamnetin. The negative-mode dataset additionally annotated baicalein and several phenolic acids. This flavonoid-rich pattern is compatible with earlier phytochemical reports on *B. tomentosa*, including studies demonstrating phenolic and flavonoid constituents and antioxidant-related activity in leaf extracts (Janaki et al., 2018; Singh et al., 2023). Reviews of the species and genus likewise describe flavonoids and phenolic compounds as important components of *Bauhinia* phytochemistry (Gudavalli et al., 2024; Kumar et al., 2025). Nevertheless, the present peak areas are detector responses, not concentrations, and neither library score nor peak area establishes pharmacological potency. Catechin, epicatechin, quercetin and isorhamnetin, which recur across both polarities, are particularly suitable for authentic-standard confirmation.

Coumarin, umbelliferone, scopoletin and esculetin were annotated in positive mode, while coumarin and esculetin were also detected in negative mode. Esculetin showed the largest positive-mode peak area among the listed positive annotations (2.120×10^6), despite a comparatively low library score (25.6), illustrating why peak area and library similarity must not be conflated. Coumarin-type metabolites have been discussed within the broader phytochemical literature on *B. tomentosa* (Shanthini Nachiar & Sameemabegum, 2023; Kumar et al., 2025). Because structurally related compounds can produce similar mass spectra, retention-time agreement with an authentic standard and diagnostic product-ion matching are required before definitive identification (Schymanski et al., 2014).

The negative-mode dataset contained palmitic, oleic, linoleic, linolenic, arachidic and behenic acid annotations. Arachidic acid produced the largest reported peak area in the entire negative-mode dataset (8.154×10^7), followed by oleic acid (1.290×10^7) and linoleic acid (8.191×10^6). These values represent detector response and cannot be interpreted as relative concentration without standards, calibration and matrix-validated quantitation. Earlier GC–MS work on *B. tomentosa* supports the occurrence of lipid-associated constituents, providing complementary context for the present fatty-acid annotations (Mini et al., 2021). Positive-mode annotations included carvacrol, eugenol, geraniol, farnesol, menthol and linalool. Since several volatile constituents are more conventionally characterized by GC–MS, their LC–MS/MS library assignments should remain screening-level observations until confirmed by authentic standards or orthogonal analytical evidence (Mini et al., 2021).

The simultaneous observation of flavonoids, phenolic acids, coumarins, fatty acids and terpenoid/volatile-type annotations suggests a chemically diverse stem extract and provides for future chemotaxonomic study. However, a single extract from one geographic population cannot establish chemotaxonomic markers. Comparative studies should use replicated sampling, standardized extraction, validated quantitative methods and multiple *Bauhinia* taxa. The wider literature documents antioxidant, antimicrobial and other biological activities for *B. tomentosa* and *Bauhinia* species (Janaki et al., 2018; Singh et al., 2023; Gudavalli et al., 2024), but these reports do not demonstrate that the present LC–MS/MS annotations are responsible for activity in the stem extract. Bioassay-guided fractionation and standard-based quantitation are therefore needed to establish biological relevance.

CONCLUSION

LC–MS/MS profiling of the ethanol extract of *Bauhinia tomentosa* L. stem revealed 22 positive-mode and 28 negative-mode putative annotations, representing 50 polarity-specific component records. The annotated profile comprised diverse chemical classes, including flavonoids and flavan-3-ols, phenolic acids, coumarins, fatty acids, organic acids, anthraquinone-related compounds, and terpenoid/volatile-type constituents. Catechin, epicatechin, quercetin, isorhamnetin, esculetin, and coumarin were detected in both ionization

modes, indicating their consistent representation within the LC–MS/MS fingerprint. The present findings provide a stem-specific chemical profile of *B. tomentosa* and complement previously reported phytochemical, GC–MS, and isolation studies of the species. Further studies should incorporate full MS/MS acquisition parameters and diagnostic product ions, authentic-standard confirmation, validated quantitative analysis, and replicated sampling across populations. Such validation will strengthen the chemical characterization and provide a more reliable basis for evaluating the phytochemical significance of *B. tomentosa* stem.

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Conflict of Interest

The author declares no conflict of interest.

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