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Research Article

Metabolic profiling of Kokilaksha kashaya, a single herb formulation through high resolution liquid chromatography mass spectrometry quadrupole time of flight (HR LCMS QTOF)

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Abstract

The single plant used to make *Kokilaksha Kashaya,* an Ayurvedic medicine that has long been used to treat arthritic conditions like osteoarthritis, gout, and rheumatoid arthritis, is *Asteracantha longifolia Nees*, also known as *Kokilaksha. Kokilaksha* is used as a food supplement and in medicinal infusions because of its well-known diuretic, hepatoprotective, and anti-inflammatory qualities. The decoction is specially advised for inflammatory diseases, liver difficulties, urinary tract problems, and infertility. Eating it as a leafy vegetable improves general health and vigor. The comprehensive investigation of small-molecule metabolites in biological systems, referred to as metabolomics, has emerged as a crucial tool in research. It is widely used in drug discovery, disease diagnostics, and nutritional research and offers important insights into drug metabolism. In this study, using extensive phytochemical profiling through HR LCMS QTOF (High-Resolution Liquid Chromatography–Mass Spectrometry Quadrupole Time of Flight), we put forward a scientific basis for the traditional uses of *Kokilaksha Kashaya*. Approximately 54 bioactive metabolites, such as flavonoids, glycosides, organic acids, and other secondary metabolites, were found by analytical results. For accurate compound identification, each compound was then outlined further using precise mass-to-charge ratios (m/z), time of retention (RT), predicted molecular formulae, and database match scores (Hits/DB). Among them notable compounds found were 1-Octen-3-yl glucoside, 10-epi-Eupatoroxin, 1-O-Sinapoylglucose and N5-Dinitrophenyl-L-ornithine methyl ester. Future pharmacological and clinical research can be built upon these findings, which offer compelling scientific evidence for the traditional use of the water-soluble extract of *Kokilaksha* and highlight its potential as a source of naturally derived medicinal compounds.

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Keywords: Metabolites, LCMS, QTOF, *Kokilaksha*, Flavonoids, Glycosides, Ayurveda.

Introduction

The World Health Organization (WHO) recognized traditional herbal medicine as an indispensable component of basic healthcare systems. About 11 percent of the WHO's 252 essential drugs originate from plants. Growing interest in natural and integrative healthcare techniques has led to a rapid increase in demand for medicinal plants. Scientific research addressing the possible medicinal benefits of numerous plant species has increased as a result of this increasing interest. (1) Metabolomics is the comprehensive study of every single metabolite that exists within an organism under specific conditions. (2) The two fundamental metabolomic research approaches are targeted and non-targeted—are differentiated by how they recognize metabolites in data processing. The targeted method is a precise and directed procedure that includes selectively investigating a predetermined group of known metabolites. Providing good specificity and sensitivity, it may be biased because it exclusively targets particular types of chemicals, such lipids or alkaloids. This limits the potential of finding novel metabolites. The non-targeted method, on the other hand, explores every metabolite that can be identified within a sample without making any inferences. This scientific approach assists in discovering new targets and boosts the potential of developing new metabolites. Although each method has positive and negative aspects, the choice relies on the research goal. (3) Ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC-QTOFMS), gas

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chromatography coupled with quadrupole time-of-flight mass spectrometry (GC-QTOF-MS), liquid chromatography coupled with electrospray-ionization quadrupole time-of-flight mass spectrometry (LC-ESIQTOF/MS), and liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) constitute some of the analytical techniques employed in metabolomics. (4) A useful method for evaluating the quality of plant-based formulations and natural medications is metabolomics. Approval and regulation of plant-based drugs using herbal ingredients depend on this procedure. (5) Plant metabolites can be broadly divided into two categories: essential (primary) and non-essential (secondary). Primary metabolites like as lipids, carbohydrates, and amino acids are required for both basic physiological functions and plant development. Secondary metabolites, notably terpenes, alkaloids, polyphenols, flavonoids, and saponins, are essential for defense mechanisms, environmental adaption, and signaling pathways, nevertheless they are not necessary for basic growth endeavors. (6) In pharmaceutical terms, extraction refers to the process of isolating the therapeutically active constituents of plants using specific solvents under standardized conditions. The resulting products are typically complex mixtures of metabolites, which may be obtained in liquid, semisolid, or dry powdered forms—depending on whether the solvent is retained or removed. These extracts are commonly formulated for oral or topical administration and are classified into various types, including decoctions, infusions, semisolid (pills) extracts, and powdered extracts. (7) In this study we aim at the untargeted metabolic profiling of decoction of *Kokilaksha* through HR LCMS QTOF. Metabolomics, particularly LC-HRMS-based approaches, has become a powerful tool for comprehensive profiling of complex herbal formulations and natural products. (8) Recent advancements in LC-HRMS-based metabolomics have significantly improved the comprehensive profiling, annotation, and standardization of complex herbal formulations and medicinal plant extracts. (9–12)

The enhancement of Ayurvedic metabolomic databases through the inclusion of species- or genotype-specific phytochemicals into well-established repositories such as KEGG, METLIN, and HMDB represents a significant advancement for integrative research. Strategic collaboration with bioinformatics platforms is essential for the creation of a dedicated spectral library, thereby supporting the systematic standardization and scientific validation of Ayurvedic medicinal systems. (13)

Materials and method

Collection of the plant

The whole Asteracantha *longifolia Nees* (*Kokilaksha*) plants were collected from field side of Tripunithura, Ernakulam, Kerala India. The whole plant was authenticated by a plant taxonomist from the Department of Botany, St. Albert's college (Autonomous), Ernakulam and preserved as a voucher specimen (SAC/BOT/HER/02/2025) in the department herbarium following standard methods (Jain and Rao,1977).

Preparation of *kashaya*

The whole plant of *Kokilaksha* was then washed and dried in sunlight. 50 gms of the crushed plant was boiled in medium flame with 800ml(16times) water and reduced to 200 ml(1/4th) as per *Sarangdhara Samhita.* (14) This aqueous extract was kept in an air tight container.10 ml of this aqueous extract (*Kokilaksha Kashaya*) was placed in a vacuum concentrator and water content was eliminated. The resulting residue was then combined with 10 ml methanol and then sonicated for 10 min and designated as Sample 1. Methanol was used as a reconstitution solvent to enhance the recovery of semi-polar metabolites and to improve ionization efficiency and chromatographic compatibility for HR-LCMS-QTOF analysis. (10) This sample was then sent to the Sophisticated Analytical Instrument Facilities (SAIF), IIT Bombay for HR LCMS QTOF Analysis.

Instrumentation

High-resolution mass spectrometry coupled with advanced spectral databases and molecular networking platforms has enhanced metabolite identification confidence in natural product research. (15) The components of *Kokilaksha kashaya* were analysed through LC-QTOF-HRMS (Liquid Chromatography Quadrupole Time-of-Flight High Resolution Mass Spectrometry) through Sophisticated analytical instrument facilities (SAIF) at IIT Bombay. The sample analysis was performed using a High-Resolution Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometer (HRLCMS-QTOF) system from Agilent Technologies (USA). Data acquisition was carried out using Agilent MassHunter Acquisition Software, and data processing was performed using Agilent MassHunter Qualitative Analysis B.06 software.

Liquid Chromatography Conditions

Separation was achieved on a Hypersil GOLD C18 column (100 mm × 2.1 mm, 3 µm particle size). The mobile phases consisted of Solvent A: 0.1% formic acid in Milli-Q water and Solvent B: Acetonitrile. The flow rate was maintained at 0.300 mL/min, with a column temperature of 40°C. The injection volume was 5.0 µL.

The gradient elution program was as follows:

- 0–1 min: 5% B
- 1–25 min: linear increase to 100% B
- 25–30 min: hold at 100% B
- 30–31 min: decrease to 5% B
- 31–35 min: re-equilibration at 5% B.

Mass Spectrometry Conditions

Mass spectrometric detection was performed using an **Electrospray Ionization (ESI)** source operating in both **positive** and **negative ion modes**. The acquisition was done in **Auto MS/MS** mode within an m/z range of **120–1200**.

Key MS parameters were

- Drying gas temperature: **250°C**
- Drying gas flow: **13 L/min**
- Sheath gas temperature: **300°C**
- Sheath gas flow: **11 L/min**
- Nebulizer pressure: **35 psi**
- Capillary voltage (VCap): **3500 V**
- Fragmentor voltage: **175 V**
- Nozzle voltage: **1000 V**
- Skimmer voltage: **65 V**
- Octopole RF Peak: **750 V**
- **MS/MS Acquisition Parameters:**
- MS and MS/MS scan rates: **1 spectra/sec**
- Isolation width: **Medium (~4 amu)**
- Collision energy was dynamically ramped between **20–40 eV** depending on precursor mass.

- Active exclusion was enabled to prevent repetitive MS/MS of the same precursor ions
- A maximum of **10 precursors** were selected per cycle based on abundance.

Calibration and Quality Control

Internal reference masses were used for mass accuracy correction during acquisition. The instrument was calibrated daily to ensure mass accuracy within **5 ppm.**

LCMS QTOF Analysis

The methanolic extract of *Asteracantha longifolia Nees* included a variety of phytoconstituents that were detected by LC-MS/MS-QTOF profiling. As seen in Figs. 1a and 1b, the chromatograms captured both the positive and negative ionization modes.

Figure 1a: Total ion chromatogram (TIC) of *Kokilaksha Kashaya* obtained in positive ionisation mode using HR LCMS-QTOF

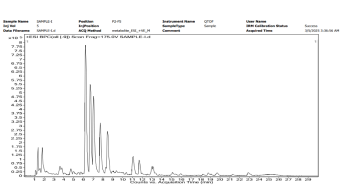


Figure 1b: Total ion chromatogram (TIC) of *Kokilaksha Kashaya* obtained in negative ionisation mode using HR LCMS-QTOF

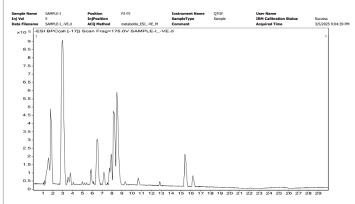


Table 1. Compounds identified by positive ionisation

Data File	SAMPLE-I.d	**Sample Name**	SAMPLE-I
Sample Type	Sample	**Position**	P2-F5
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ESI_+VE_MSMS.m	**Acquired Time**	3/5/2025 3:36:56 AM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3)

Sl.No	Name	MFG Formulae	DB Formulae	Db Diff (ppm)	Hits (DB)	RT	Mass	Abund
1	8-Hydroxy-2-chlorodibenzofuran	$C_{12}H_7ClO_2$	$C_{12}H_7ClO_2$	3.11	2	1.131	218.0128	
2	(+/-)-3-[(2-methyl-3-furyl)thio]-2-butanone	$C_9H_{12}O_2S$	$C_9H_{12}O_2S$	-10.16	1	1.135	184.0577	34587
3	8-Hydroxyadenine	$C_5H_5N_5O$	$C_5H_5N_5O$	3.49	3	1.763	151.0489	27167
4	Nitisinone	$C_{14}H_{10}F_3NO_5$	$C_{14}H_{10}F_3NO_5$	5.16	6	1.813	329.0494	
5	Pirbuterol	$C_{12}H_{20}N_2O_3$	$C_{12}H_{20}N_2O_3$	9.11	1	1.904	240.1452	19702
6	Neuraminic acid	$C_9H_{17}NO_8$	$C_9H_{17}NO_8$	5.32	7	2.05	267.094	19943
7	Pirbuterol	$C_{12}H_{20}N_2O_3$	$C_{12}H_{20}N_2O_3$	9.35	1	2.21	240.1451	16920
8	Lilaline	$C_{20}H_{17}NO_7$	$C_{20}H_{17}NO_7$	-6.75	3	3.998	383.1031	10729
9	Methyl N-methylanthranilate	$C_9H_{11}NO_2$	$C_9H_{11}NO_2$	-6.14	10	4.753	165.08	19711
10	N-gamma-L-Glutamyl-L-phenylalanine	$C_{14}H_{18}N_2O_5$	$C_{14}H_{18}N_2O_5$	11.06	9	5.332	294.1183	10947
11	Niazimicin A	$C_{16}H_{23}NO_6S$	$C_{16}H_{23}NO_6S$	-12.35	1	5.549	357.129	13504
12	Melanostatin	$C_{13}H_{24}N_4O_3$	$C_{13}H_{24}N_4O_3$	8.09	4	6.098	284.1825	11470
13	Geissoschizoline	$C_{19}H_{26}N_2O$	$C_{19}H_{26}N_2O$	12.02	3	6.208	298.2009	
14	12alpha-Fluoro-11beta,17beta-dihydroxyandrosta-1,4-dien-3-one	$C_{19}H_{25}FO_3$	$C_{19}H_{25}FO_3$	-9.27	1	6.555	320.1817	
15	1-Octen-3-yl glucoside	$C_{14}H_{26}O_6$	$C_{14}H_{26}O_6$	1.87	1	6.712	290.1724	
16	12alpha-Fluoro-11beta,17beta-dihydroxyandrosta-1,4-dien-3-one	$C_{19}H_{25}FO_3$	$C_{19}H_{25}FO_3$	-11.71	3	6.936	320.1825	
17	1-Octen-3-yl glucoside	$C_{14}H_{26}O_6$	$C_{14}H_{26}O_6$	6.16	1	7.093	290.1712	

18	12alpha-Fluoro-11beta,17beta-dihydroxyandrosta-1,4-dien-3-one	$C_{19}H_{25}FO_3$	$C_{19}H_{25}FO_3$	-9.54	3	7.324	320.181	
19	1-Octen-3-yl glucoside	$C_{14}H_{26}O_6$	$C_{14}H_{26}O_6$	3.69	1	7.726	290.1719	121978
20	12alpha-Fluoro-11beta,17beta-dihydroxyandrosta-1,4-dien-3-one	$C_{19}H_{25}FO_3$	$C_{19}H_{25}FO_3$	-6.93	1	7.736	320.1818	
21	3,5-Dihydroxyphenyl 1-O-(6-O-galloyl-beta-D- glucopyranoside)	$C_{19}H_{20}O_{12}$	$C_{19}H_{20}O_{12}$	4.87	6	7.748	440.0933	
22	1-Octen-3-yl glucoside	$C_{14}H_{26}O_6$	$C_{14}H_{26}O_6$	6.31	1	8.088	290.1711	33123
23	3-Glucosyl-2,3',4,4',6-pentahydroxybenzophenone	$C_{19}H_{20}O_{11}$	$C_{19}H_{20}O_{11}$	2.12	9	8.462	424.0997	
24	Coriandrone E	$C_{13}H_{12}O_5$	$C_{13}H_{12}O_5$	0.78	10	8.504	248.0683	33879
25	Cajanin	$C_{16}H_{12}O_6$	$C_{16}H_{12}O_6$	9.88	10	8.602	300.0604	
26	Diosmetin 7-O-beta-D-glucuronopyranoside	$C_{22}H_{20}O_{12}$	$C_{22}H_{20}O_{12}$	10.29	1	8.657	476.0906	23503
27	3-Glucosyl-2,3',4,4',6-pentahydroxybenzophenone	$C_{19}H_{20}O_{11}$	$C_{19}H_{20}O_{11}$	5.01	8	8.853	424.0984	23262
28	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	$C_{18}H_{29}NO_3$	18.51	3	10.022	307.2091	10968
29	Gabapentin	$C_9H_{17}NO_2$	$C_9H_{17}NO_2$	-5.86	10	15.179	171.1269	

Table 2: Compounds identified by negative ionisation

Data File	SAMPLE-I_-VE.d	**Sample Name**	SAMPLE-I
Sample Type	Sample	**Position**	P2-F5
Instrument Name	QTOF	**User Name**	
Acq Method	metabolite_ESI_-VE_MSMS.m	**Acquired Time**	3/5/2025 9:04:39 PM
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3)

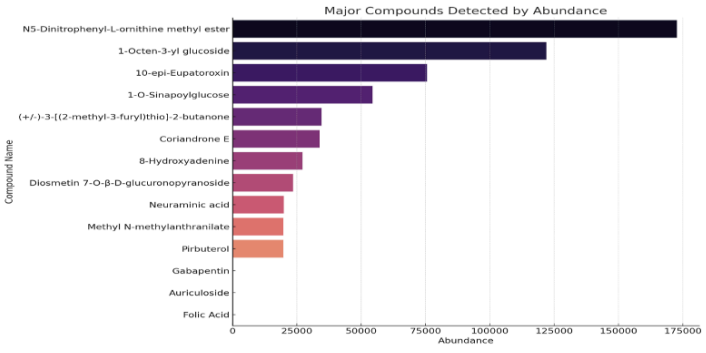
Sl.No	Name	MFG Formulae	DB Formulae	Db Diff (ppm)	Hits (DB)	RT	Mass	Abund
1	Fentin hydroxide	$C_{18}H_{16}OSn$	$C_{18}H_{16}OSn$	-11.23	1	1.785	360.029	
2	Isocitrate	$C_6H_8O_7$	$C_6H_8O_7$	3.02	10	1.879	192.0264	
3	N-Acetyl-L-glutamic acid	$C_7H_{11}NO_5$	$C_7H_{11}NO_5$	4.28	10	1.889	189.0629	
4	Quinic acid	$C_7H_{12}O_6$	$C_7H_{12}O_6$	3.51	10	2.846	192.0627	
5	Rotenonone	$C_{23}H_{18}O_7$	$C_{23}H_{18}O_7$	-4.9	3	2.918	406.1072	
6	Quinic acid	$C_7H_{12}O_6$	$C_7H_{12}O_6$	3.16	10	3.207	192.0628	
7	XMP	$C_{10}H_{13}N_4O_9P$	$C_{10}H_{13}N_4O_9P$	-8.07	1	3.692	364.045	
8	Caryoptosidic acid	$C_{16}H_{24}O_{11}$	$C_{16}H_{24}O_{11}$	2.92	10	3.73	392.1307	
9	(±)-Flufenprox	$C_{24}H_{22}ClF_3O_3$	$C_{24}H_{22}ClF_3O_3$	-14.68	5	4.959	450.1276	
10	1-O-Sinapoylglucose	$C_{17}H_{22}O_{10}$	$C_{17}H_{22}O_{10}$	-11.23	1	5.903	386.1236	54362
11	N5-Dinitrophenyl-L-ornithine methyl ester	$C_{18}H_{20}N_4O_6$	$C_{18}H_{20}N_4O_6$	-2.32	10	6.446	388.1392	172693
12	trans-O-Methylgrandmarin	$C_{16}H_{18}O_6$	$C_{16}H_{18}O_6$	-5.97	10	6.479	306.1122	
13	Luteolin 7-O-[β-D-glucuronosyl-(1->2)-β-D-glucuronide]-4'-O-β-D- glucuronide	$C_{33}H_{34}O_{24}$	$C_{33}H_{34}O_{24}$	2.84	1	6.486	814.1417	
14	N5-Dinitrophenyl-L-ornithine methyl ester	$C_{18}H_{20}N_4O_6$	$C_{18}H_{20}N_4O_6$	-1.49	10	6.693	388.1389	112496
15	3-Methylellagic acid 8- rhamnoside	$C_{21}H_{18}O_{12}$	$C_{21}H_{18}O_{12}$	3.53	7	7.781	462.0782	
16	10-epi-Eupatoroxin	$C_{20}H_{24}O_8$	$C_{20}H_{24}O_8$	-4.85	5	7.798	392.149	75575

17	10-epi-Eupatoroxin	$C_{20}H_{24}O_8$	$C_{20}H_{24}O_8$	-4.63	5	8.036	392.1489	
18	Auriculoside	$C_{22}H_{26}O_{10}$	$C_{22}H_{26}O_{10}$	-7.72	10	8.176	450.1561	
19	Auriculoside	$C_{22}H_{26}O_{10}$	$C_{22}H_{26}O_{10}$	-6.42	10	8.218		
20	3,3'-Di-O-galloylprodelphinidin B5	$C_{44}H_{34}O_{22}$	$C_{44}H_{34}O_{22}$	4.74	3	8.483	450.1555	
21	Genistein 4'-O-glucuronide	$C_{21}H_{18}O_{11}$	$C_{21}H_{18}O_{11}$	1.71	10	8.5	914.1498	
22	Folic acid	$C_{19}H_{19}N_7O_6$	$C_{19}H_{19}N_7O_6$	-1.42	2	8.682	441.1403	
23	Diosmetin 7-O-beta-D-glucuronopyranoside	$C_{22}H_{20}O_{12}$	$C_{22}H_{20}O_{12}$	4.43	7	8.722	476.0934	
24	Genistein 5-O-glucuronide	$C_{21}H_{18}O_{11}$	$C_{21}H_{18}O_{11}$	3.42	10	8.907	446.0834	
25	Momordin I	$C_{41}H_{64}O_{13}$	$C_{41}H_{64}O_{13}$	0.96	4	15.41	764.434	

Result

The chromatograms acquired from HRLCMS-QTOF revealed multiple peaks representing different metabolites. The total ion chromatograms (TICs) obtained in both positive and negative ionization modes showed clear separation and reproducibility of the detected compounds. **In positive ionisation mode**, major peaks were observed between 3–10 minutes, with the highest intensity around 7 minutes. **In negative ionisation mode**, intense peaks were observed between 2–9 minutes. The mass spectrometry analysis allowed identification of various metabolites based on accurate mass measurements and isotopic distribution matching. LC-MS QTOF analysis of the sample revealed the presence of a diverse set of metabolites and compounds with varying chemical structures and functional groups. A total of over 54 compounds were identified from both the positive and negative ionisation modes, including flavonoids, glycosides, organic acids, and other secondary metabolites. Each compound was characterized based on accurate mass (m/z), retention time (RT), molecular formula, and database match quality (Hits/DB).

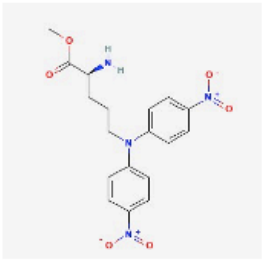
Table 3: Bar graph depicts the abundance levels of the major compounds



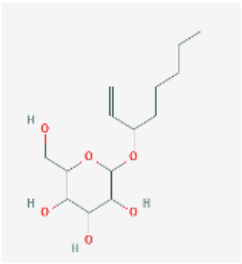
Among them, 29 compounds were identified by High - Resolution LC-QTOF-MS/MS analysis in the positive ionisation mode (Table 1) and 25 compounds were identified by High-Resolution LC-QTOF-MS/MS analysis in the negative ionisation mode (Table 2). The table lists the retention times (RT), accurate mass-to-charge ratios (m/z), molecular formulae, and tentative compound names. The identified metabolites include triterpenoids, flavonoids, glycosides, organic acids, and other phytochemical derivatives, indicating the complex chemical profile of the sample. The HR LCMS QTOF spectra confirming the tentative identification of the major metabolites are shown in Figs 3, 4 and 5. Among them, *N5-Dinitrophenyl-L-ornithine methyl ester* demonstrated the highest abundance. Additionally, compounds such as *1-Octen-3-yl glucoside*, *10-epi-Eupatoroxin*, and *1-O-Sinapoylglucose* also showed notably high abundance, indicating their substantial presence in the sample. The bar graph (Table 3) presents the abundance levels of the major compounds identified in the analysis. The major phytoconstituents detected in the methanolic extract were structurally characterized, and their two-dimensional chemical structures from PubChem database are illustrated in Figure 2.

Figure 2: 2D chemical structures of the major detected compounds

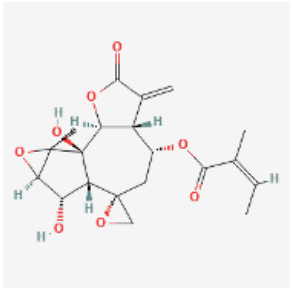
N5-Dinitrophenyl-L-ornithine methyl ester



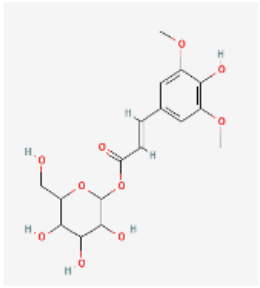
1-Octen-3-yl glucoside



10-epi-Eupatoroxin



1-O-Sinapoylglucose



Discussion

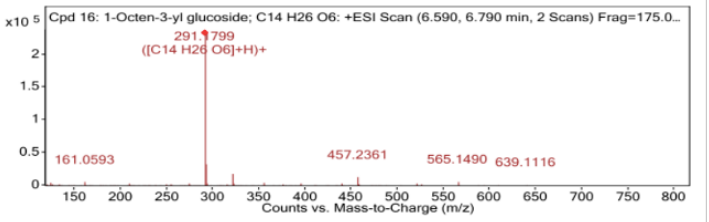
The HR-LCMS-QTOF method provided high-resolution separation and accurate mass detection, enabling reliable identification of a wide range of secondary metabolites. The use of ESI positive and negative modes ensured comprehensive coverage of different classes of compounds. The chromatographic method using a Hypersil GOLD C18 column with a water/acetonitrile gradient provided good peak shape and retention for both polar and non-polar metabolites. The MS/MS fragmentation data confirmed the tentative identification of compounds through library matching and mass accuracy criteria. **The identified compounds exhibit notable bioactivity, demonstrating anti-inflammatory, anticancer, and antimicrobial properties. The**

integration of HR-LCMS-based metabolomics with phytochemical databases supports the scientific validation, quality control, and bioactivity correlation of traditional herbal formulations. (16–18)

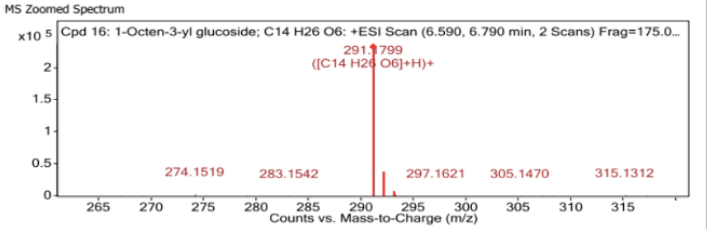
1-Octen-3-yl glucoside **-a bioactive compound naturally synthesized by plants**. a **non-volatile storage form** of **1-octen-3-ol**, a compound known for its role in plant defense and aroma. Upon plant tissue damage, this glucoside can be enzymatically hydrolyzed to release 1-octen-3-ol, which acts as a **defense signal** and antimicrobial agent. It also plays a role in plant–microbe interactions and is studied for potential impacts on human health due to its biological activity. *(19)*

Figure 3: HR-LCMS-QTOF MS/MS spectrum showing the identification of 1-Octen-3-yl glucoside based on accurate mass and fragmentation pattern

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 16: 1-Octen-3-yl glucoside; C14 H26 O6	**1-Octen-3-yl glucoside**	291.1799	6.712	Auto MS/MS	290.1724



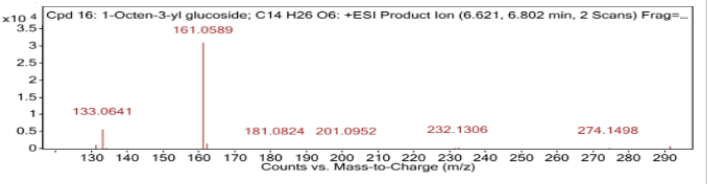
MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
124.0857				4293.83		
161.0593				5597.8		
291.1799	291.1802	1.19	1	237422.77	C14 H26 O6	(M+H)+
292.1819	292.1836	6.12	1	33113.87	C14 H26 O6	(M+H)+
293.1836	293.1857	7.32	1	3331.33	C14 H26 O6	(M+H)+
321.1885			1	17986.13		
355.1691				4668.98		
439.152			1	4226.7		
457.2361			1	13105.32		
565.149			1	6361.6		

MSMS Spectrum



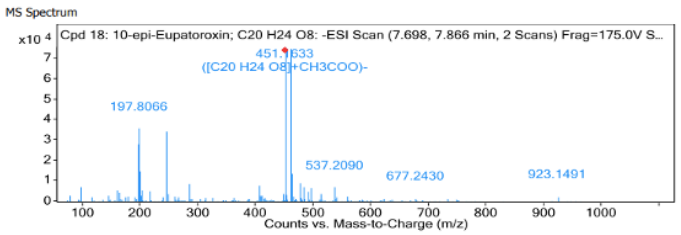
10-epi-Eupatoroxin: is a naturally occurring **sesquiterpene lactone** isolated from certain plants has been recognized for its notable cytotoxic potential. It is found in natural sources such as Greek heather honey, contributing to the product's distinct chemical characteristics. Studies using quantitative structure-activity relationship (QSAR) analysis have emphasized its possible pharmacological value, particularly in anticancer research. (20)

1-O-Sinapoylglucose - is a plant-derived phenolic compound formed by combining sinapic acid and glucose. It plays a key role in plant metabolism and defense, mainly by serving as a precursor for sinapate esters like sinapoylmalate and sinapoylcholine, which help protect plants from UV radiation, oxidative stress, and pathogens. (21) Research has shown that sinapic acid, a hydroxycinnamic acid found in various plants and a precursor to compounds like 1-O-sinapoylglucose, exhibits several bioactive properties—notably anti-inflammatory and anticancer effects. (22)

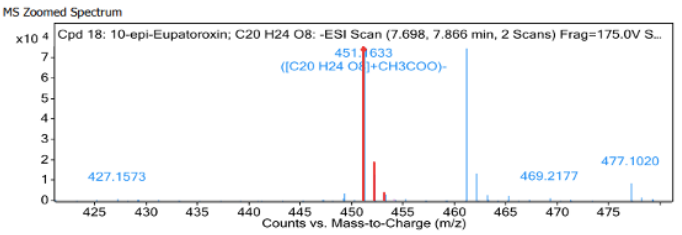
Fig. 4 HR-LCMS-QTOF MS/MS spectrum showing the identification of 10-epi-Eupatoroxin based on accurate mass and fragmentation pattern

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 18: 10-epi-Eupatoroxin; C20 H24 O8	**10-epi-Eupatoroxin**	451.1633	7.798	Auto MS/MS	392.149

MS Spectrum



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
195.8094				27887.67		
197.8066				35837.83		
199.8038				14973.67		
245.0922			1	34453.26		
451.1633	451.161	-5.08	1	75575.13	C20 H24 O8	(M+CH3COO)-
452.1655	452.1644	-2.51	1	13320.03	C20 H24 O8	(M+CH3COO)-
453.1633	453.1667	7.63	1	3654.05	C20 H24 O8	(M+CH3COO)-
461.071			1	74889.42		
462.0739			1	13616.84		
477.102			1	8769.22		

MSMS Spectrum

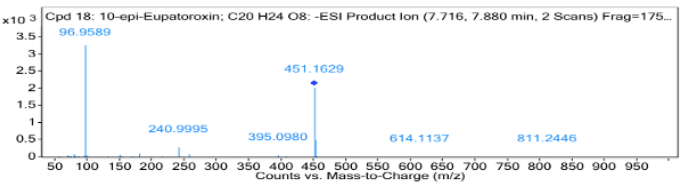
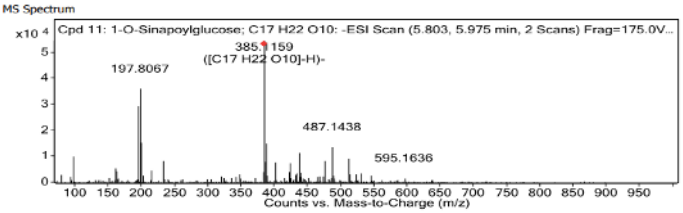
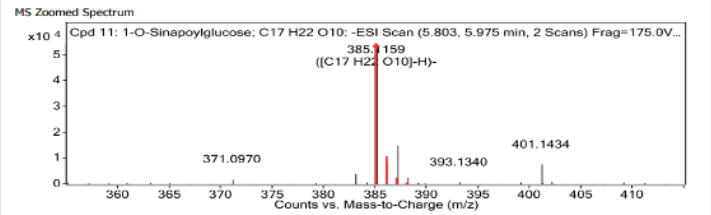


Figure 5: HR-LCMS-QTOF MS/MS spectrum showing the identification of 1-O-Sinapoylglucose based on accurate mass and fragmentation pattern

Compound Label	Name	*m/z*	RT	Algorithm	Mass
Cpd 11: 1-O-Sinapoylglucose; C17 H22	**1-O-Sinapoylglucose**	385.1159	5.903	Auto MS/MS	386.1236

MS Spectrum

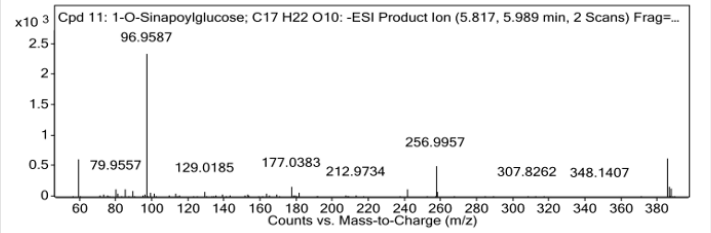




MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
195.8095				29303.69		
197.8067				36092.06		
199.8039				15520.91		
385.1159	385.114	-4.88	1	54361.58	C17 H22 O10	(M-H)-
386.1187	386.1174	-3.32	1	8136.74	C17 H22 O10	(M-H)-
387.1267	387.1194	-18.71	1	5600.45	C17 H22 O10	(M-H)-
387.1643			1	15137.88		
388.1301	388.1222	-20.5	1	767.05	C17 H22 O10	(M-H)-
437.1202			1	11375.66		
487.1438			1	13701.61		

MSMS Spectrum



The technique demonstrated its suitability for complex biological sample analysis by proving effective overall for untargeted metabolite profiling. In order to enable structural elucidation, key parameters such as molecular weight, chemical structure, base peak intensity, and ion fragmentation patterns were recorded. Researchers who are assessing the complex structure of phytochemicals of Ayurvedic medicines can gain a lot from this data repository that is structured for comparative metabolomics, quality control, and pharmacological investigation. The combined raw data includes chromatograms, peak intensities, spectral fingerprints, and molecular fragmentations for subsequent virtual modeling and authentication. Additionally, the dataset can help with network pharmacology research, Ayurvedic formulation standardization, and future drug discovery initiatives, guaranteeing reproducibility and promoting integrative studies in traditional medicine.

Conclusion

To identify the plant's active ingredients and assess their potential for therapeutic application, further investigations are required. To support the development of these phytochemicals for pharmaceutical applications, in vivo studies should place a strong emphasis on evaluating their bioavailability and bio-accessibility.

Conflict of interest statement: The authors declare there was no conflict of interest.

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References

1. Kumar S, Shakya A, Shakya AK. Medicinal plants: Future source of new drugs. *International Journal of Herbal Medicine*. 2016;4(4):59–64.
2. Fiehn O. Metabolomics: The link between genotypes and phenotypes. *Plant Molecular Biology*. 2002; 48:155–171. DOI: https://doi.org/10.1023/A:1013713905833
3. Pande J, Chanda S. Metabolic profiling of bioactive compounds from different medicinal plants: An overview. *International Journal of Chemical Studies*. 2020;8(5):240–246. DOI: https://doi.org/10.22271/chemi.2020.v8.i5d.10303
4. Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. *Mass Spectrometry Reviews*. 2007; 26(1):51–78. DOI: 10.1002/mas.20108
5. Schwab W. Metabolome diversity: Too few genes, too many metabolites? *Phytochemistry*. 2003; 62:837–849. DOI: https://doi.org/10.1016/S0031-9422(02)00723-9
6. Wolfender JL, Marti G, Thomas A, Bertrand S. Current approaches and challenges for the metabolite profiling of complex natural extracts. *Journal of Chromatography A*. 2015; 1382:136–164. DOI : https://doi.org/10.1016/j.chroma.2014.10.091
7. Longo G. Extraction technologies for medicinal and aromatic plants [Internet]. 2008. Available from: http://www.di.ens.fr/users/longo/download.html Accessed on: 15 September 2025; 10:30 AM.
8. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems-level studies of mammalian metabolomes: The roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chemical Society Reviews*. 2021;50(2):330–369. DOI : https://doi.org/10.1039/b906712b
9. Ivanisevic J, Want EJ. From samples to insights into metabolism: Uncovering biologically relevant information in LC-HRMS metabolomics data. *Metabolites*. 2019;9(12):308. DOI: https://doi.org/10.3390/metabo9120308
10. Naz S, Moreira-Dos-Santos DC, Garcia A, Barbas C. Analytical protocols based on LC-MS, GC-MS and NMR for metabolomics studies of medicinal plants. *Phytochemical Analysis*. 2020;31(6):706–721. DOI: https://doi.org/10.4155/bio.14.119.
11. Yu K, Ji J, Wang H, Xue X, Zhang F. Advances in metabolomics analysis of traditional herbal medicines using LC-HRMS. *Journal of Ethnopharmacology*. 2021; 273:113998. DOI: 10.1016/j.molp.2022.09.007
12. Vinaixa M, Schymanski EL, Neumann S, Navarro M, Salek RM, Yanes O. Mass spectral databases for LC/MS- and GC/MS-based metabolomics: State of the field and future prospects. *Trends in Analytical Chemistry*. 2019; 120:115324. DOI: https://doi.org/10.1016/j.trac.2015.09.005
13. Gowda H, Ivanisevic J, Johnson CH, Kurczy ME, Benton HP, Rinehart D, et al. Interactive XCMS online: Simplifying advanced metabolomic data processing and subsequent statistical analyses. *Analytical Chemistry*. 2014;86(14):6931–6939. DOI: 10.1021/ac500734c
14. Prabhakar Rao. *Sharangadhara Samhita: Madhyama Khanda*. 2018 ed. Varanasi: Chaukhambha Sanskrit Sansthan; 2018. Chapter 2/1.
15. Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking (GNPS). *Nature Biotechnology*. 2020; 38:208–213. DOI: https://doi.org/10.1038/nbt.3597

16. Li Y, Kong D, Fu Y, Sussman MR, Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*. 2020; 148:80–89. DOI: https://doi.org/10.1016/j.plaphy.2020.01.006
17. Patel S, Goyal A. Applications of metabolomics in quality control and standardization of herbal medicines. *Journal of Pharmaceutical and Biomedical Analysis*. 2022; 215:114741.
18. Abdullah A, Lee JY, Kang JH, Kim YH. LC-QTOF-MS-based metabolomic profiling for identification of bioactive compounds from medicinal plants. *Molecules*. 2023;28(5):2149.
19. Ntoruru JM, Ohnishi T, Katsumata F, Koeduka T, Matsui K. 1-Octen-3-ol is formed from its primeveroside after mechanical wounding of soybean leaves. *Plant Molecular Biology*. 2022;109(4–5):551–561. DOI: 10.1007/s11103-021-01226-9
20. Scotti MT, Fernandes MB, Ferreira MJP, Emerenciano VP. Quantitative structure–activity relationship of sesquiterpene lactones with cytotoxic activity. *Bioorganic & Medicinal Chemistry*. 2007;15(8):2927–2934. DOI: 10.1016/j.bmc.2007.02.005
21. Fraser CM, Chapple C. The phenylpropanoid pathway in *Arabidopsis*. *The Arabidopsis Book*. 2011;9: e0152. DOI: 10.1199/tab.0152
22. Chen C. Sinapic acid and its derivatives as medicine in oxidative stress-induced diseases and aging. *Oxidative Medicine and Cellular Longevity*. 2016;2016: Article ID 3571614. DOI: https://doi.org/10.1155/2016/3571614

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