

Untargeted LC–MS Metabolite Profiling and Antibacterial Activity of *Lasianthus verticillatus* (Lour.) Merr. Leaf Extracts

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ABSTRACT

Background:

Lasianthus verticillatus (Rubiaceae) has a long history in traditional medicine, yet its chemical composition and antibacterial potential have not been fully explored. This study aimed to identify the bioactive compounds in plant extract and evaluate their effectiveness against common bacterial strains.

Methods:

Leaves were extracted with methanol, acetone, and chloroform and then analyzed via Liquid chromatography-mass spectroscopy (LC–MS) in positive and negative ion modes. The compounds were tentatively annotated based on their retention times, mass-to-charge ratios, and database comparisons. Antibacterial activity was tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Pseudomonas aeruginosa* via agar well diffusion at concentrations of 2.5, 5, and 10 µg/mL. One-way ANOVA followed by Tukey's HSD test was used to determine statistical significance ($p < 0.05$).

Results:

The analysis revealed a diverse range of phytochemicals, including naphthoquinones (juglone, isoplumbagin, lawsone), flavonoids (kaempferol derivatives, chlorogenic acid), triterpenoids, coumarins, fatty acids, and several antibiotic-like compounds (clindamycin, mupirocin, capreomycin). Antibacterial tests clearly revealed dose-dependent effects: *B. subtilis* and *P. aeruginosa* were the most sensitive, *S. mutans* showed moderate sensitivity, and *S. aureus* was relatively resistant.

Conclusions:

This study highlights that *L. verticillatus* contains a rich variety of bioactive compounds capable of inhibiting both gram-positive and gram-negative bacteria. These findings support the traditional medicinal use of *L. verticillatus* and reveal promising antibacterial compounds, particularly quinones and flavonoids, warranting further mechanistic studies.

Keywords: *Phytochemical composition; LC–MS profiling; Electrospray ionization; Inhibition; antibacterial activity*

Introduction

Medicinal plants offer diverse bioactive secondary metabolites, driving interest in underexplored species [1]. *Lasianthus verticillatus* (Lour.) Merr. (syn. *Lasianthus truncatus* Bedd.), locally known as *Lasianthus truncatus*, is a medicinal plant belonging to the family Rubiaceae. It is traditionally valued for anti-inflammatory and wound-healing properties, yet its phytochemical composition and biological activities remain insufficiently studied.

Liquid chromatography-mass spectrometry (LC-MS), especially coupled with tandem MS and high resolution, has become a powerful tool for plant metabolite profiling [2], allowing sensitive, selective detection and tentative annotation of complex phytochemical mixtures. Untargeted LC–MS metabolomics has been widely applied for the characterization of plant secondary metabolites, the exploration of metabolic diversity, and the assessment of biochemical responses to environmental or physiological stimuli [3]. Retention-time data and spectral libraries, combined with accurate mass measurements, further increase the confidence in metabolite annotation in complex plant matrices.

Increased interest in antimicrobial compounds from plants has also been attributed to increased antibiotic resistance [4]. The chemical diversity of secondary metabolites in medicinal plants, which can exert their effects by membrane disruption, inhibition of enzymes, or altered cellular transport, still makes them a promising source of antibacterial agents [5]. Nevertheless, the comprehensive LC–MS profile of *L. verticillatus* and its relation to antibacterial activity remains poorly explored. Previous studies have demonstrated that the antibacterial activity

is highly dependent on the extraction solvent, phytochemical composition, and screening method, highlighting the significance of correlating the chemical profiles with the biological outcomes.

Therefore, the study was designed to (i) build an LC–MS-based phytochemical profile of *L. verticillatus* leaf extracts in solvents of varying polarity and (ii) evaluate their antibacterial activity against Gram-positive (*B. subtilis*, *S. aureus*, *S. mutans*) and Gram-negative (*P. aeruginosa*) strains [6], offering a chemical foundation for the traditional use of *L. verticillatus* and one of the first integrations of LC–MS profiling with antibacterial screening in this species.

Materials and Methods

Collection of plant material

The fresh leaves of *Lasianthus verticillatus* (Lour.) Merr. (syn. *Lasianthus truncatus* Bedd.) were collected from Minumuluru, Paderu, Andhra Pradesh, India (latitude: 18.041292°, longitude: 82.707041°), and transported aseptically to the laboratory for extract preparation. The samples were carefully rinsed under tap water, followed by washing with distilled water to remove soil particles and other surface contaminants. The leaves were then shade-dried at room temperature and stored under laboratory conditions until extraction.

Extraction

The dried plant material was powdered and extracted with different solvents by using Soxhlet apparatus. Approximately 100 g of powder was placed in the thimble chamber, and 250 mL of solvent (methanol, chloroform or acetone) was cycled continuously through the material. The extraction was continued for 24 h or until the solvent in the syphon tube was colourless.

The resulting extracts were concentrated by solvent evaporation at the respective solvent boiling point for 8–14 hours, yielding viscous concentrates, which were further dried and stored at 4 °C until analysis. Qualitative phytochemical screening of each extract was performed following established classical protocols [7]. Extraction temperature corresponded to the boiling point of the respective solvents.

LC–MS analysis

Extracts (dissolved in HPLC-grade methanol, 0.22 µm filtered) were analyzed on an Agilent 6545 Q-TOF LC–MS at SAIF, IIT Bombay (supported by I-STEM, Government of India) [8]. Mobile phases were 0.1% formic acid in water (A) and acetonitrile (B), gradient 5–95% B over 30 min at 0.3 mL/min, with positive/negative ESI scanning *m/z* 100–1000. Data (MassHunter software) were tentatively annotated using accurate mass (± 10 ppm), retention time, isotopic pattern, and METLIN/PubChem matching; no reference-standard confirmation was performed, and calibration was routinely verified.

Antibacterial activity

Antibacterial activity against *B. subtilis*, *S. aureus*, *S. mutans*, and *P. aeruginosa* was assessed via agar well diffusion. Bacterial suspensions (1×10^6 CFU/mL) were plated, and wells (6 mm) received 100 µL extract (2.5, 5, 10 µg/mL in DMSO) [9], with DMSO as negative and ampicillin (same concentration throughout) as positive control. Plates were incubated (37°C, 24 h) and inhibition zones measured in triplicate (mean $\pm$ SD), analyzed via one-way ANOVA with Tukey's post hoc test ($p < 0.05$) [10].

Quality Assurance and Quality Control

All studies followed defined protocols using calibrated instruments and analytical-grade reagents. Assays were performed in triplicate with appropriate controls. LC–MS annotation relied on accurate mass, isotopic pattern, retention time, and database matching, while antibacterial assays used standard antibiotics for validation.

Results and Discussion

LC-MS Analysis

The LC–MS study of *L. verticillatus* leaf extracts (acetone, chloroform, and methanol) in positive and negative ESI modes resulted in the tentative annotation of 54 compounds [11], based on retention time, accurate *m/z* (± 10 ppm), isotopic pattern, and database matching [12]. Table 1 shows the mass spectral/chromatographic data; Table 2 and the supplementary materials summarize the class-wise distribution (quinones, flavonoids, phenolics, alkaloids, terpenoids, neolignans, fatty acids, glycosides, esters, lipid-derived chemicals) across extracts and modes.

Positive ESI mode detected 21 tentatively annotated compounds (acetone: 9, chloroform: 6, methanol: 6), ranging from low/medium molecular weight chemicals to high-mass quinones, alkaloids, lignans, and terpenoids. Early-eluting peaks were tentatively associated with aromatic and quinonoid compounds, followed by annotations corresponding to alkaloids, terpenoids, and other complex metabolites [13]. Increased signal intensities correlated with higher relative abundance and consistent detection across runs supported reproducibility [14], validating the utility of dual mode profiling.

In negative ESI mode 33 tentatively annotated compounds were detected (acetone: 12, chloroform: 9, methanol: 12) giving a broader coverage than positive mode [15] and including phenolic acids, glycosylated metabolites, fatty acids and lipids, with early peaks mainly organic acids/conjugated phenolics and later peaks oxylipins and higher-mass secondary metabolites. This mode also enabled the detection of glycosylated and esterified metabolites, with flavonoids and phenolic compounds detected across the extracts, particularly in methanol and acetone extracts [16]. Combined use of multiple solvents and dual ESI modes provided broader metabolite coverage. Quinone and naphthoquinone derivatives were detected across multiple extracts and ionization modes. Annotations, however, remain uncertain in the absence of isolated-compound confirmation; repeated detections at various retention durations are likely to be isomers, and annotations with higher mass errors were interpreted with caution.

Table 1. Tentative LC-MS annotation of compounds in *Lasianthus verticillatus* leaf extracts obtained using acetone, chloroform, and methanol.

LA1 ESI +VE							
S.NO	Name	Formula	RT	Mass	Abundance	DB Diff (ppm)	Hits (DB)
1.	Benzaldehyde	C7 H6 O	1.241	106.0423	116836	-3.66	1
2.	Juglone	C10 H6 O3	3.654	174.0316	231706	0.35	8
3.	Isoplumbagin	C11 H8 O3	4.541	188.047	-	1.59	10
4.	Bruceantinol	C30 H38 O13	5.125	606.226	-	8.65	1
5.	Myristicanol B	C22 H28 O7	8.341	404.1805	440528	7.47	10
6.	Cinnassiol C3	C20 H30 O7	8.344	382.1984	-	2.03	10
7.	Ganoderic acid F	C32 H42 O9	18.11 3	570.2816	-	1.82	2
8.	Ganoderic acid F	C32 H42 O9	18.50 2	570.2818	379011	1.82	2
9.	Haplophytine	C37 H40 N4 O7	19.34 2	652.2855	-	6.47	2
LA1 ESI -VE							
10.	Citric acid	C6 H8 O7	1.239	192.0264	3088	2.93	
11.	Dihydroferulic acid 4-Oglucuronide	C16 H20 O10	1.508	372.1038	-	4.93	6
12.	Apiosylglucosyl 4-hydroxybenzoate	C18 H24 O12	3.842	432.1255	-	2.96	8
13.	trans-Chlorogenic acid	C16 H18 O9	4.409	354.0935	-	4.51	10
14.	trans-Caffeic acid [apiosyl-(1->6)-glucosyl] ester	C20 H26 O13	6.578	474.135	-	4.91	5
15.	Azelaic acid	C9 H16 O4	7.543	188.1038	-	5.78	10
16.	alpha-Crocetin glucosyl ester	C26 H34 O9	8.845	490.219	-	2.53	7
17.	Thalidasine	C39 H44 N2 O7	15.65 3	652.318	-	-4.84	4
18.	9-HOTE	C18 H30 O3	15.66	294.2177	-	5.98	10
19.	α -Linolenic Acid	C18 H30 O2	17.19 7	278.2226	-	6.95	10
20.	Petroselinic acid	C18 H34 O2	19.48 2	282.2536	-	8.17	6

21.	C-13(2)-Carboxypyrrophenorbide a	C34 H34 N4 O5	21.056	578.2501	-	4.92	5
LC1 ESI +Ve							
22.	Lawson	C10 H6 O3	3.993	174.031	129765	4.18	10
23.	Isoplumbagi	C11 H8 O3	4.565	188.0465	-	4.53	10
24.	(+)-Carnegine	C13 H19 N O2	4.8	221.1416	58976	-0.24	9
25.	Myristicanol B	C22 H28 O7	8.355	404.1801	314901	8.46	10
26.	(S)-Auraptanol	C15 H16 O4	10.558	260.1051	-	-0.99	10
27.	D8'-Merulinic acid A	C24 H38 O4	20.143	390.2732	-	9.8	10
LC1 ESI -Ve							
28.	Asperuloside	C18 H22 O11	4.246	414.1162	-	0.06	2
29.	trans-Chlorogenic acid	C16 H18 O9	4.438	354.0942	6510	2.56	
30.	m-Coumaric acid	C9 H8 O3	5.544	164.0469	417	2.43	
31.	Benzyl benzoate	C14 H12 O2	11.817	212.0816	14622	9.92	10
32.	9(S)-HpOTrE	C18 H30 O4	14.567	310.2131	-	4.25	10
33.	9-HOTE	C18 H30 O3	15.688	294.2184	-	3.71	10
34.	α -Linolenic Acid	C18 H30 O2	16.965	278.2233	-	4.76	10
35.	Pheophorbide a	C35 H36 N4 O5	21.729	592.2669	-	2.86	3
36.	Rapanone	C19 H30 O4	27.071	322.2162	-	-5.61	10
LM1 ESI +VE							
37.	Lawson	C10 H6 O3	3.129	174.0309	65062	4.52	10
38.	Vanillactic acid	C10 H12 O5	3.607	212.069	-	-2.49	10
39.	Vanillactic acid	C10 H12 O5	3.949	212.0688	-	-1.32	10
40.	Isoplumbagin	C11 H8 O3	4.527	188.0469	-	2.41	10
41.	Myristicanol B	C22 H28 O7	8.323	404.1805	-	7.47	10
42.	D8'-Merulinic acid A	C24 H38 O4	20.168	390.2732	-	9.74	10
LM1 ESI-VE							
43.	Monotropein	C16 H22 O11	1.342	390.1148	-	3.62	7
44.	Asperuloside	C18 H22 O11	4.288	414.1156	-	1.47	2
45.	Kaempferol 3- [2"-glucosyl-6"- acetyl-galactoside] 7-glucoside	C35 H42 O22	6.741	814.2155	-	1.55	2
46.	Erinapyrone C	C8 H10 O5	7.005	186.0519	-	4.76	10
47.	9(S)-HpOTrE	C18 H30 O4	13.991	310.2125	-	6.01	10
48.	9-HOTE	C18 H30 O3	15.689	294.2176	-	6.36	10
49.	α -Linolenic Acid	C18 H30 O2	16.973	278.2223	-	8.36	10
50.	5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-3- heptanone	C19 H22 O3	18.631	298.1574	22417	-1.8	7
51.	5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-3- heptanone	C19 H22 O3	19.012	298.1575	-	-2.16	7

52.	C-13(2)-Carboxypyropheophorbide a	C34 H34 N4 O5	21.104	578.2497	-	5.62	5
53.	Petroselinic acid	C18 H34 O2	22.772	282.2542	-	6.08	6
54.	Stearic acid	C18 H36 O2	24.462	284.2696	-	6.66	1

Anti-bacterial activity

The antibacterial activity of *L. verticillatus* leaf extracts was evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans* and *Pseudomonas aeruginosa* using agar well diffusion method at the concentrations of 2.5, 5 and 10 µg/mL [17]. Evaluation of extracts made in methanol, acetone, chloroform extract. Zones of inhibition are represented as mean ± SD. Statistical analysis was done using one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

Across all studied bacterial strains, significant variations in antibacterial activity were found based on solvent type and concentration ($p < 0.05$). Differential susceptibility patterns among different bacterial species were observed.

Bacillus subtilis

All the extracts showed significant antibacterial activity against *B. subtilis*, and the zone of inhibition increased with the concentration ($F(2,33) = 33.59$, $p < 0.001$). Average inhibition zones of 9.00 ± 5.80 mm (2.5 µg/mL), 15.67 ± 2.93 mm (5 µg/mL) and 24.08 ± 4.36 mm (10 µg/mL) were observed [18].

Post hoc analysis revealed significant variations between the lowest and the highest concentrations. A solvent-dependent effect was also observed ($F(3,32) = 4.38$, $p = 0.011$).

Pseudomonas aeruginosa

P. aeruginosa was significantly influenced by solvent type ($F(3,32) = 7.97$, $p < 0.001$) and concentration ($F(2,33) = 17.92$, $p < 0.001$). The acetone, chloroform, and methanol extracts showed increasing inhibition with increasing concentration, with the highest inhibition observed at 10 µg/mL [19]. The inhibitory zones increased with concentration to 19.92 ± 6.11 mm at 10 µg/mL.

Streptococcus mutans

A substantial effect of solvent was detected for *S. mutans* ($F(3,32) = 16.56$, $p < 0.001$).

The maximum inhibition was observed with the acetone extract at 10 µg/mL, followed by the chloroform extract, whereas methanolic extract showed detectable activity (11.33 ± 0.58 mm). No inhibition was seen for methanol at lower quantity [20].

L. verticillatus showed statistically significant, dose-dependent antibacterial activity against *B. subtilis* and *P. aeruginosa* retaining strongest activity, suggesting synergistic phytochemical interactions.

Table 2.: Comparison of the antibacterial activity (zone of inhibition in mm) of acetone, chloroform, and methanol extracts of *Lasianthus verticillatus* against the bacterial strains tested at different concentrations.

Bacterium	Concentration (µg/mL)	Acetone (mm)	Chloroform (mm)	Methanol (mm)	Control (mm)
<i>Bacillus subtilis</i>	2.5	9.00 ± 5.80^b	8.67 ± 6.11^b	9.33 ± 5.22^b	21.67 ± 6.61^a
	5	15.67 ± 2.93^a	15.33 ± 3.10^a	15.00 ± 2.89^a	22.10 ± 5.60^a
	10	24.08 ± 4.36^a	23.75 ± 4.20^a	24.33 ± 3.90^a	25.00 ± 4.01^a

<i>Pseudomonas aeruginosa</i>	2.5	8.00 ± 4.33^c	7.75 ± 3.99^c	8.25 ± 4.05^c	21.67 ± 6.61^a
	5	14.33 ± 3.92^b	14.00 ± 3.75^b	13.67 ± 3.90^b	22.33 ± 6.22^a
	10	19.92 ± 6.11^a	20.08 ± 5.90^a	19.67 ± 5.80^a	23.10 ± 6.00^a
<i>Streptococcus mutans</i>	—	11.44 ± 5.20^b	9.78 ± 4.15^b	3.78 ± 5.67^c	21.67 ± 6.61^a
<i>Staphylococcus aureus</i>	—	3.75 ± 6.78^a	6.42 ± 8.54^a	9.92 ± 12.80^a	12.10 ± 11.90^a

The values are expressed as means $\pm$ standard deviations (n = 3). Different superscript letters within a row indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test.

Conclusion

The present investigation demonstrated that the antibacterial activity of *Lasianthus verticillatus* is strongly influenced by the choice of extraction solvent, reflecting solvent-specific differences in phytochemical composition and polarity. The methanolic and chloroform extracts exhibited notable inhibitory effects against both gram-positive and gram-negative bacteria. Among the tested strains, *Bacillus subtilis*, *Streptococcus mutans*, and *Pseudomonas aeruginosa* presented clear zones of inhibition. Among the tested strains, *Bacillus subtilis*, *Streptococcus mutans*, and *Pseudomonas aeruginosa* exhibited clear zones of inhibition, whereas *Staphylococcus aureus* showed comparatively lower antibacterial susceptibility.

Overall, the findings highlight the selective antibacterial spectrum of *L. verticillatus* and emphasize the critical influence of solvent extraction efficiency and the bacterial cell wall structure on antimicrobial activity. The LC–MS tentatively annotated compounds—such as lawsone, isoplumbagin, and kaempferol derivatives—are likely contributors to the observed activity. These insights reinforce the ethnomedicinal significance of the species and pave the way for future work focusing on bioactive fractionation, molecular docking, and mechanistic studies to elucidate specific antibacterial pathways in this Rubiaceae member.

Artificial Intelligence Statement

The authors used ChatGPT (OpenAI, USA) solely for language refinement and manuscript editing assistance. No data analysis, interpretation, or scientific content generation was performed using artificial intelligence tools.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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