



ORIGINAL RESEARCH ARTICLE

Metabolic profiling of milk-based decoction prepared with roots of *Desmodium gangeticum* (L) DC (*Prisniparni ksheerapaka*) through HR-LCMS QTOF

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Abstract

Background

Prisniparni ksheerapaka, a milk-based formulation using roots of *Desmodium gangeticum* (L.) DC is traditionally used in Ayurveda to manage various cardiovascular conditions. Its cardioprotective effects have been demonstrated in isolated frog heart, H9C2 cell models, and in a clinical study on stable angina patients. However, data on its detailed phytochemical composition and mechanisms of action remain limited, hindering its scientific validation.

Objective:

This study aimed untargeted profiling of chemical constituents of *Prisniparni ksheerapaka* using HPTLC and HR-LCMS QTOF.

Material and Methods:

Ksheerapaka was prepared as per *Śārīngadhara Samhitā* (Madhyama Khanda). Prominent bands observed in the HPTLC chromatogram were scraped and subjected to HR-LCMS QTOF analysis. Both positive and negative ESI modes were employed, and MS/MS fragmentation was used for compound identification. Acquisition parameters were saved in a dedicated method file.

Results:

A total of 90 phytoconstituents were detected in positive mode and 6 in negative mode; 60 were successfully identified through spectral library matching. These included alkaloids, steroids, saponins, amino compounds, glycosides, and dipeptides.

Conclusion:

The formulation showed a diverse phytochemical profile supporting its therapeutic use. The current solvent system favoured low to moderately polar compounds. Future studies using solvents of varying polarity are recommended to identify additional, especially highly polar, constituents for a more complete profile

Introduction

Prisniparni ksheerapaka, a milk-based Ayurvedic formulation prepared from roots of *Desmodium gangeticum* (L.) DC is widely recognized among Ayurvedic practitioners for its efficacy in managing a range of cardiovascular disorders. Classical Ayurvedic authorities such as *Charaka* and *Vagbhata* have documented the use of formulation in the management of *Hridayagata Vata* (cardiac disorders of *Vata* origin) within the context of *Vatavyadhi Chikitsa*^{[1],[2]} Additionally, *Chikitsa Manjari* a renowned regional text widely followed by physicians in Kerala also recommends its application in the treatment of *Vatika Hridroga* (cardiac disorders of *Vata* origin).^[3] Experimental studies have demonstrated its cardioprotective efficacy in isolated frog heart models^[4] and H9C2 cardio myoblast cells^[5] while clinical evaluation in patients with stable angina has shown significant alleviation of anginal pain.^[6] Despite these encouraging findings and its extensive clinical use, comprehensive information on its phytochemical profile and underlying molecular mechanisms remains limited, constraining its scientific validation and translational potential. To bridge this gap, the present study employed an innovative analytical approach in which *Prisniparni ksheerapaka* was subjected to High-Resolution Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (HRLCMS-QTOF) analysis using bands scraped from High-Performance Thin Layer Chromatography (HPTLC) plates. The primary objective was to perform an untargeted phytochemical profiling of the formulation to elucidate its active constituents, thereby contributing to a mechanistic understanding of its cardioprotective and pharmacological attributes.

Materials and Methods

Collection and authentication of drug

The whole plant of *Desmodium gangeticum* (L.) DC, commonly known as *Prisniparni*, was collected from Tripunithura, located in the Ernakulam district of Kerala, India. The plant material was taxonomically identified and authenticated by an expert taxonomist from the Department of Botany, St. Albert's College (Autonomous), Ernakulam. A voucher specimen has been deposited in the institution's certified herbarium for future reference (Voucher No: SAC622).

Preparation of *Ksheerapaka*

Fresh cow's milk was collected from a certified dairy in Tripunithura, Ernakulam district, Kerala, India. The dried, crushed roots of the authenticated *Desmodium gangeticum* (L.) DC. were utilized for the preparation of the *Ksheerapaka*. The formulation was prepared in accordance with the classical

procedure described in the *Śārṅgadhara Saṃhitā* (Madhyama Khanda).^[7] In brief, one part of the dried root material was placed in an earthen vessel and combined with eight parts of cow's milk and thirty-two parts of water. The mixture was heated over a medium flame on a gas stove and boiled until the total volume was reduced to that of the original quantity of milk, following the traditional method.

HPTLC Analysis

Sample preparation

10 ml of *Prisniparni ksheerapaka* was placed in a vacuum concentrator to remove its water content. The resulting residue was dissolved in 10 ml of methanol and subjected to sonication for 10 minutes. The mixture was then centrifuged and stored at -20°C . The supernatant obtained was subsequently used for HPTLC analysis.

HPTLC Protocol

Materials and Instrumentation

High-Performance Thin-Layer Chromatography (HPTLC) was performed using 20×10 cm precoated silica gel 60 F₂₅₄ TLC plates (E. Merck, Germany) with a layer thickness of 0.2 mm and aluminium backing. Samples were applied as bands using a CAMAG Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland) equipped with a 100 μL Hamilton syringe. Plate development was carried out in a CAMAG glass twin-trough chamber (20×10 cm). Densitometric scanning was conducted using a CAMAG TLC Scanner 3, operated via *winCATS* software (Camag, Switzerland).

Procedure

The development chamber was pre-saturated with the mobile phase consisting of chloroform: ethyl acetate: formic acid (5:4:1, v/v/v) for 30 minutes prior to development. The Hamilton syringe was rinsed twice with methanol before sample application. After plate development, the TLC plate was air-dried and subjected to densitometric scanning.

Scanning was performed under both absorption-reflection and fluorescence-reflection modes. In the absorption-reflection mode, the plate was scanned at 254 nm using deuterium and tungsten lamps. For fluorescence-reflection mode, scanning was conducted at 366 nm using a mercury lamp. Tracks were scanned, and UV spectra of individual spots were recorded using the spectrum display feature of the *winCATS* software. Chromatographic fingerprints were generated, and spectral comparisons were made across spots. Prominent bands were observed at R_f values of 0.22, 0.80, and 0.91. All HPTLC procedures were carried out at the Analytical Chemistry Laboratory, CARE Keralam Ltd., Koratty, Thrissur, Kerala, India.

HR LCMS -QTOF analysis

Sample Preparation

Since it is a complex formulation, targeted extraction of specific zones from the HPTLC plate was done to simplify the LC-MS matrix and enhance the precision of compound identification based on mass to charge (m/z) ratios. Bands corresponding to R_f values of 0.80, 0.91, and 0.22 were carefully scraped from the developed TLC plate using a clean, sharp scalpel. These were designated as Sample 1 (R_f 0.80), Sample 2 (R_f 0.91), and Sample 3 (R_f 0.22), respectively.

The scraped silica gel was collected and transferred into sterile 1.5 mL Eppendorf tubes. To each tube, 1 mL of LC-MS grade methanol was added, and the contents were thoroughly mixed to facilitate extraction of analytes. The samples were sonicated for a few minutes to enhance compound solubilization, followed by centrifugation to separate the supernatant from the silica matrix. The supernatant was carefully decanted and filtered through a 0.2 μ m membrane to remove residual particulate matter. The clarified extract was then collected in airtight Eppendorf tubes and stored under appropriate conditions for subsequent LC-MS analysis.

All procedures were carried out in the Analytical Chemistry Laboratory at CARE Keralam Ltd., Koratty, Thrissur, Kerala, India.

HR LCMS -QTOF Protocol: HR-LCMS-QTOF analysis was performed at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT) Bombay. Metabolite profiling was carried out using Electrospray Ionization (ESI) in both positive and negative ionization modes, coupled with tandem mass spectrometry (MS/MS fragmentation) to enable structural elucidation of the compounds. Data acquisition was performed under predefined parameters, which were saved in a method file for reproducibility.

Instrument Method for HR-LCMS-QTOF Analysis

High-resolution LC-MS analysis was performed using an Agilent 6545 Q-TOF LC/MS system equipped with a Dual AJS ESI source. Data were acquired in both positive and negative ionization modes using AutoMS² acquisition. Agilent Mass Hunter Acquisition Software was used for data acquisition, while Agilent Mass Hunter Qualitative Analysis B.06 was employed for data processing and spectral interpretation. Details and Mass spectrometric conditions are attached as Supplementary file 1.

Results

Total Ion Chromatograms (TICs) for Samples 1, 2, and 3 were generated using LC-MS/MS analysis with ESI in both positive and negative ionization modes. Distinct peaks corresponding to various phytoconstituents were observed and analysed using high-resolution QTOF-based MS/MS fragmentation. A total of 90 compounds were detected across the three samples under positive ion mode, while 6 compounds were identified in negative mode. Among these, 60 constituents were successfully annotated based on spectral library matching, accurate mass, retention time, and fragmentation patterns.

High-resolution LC-MS/MS analysis was performed on three HPTLC-derived samples corresponding to bands at R_f 0.80 (Sample 1), R_f 0.91 (Sample 2), and R_f 0.22 (Sample 3) to elucidate their phytochemical composition. ESI in positive mode coupled with AutoMS² fragmentation enabled the tentative annotation of 30 metabolites in each sample. Identification was based on accurate mass, retention time (RT), molecular formula, and MS/MS spectral data. Additionally, 2 compounds were annotated in each sample using negative ionization mode. Compound identification was facilitated by spectral database matching with acceptable mass errors (± 15 ppm) and consistent isotope patterns. The qualitative compound reports for Samples 1, 2, and 3 are presented in Tables 1, 2, and 3 respectively. LC MS/MS chromatograms of sample 1, 2 and 3 in both positive and negative ionization mode are given in Figure 1 and 2.

Discussion

A single medicinal herb contains diverse chemical constituents with distinct therapeutic effects, which may undergo chemical transformations during formulation due to inter-component interactions. These interactions can enhance or diminish overall bioactivity, with efficacy often attributed to newly formed compounds rather than the original constituents. Characterization of such bioactive compounds using advanced hyphenated techniques like LC-MS is essential, although chromatographic profiles may vary with raw material quality, storage conditions, and manufacturing processes.^[8]

In this study, high-resolution LC-MS QTOF analysis was utilized to elucidate the complex phytochemical composition of *Ksheerapaka of Prisiniparni*. Under positive ionization mode, thirty phytoconstituents were detected in sample 1, with 15 compounds identified via library matching, including alkaloids, steroids, saponins, amino compounds, and dipeptides, some

Table 1. Qualitative HR-LCMS-QTOF Compound Profile of Sample 1 (Rf 0.80 Zone)

Ionization mode	Compound Label	Name	Mass	m/z	RT
Positive	1	Unidentified analyte	—	231.1638	1.424
	2	2-Methylpiperidine;	296.0442	122.0926	1.497
	3	Enilconazole	296.0442	319.0334	8.395
	4	Unidentified analyte	—	190.0381	8.41
	5	Unidentified analyte	—	246.2357	11.23
	6	Unidentified analyte	—	244.0643	11.852
	7	Unidentified analyte	—	242.2772	12.005
	8	5alpha-Ethoxy-6beta hydroxy-5,6-dihydrophysalin B;	572.2308	573.2384	12.087
	9	3-Deoxy-D-manno octulosonate	238.0673	239.0745	12.487
	10	Unidentified analyte	—	274.2668	13.055
	11	Unidentified analyte	—	290.2605	13.352
	12	Moschamindole	350.1274	373.1167	13.406
	13	Unidentified analyte	—	682.3988	13.5
	14	Hydroxypropyl-glutamate	260.0997	283.089	13.826
	15	Quinacridone	312.0907	313.098	14.034
	16	Hydroxypropyl-glutamate	260.0993	283.0885	14.133
	17	Unidentified analyte	—	250.1731	14.403
	18	Quinacridone	312.0907	313.098	14.628
	19	O-Desmethylquinine	310.1681	311.1758	14.928
	20	(-)-Ormosanine	317.2836	318.291	15.145
	21	Unidentified analyte	—	432.2259	15.145
	21	Ivacaftor	392.2109	415.2	15.773
	22	Unidentified analyte	—	330.3273	16.676
	23	Unidentified analyte	—	358.3572	18.659
	24	Demethylcitalopram	310.1476	311.1547	18.807
	25	Unidentified analyte	—	279.1509	19.895
	26	(-)-Fumigaclavine B	256.1611	279.1505	20.152
	27	Demethylcitalopram	310.1485	311.1556	23.006
	28	Unidentified analyte	—	741.2877	24.736
	29	Unidentified analyte	—	758.3131	24.772
Negative	1	Medicagenic acid 3 O-beta-D-glucoside	664.3889	663.3818	13.559
	2	Cucurbitacin I 2-glucoside	676.3389	721.3392	13.562

Table 2. Qualitative HR-LCMS-QTOF Compound Profile of Sample 2 (Rf 0.91 Zone)

Ionization mode	Compound Label	Name	Mass	m/z	RT
Positive	1	Unidentified analyte	—	166.9921	1.46
	2	Enilconazole	296.044	319.0333	8.335
	3	Levofuraltadone	324.1094	325.1167	10.727
	4	9-Chloro-11beta,17beta dihydroxy-17-methylandrosterone-4-en-3-one	352.1826	375.1713	11.135
	5	Unidentified analyte	—	246.2356	11.198
	6	Unidentified analyte	—	262.2302	11.394
	7	Unidentified analyte	—	242.2772	11.976
	8	5alpha-Ethoxy-6beta-hydroxy 5,6-dihydrophysalin B	572.231	573.2383	12.057
	9	Americine	545.294	568.2835	12.061
	10	3-Deoxy-D-manno octulosonate	238.0672	239.0745	12.475
	11	Unidentified analyte	—	274.2666	13.026
	12	Unidentified analyte	—	230.2408	13.155
	13	Unidentified analyte	—	290.2609	13.267
	14	Unidentified analyte	—	274.2659	13.398
	15	Unidentified analyte	—	272.2503	13.456
	16	Unidentified analyte	—	250.1724	14.392
	17	O-Desmethylquinine	310.1676	311.1753	14.903
	18	(-)-Ormosanine	317.2833	318.2906	15.079
	19	Icaceine	375.2411	376.2484	15.187
	20	Ivacaftor	392.2106	415.2	15.749
	21	Unidentified analyte	—	432.2256	15.806
	22	Unidentified analyte	—	330.3269	16.641
	23	Unidentified analyte	—	356.3415	17.197
	24	Unidentified analyte	—	358.9689	18.16
	25	Unidentified analyte	—	358.3572	18.58
	26	Demethylcitalopram	310.1478	311.1549	18.831
	27	(-)-Fumigaclavine B	256.1614	279.1506	19.887
	28	Unidentified analyte	—	454.3034	20.431
	29	Demethylcitalopram	310.148	311.1553	22.934
	30	Unidentified analyte	—	368.414	24.146
Negative	1	Butylparaben	194.095	193.0885	13.513
	2	Medicagenic acid 3-O-beta-D glucoside	664.3873	663.3802	13.541

Table 3. Qualitative HR-LCMS-QTOF Compound Profile of Sample 3 (Rf 0.22 Zone)

Ionization mode	Compound Label	Name	Mass	m/z	RT
Positive	1	Unidentified analyte	—	122.0923	1.488
	2	Unidentified analyte	—	319.033	8.31
	3	Unidentified analyte	—	246.2349	11.204
	4	5alpha-Ethoxy-6beta-hydroxy 5,6-dihydrophysalin B	572.2306	573.2379	12.061
	5	3-Deoxy-D-manno octulosonate	238.0669	239.0741	12.461
	6	Unidentified analyte	—	274.2665	13.048
	7	Unidentified analyte	—	230.2408	13.161
	8	Unidentified analyte	—	290.2601	13.29
	9	Unidentified analyte	—	274.2659	13.326
	10	Unidentified analyte	—	272.2499	13.471
	11	O-Desmethylquinine	310.1676	311.1755	14.911
	12	Unidentified analyte	—	432.2253	15.711
	13	Ivacaftor	392.2105	415.1997	15.754
	14	Unidentified analyte	—	330.3267	16.645
	15	Unidentified analyte	—	358.3569	18.57
	16	Paederoside	446.0868	469.0762	18.813
	17	Demethylcitalopram	310.1475	311.1545	18.911
	18	Unidentified analyte	—	279.1507	19.878
	19	Unidentified analyte	—	121.0245	19.918
	20	Demethylcitalopram	310.1474	311.1544	23.011
	21	Unidentified analyte	—	537.5201	26.241
	22	Unidentified analyte	—	537.5207	26.54
	23	(23S)-23,25-dihydroxy-24 oxovitamin D3 23-(beta glucuronide)	606.3387	629.3281	26.59
	24	Westiellamide	546.3189	569.308	26.608
	25	Istamycin KL1	336.1987	337.2058	26.718
	26	Unidentified analyte	—	391.2725	26.782
	27	Unidentified analyte	—	391.2729	26.822
	28	Unidentified analyte	—	677.4002	26.822
	29	Cerberin	576.3296	599.3191	26.827
	30	Unidentified analyte	—	594.3634	26.881
Negative	1	Jasmine ketolactone	208.1115	207.1043	13.345
	2	Butylparaben	194.0957	193.0885	13.531

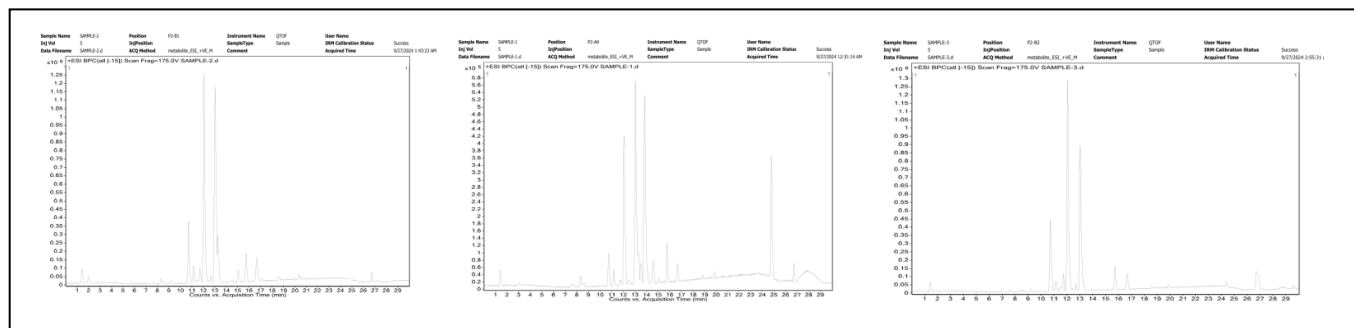


Figure 1. LC-MS/MS chromatogram of *Prinsiparni Ksheerapaka* in Positive ionization mode

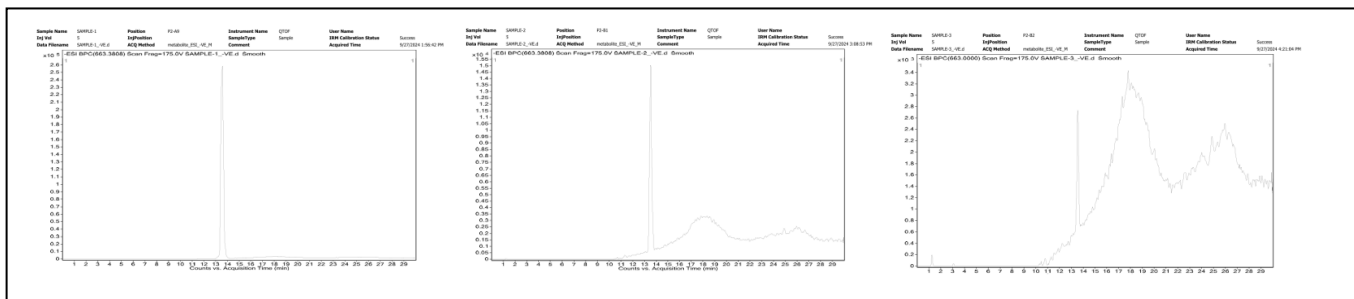


Figure 2. LC-MS/MS chromatogram of *Prinsiparni Ksheerapaka* in Negative ionization mode

reflecting incomplete protein metabolism. Among them, 5 α -Ethoxy-6 β -hydroxy-5,6-dihydrophysalin B is a physalin-type withanolide closely related to physalin B. Physalin B shows strong anti-inflammatory activity through NF- κ B inhibition, suggesting this analogue may contribute to similar anti-inflammatory and cytoprotective effects.^[9] O-Desmethylquinine is a demethylated metabolite of quinine that shares weak antioxidant and anti-inflammatory properties reported for quinine derivatives.^[10] Negative ionization mode further enabled the identification of two triterpenoid saponins: Medicagenic acid 3-O- β -D-glucoside and Cucurbitacin I 2-glucoside. Medicagenic acid 3-O- β -D-glucoside is a triterpenoid saponin with anti-inflammatory activities.^[11] Saponins contribute to antioxidant defense through the modulation of endogenous antioxidant enzymes, including SOD, CAT, and GPx. They also exhibit hypocholesterolemic activity by enhancing the excretion of cholesterol and bile acids and by regulating key enzymes involved in cholesterol metabolism.¹² Cucurbitacin I 2-glucoside is a glycosylated cucurbitacin with notable anti-inflammatory effects, mainly by blocking JAK/STAT and NF- κ B pathways.^[13]

In sample 2, thirty phytoconstituents were detected under positive ionization mode, with 13 compounds identified through spectral library matching, including alkaloids, steroids, and amino compounds. Among them, Americine is an oleanane-type triterpenoid saponin with likely anti-inflammatory and antioxidant effects similar to related saponins.^[14] Paederoside is an iridoid glycoside with demonstrated anti-inflammatory and antioxidant activity in experimental studies.^[15] In negative ionization mode, Medicagenic acid 3-O- β -D-glucoside (a triterpenoid saponin)

and Butylparaben (a para-hydroxybenzoic acid ester) were identified. Sample 3 similarly revealed thirty phytoconstituents in positive ionization mode, with 11 compounds matched, including alkaloids, steroids, amino compounds, and glycosides. Significant detections included 23(S)-23,25-Dihydroxy-24-oxovitamin D3 23-(β -glucuronide), a vitamin D3 catabolic intermediate potentially formed during milk heating. This vitamin D metabolite may contribute to cardiovascular protection through its reported effects on endothelial function, nitric oxide bioavailability, and inflammatory regulation¹⁶.

Westiellamide, a cyclic peptide with cardioprotective relevance^[17] and Cerberin is a potent plant-derived cardiac glycoside that inhibits the Na⁺/K⁺-ATPase pump. Cerberin produces strong cardiotonic effects but is highly toxic.^[18] Under negative ionization mode, Jasmine ketolactone (a lactone) and Butylparaben were also detected.

Phytoconstituents in a formulation contribute not only through their direct bioactivity but also by serving as structural scaffolds, chemical frameworks that enhance target binding and molecular interactions. When several compounds possess complementary scaffolds, they can influence multiple pathways together, creating synergy that amplifies the overall therapeutic effect of the formulation.^[19] 2-Methylpiperidine, reported in this study functions as a structural scaffold, with its piperidine ring offering a stable backbone that enhances target binding and supports synergistic biological activity.^[20] Similarly, the formulation may contain multiple structural scaffolds that, together, enhance overall therapeutic efficacy through synergistic interactions.

Prisniparni ksheerapaka contains both lipophilic and hydrophilic phytoconstituents with varying polarities. In the present study, samples were derived from HPTLC bands developed using a relatively low-polarity mobile phase comprising chloroform: ethyl acetate: formic acid in the ratio 5:4:1. As a result, the phytoconstituents identified through subsequent analysis are likely to be predominantly low-polar compounds. To achieve a more comprehensive phytochemical profile, further chromatographic studies employing solvent systems of higher polarity are warranted to detect highly polar constituents. Moreover, it is important to note that compound identification in this study was solely based on library matching, which presents certain limitations, particularly in the absence of reference standards or confirmatory spectral data.

Conclusion

Evidence from the research underscores the effectiveness of high-resolution LC-MS/MS coupled with HPTLC in profiling the complex phytochemical composition of *Prisniparni Ksheerapaka*. A total of 96 compounds were detected across three samples, with 60 phytoconstituents tentatively identified based on accurate mass, retention time, and fragmentation data. The identified metabolites comprised diverse bioactive classes, including alkaloids, glycosides, saponins, steroids, amino compounds, and peptides, some of which may arise from transformations during *Ksheerapaka* preparation. Several constituents possess reported antioxidant, anti-inflammatory, hypolipidemic, and cardioprotective activities, providing a phytochemical basis for the traditional therapeutic applications of *Prisniparni Ksheerapaka* and its potential role in mitigating oxidative stress and inflammation associated with cardiovascular disorders. The predominance of low-polar compounds is attributed to the use of a low-polarity mobile phase during HPTLC band isolation. Future studies incorporating higher-polarity solvent systems and confirmatory techniques are warranted to fully elucidate the phytochemical complexity and therapeutic relevance of such traditional formulations.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Institutional and technical collaborations mentioned above were purely academic and did not involve any commercial or financial interests.

Supplementary File

Instrument Method for HR-LCMS-QTOF Analysis

High-resolution LC-MS analysis was carried out on an Agilent 6545 Q-TOF LC/MS system equipped with a Dual AJS ESI source, with data acquired in both positive and negative ionization modes using AutoMS². Data acquisition and processing were performed using Agilent MassHunter Acquisition Software and MassHunter Qualitative Analysis B.06, respectively, for spectral interpretation. The separation

was carried out on a Hypersil GOLD C18 column (100 × 2.1 mm, 3 µm particle size) maintained at 40 °C. The mobile phase consisted of Solvent A (0.1% formic acid in Milli-Q water) and Solvent B (acetonitrile), delivered at a flow rate of 0.3 mL/min. LC Gradient program is listed in Table 2. Injection volume was 5 µL with needle wash between injections. The sample tray was maintained at 0.8 °C. Mass Spectrometric Operating Conditions for HR-LCMS-QTOF Analysis are listed in Table 3.

Table 2: LC Gradient Program for HR-LCMS-QTOF Analysis

Time (min)	%A	%B
0.00	95	5
1.00	95	5
25.00	0	100
30.00	0	100
31.00	95	5
35.00	95	5

Table 3: Mass Spectrometric Operating Conditions for HR-LCMS-QTOF Analysis

Parameter	Value
Ion Source	Dual AJS ESI
Drying Gas Temperature	250 °C
Drying Gas Flow	13 L/min
Nebulizer Pressure	35 psig
Sheath Gas Temperature	300 °C
Sheath Gas Flow	11 L/min
Capillary Voltage (Vcap)	3500 V
Nozzle Voltage	1000 V
Fragmentor Voltage	175 V
Skimmer Voltage	65 V
Octopole RF	750
Mass Range	<i>m/z</i> 120–1200
MS/MS Scan Rate	1 spectrum/sec
Collision Energy	Ramped, medium isolation width (~4 amu)
Precursor Selection	By abundance with active exclusion (1 spectrum, 0.2 min release)

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