IJPSR (2025), Volume 16, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 October 2024; received in revised form, 10 November 2024; accepted, 14 November 2024; published 01 March 2025

HR LC-MS FOR THE MANAGEMENT OF SYSTEMIC HYPERTENSION (RATHTHA KOTHIPPU NOI)

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Keywords:

Vaasathi Kashaym, HR-LCMS, Siddha Herbal Formulation, Raththa kothippu noi, Systemic hypertension, Metabolite profiling

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ABSTRACT: The delineation, characterization, and fingerprint profiling of metabolites derived from Siddha formulations are pivotal in advancing drug development and substantiating time-honored evidence-based medicinal practices. This study employs an untargeted metabolomics approach utilizing High Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) to investigate the Siddha herbal formulation 'Vaasathi kashayam,' as documented in the classical text 'Agasthiyar 2000,' specifically indicated for Raththa kothippu noi (systemic hypertension). Among the 32 categories of internal medicines, Kashayam or Kudineer is a decoction characterized by a limited shelf life of merely three hours. Vaasathi kashayam is freshly prepared from equal proportions of *Justicia adhatoda* L. (Acanthaceae) leaves and Vitis vinifera (Vitaceae) dried fruits. Compounds isolated via Q-TOF liquid chromatography were analyzed through mass spectrometry, with their mass values confirmed through database searches. Approximately 20 metabolites were identified in both solvent A (0.1% formic acid aqueous solution) and solvent B (acetonitrile), including notable compounds such as Peganine (a vasicine-quinazoline alkaloid), Jatrophone (a macrocyclic diterpenoid), Kuwanon Z (a flavan), and Isoacteoside (a hydrocinnamic acid). The classification of these metabolites and their corresponding therapeutic properties was conducted through a comprehensive literature review utilizing databases such as PubChem, Google Scholar, and PubMed, revealing antihypertensive, antidepressant, and vasorelaxant activities mediated by PKC-dependent mechanisms, as well as properties such as ACE inhibition, reduction of oxidative stress, enhancement of coronary reserve, sedative-hypnotic effects, and antagonism of adrenergic receptors. In conclusion, the detailed metabolomic analysis of Vaasathi kashayam highlights its potential therapeutic applications in managing systemic hypertension and related disorders.

INTRODUCTION: With the emergence of integrative medicine, the demand now exists for a more rigorous scientific substantiation of such concepts.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.16(3).741-51

This article can be accessed online on
www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(3).741-51

Metabolite profiling via High Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) is the most effective method of doing this, possessing unique capabilities to separate, identify, and characterize complex mixtures of metabolites with high sensitivity and accuracy. This technology serves to aid in the identification of bioactive ingredients within Siddha herbal formulations and helps to determine the metabolites that are responsible for certain pharmacological properties and their mechanisms. Increasingly, metabolomics is being recognized as a crucial aspect of drug

discovery, as well as, customizing medicine to an individual's need. Researchers, by placing maps of the metabolic profiles behind use of certain traditional remedies, hotspot active compounds, which validates the therapeutic claims.

Moreover, the World Health Organization encourages development of traditional and complementary medicine in different countries, which emphasizes the need for comprehensive and scientific assessments. In that light, HR-LCMS provides quite an essential tool in understanding how Siddha formulations are made. The comprehensive identification of secondary metabolites represents a fundamental step for the assessment of bioactivities and pharmacological properties of traditional herbal drugs [1]. Although untargeted LC-MS analysis has been applied to specific medicinal herbs, many traditional preparations comprise multiple herbs, so the abundance of specific bioactive compounds can vary due to the mixing ratio as well as the effect of different environments on the individual herbal components [2]. The high selectivity, sensitivity and versatility of LC-MS analysis makes it ideal also for such complex herbal medicines [3]. The Siddha system of medicine recognizes approximately 32 types of internal remedies. Among these, Kashayam, also known as Kudineer, is a decoction made by reducing raw ingredients to one-fourth, one-eighth, or one-sixteenth of their original volume.

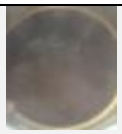
Vaasathi Kashayam is specifically indicated for Raththa Kothippu Noi (systemic hypertension), a globally concerning non-communicable disease. This study is the screening of Vaasathi kashayam using HR-LCMS method for metabolite profiling and fingerprinting.

MATERIALS AND METHODS:

Materials:

Preparation of Vaasathi Kashayam: Vaasathi Kashayam is a decoction formula stated in literature Agathiyar 2000 specifically for Rathatha Pitham. The fresh decoction 'Vaasathi kashayam' was concocted from the leaves of Justicia adhatoda and dried fruits of *Vitis vinifera*. The leaves and black raisins were collected and cleaned of dust from Thakkalai town, Kalkulam Taluk, Kanyakumari district in the month of June and were identified and authenticated by Medicinal Botanist, The Department of Postgraduate Gunapadam (Pharmacology), Government Siddha medical college, Palayamkottai, Tirunelveli District dated on 12.06.2023. Purified drugs are soaked in water (8 times the amount of drug) overnight. Equal parts of the leaves of Justicia adhatoda and dry fruits of Vitis vinifera (5 grams each) were ground and about 240 ml of water was added to this mixture. This mixture was boiled to 30 ml using a hot plate/heating mantle covered with aluminium foil and this fresh extract of decoction was used after filtering the residues through strainer/Whatman filter paper.

TABLE 1: INGREDIENTS AND PREPARATION OF VAASATHI KASHAYAM

			
Leaves of *Justicia adhatoda L. (Acanthaceae)*	Dried fruits of *Vitis vinifera L. (Vitaceae)*	Preparation of *Vaasathi Kashayam*	*Vaasathi Kashayam*

Methods: In this study, VK was prepared at the Government Siddha Medical College in Palayamkottai, Tirunelveli, and analyzed using HR-LCMS at the SAIF (Sophisticated Analytical Instrument Facility) laboratory at IIT Bombay.

The equipment used included the Agilent Technologies HR LCMS – Q-TOF system, 1290 Infinity UHPLC, 1260 Infinity Nano HPLC with Chipcube, and 6550 iFunnel Q-TOFs, all equipped with a Hypersil GOLD C18 column (100 x 2.1 mm, 3 MICRON). A sample volume of 5.00 µL of syrup was analysed under the following conditions: mobile phase Solvent A consisted of 0.1% formic acid in water, while Solvent B was a mixture of 90% acetonitrile, 10% Milli-Q water, and 0.1% formic acid.

The column pressure was set to a maximum of 1200.00 bar, with a flow rate of 0.300 mL/min. The sample was injected at a volume of 3.00 µL with an eject speed of 100.0 µL/min, preceded by a needle

wash, and the stop time was set to 35.00 minutes at a temperature of 40.00 °C. The HR-LCMS results were compared with spectra from the National Institute of Standards and Technology (NIST) database, and the identified compounds were subjected to further analysis.

Data Acquisition Method: Data acquisition method is detailed in **Table 2**. The solvent composition is tabulated in **Table 3** and the running timetable is noted in **Table 4.**

TABLE 2: DATA ACQUISITION METHOD

Method name	**Metabolite_ESI_+VE_MSMS.m**		
Method Path	**D:\MassHunter\methods\2022\metabolite_ESI_+VE_MSMS.m**		
TOF/Q-TOF Mass Spectrometer		MS Q-TOF G6550A	
Ion Source		Dual AJS ESI	
MS Abs. threshold	200	MS Rel. threshold (%)	0.010
MS/MS Abs. threshold	5	MS/MS Rel. threshold (%)	0.010
Acquisition Mode Auto MS 2			
MS Min Range (m/z)	120	MS Scan Rate (spectra/sec)	1.00
MS Max Range (m/z)	1200	MS/MS Scan Rate (spectra/sec)	1.00
Gas Temp (°C)	250	V cap	3500
Gas Flow (I/min)	13	Nozzle Voltage (V)	1000
Nebulizer (psig)	35	Fragmentor	175
Sheath Gas Temp	300	Skimmer1	65
Sheath Gas Flow	11	Octopole RF Peak	750
Chrom Type	Label	Offset	Y-Range
TIC	TIC	15	10000000

TABLE 3: SOLVENT COMPOSITION

	Channel	**Ch. 1 Solv.**	**Name 1**	**Ch2 Solv.**	**Name 2**	**Selected**	**Used**	**Percent**
1	A	100.0% Water V.02	0.1% FA in water	100.0 % Water V.02	0.1% FA in water	Ch.2	Yes	95.00 %
2	B	100.0% Methanol V.03		100.0 % Acetonitrile V.02		Ch.2	Yes	95.00 %

TABLE 4: TIMETABLE

	Time	**A**	**B**	**Flow**	**Pressure**
1	1.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar
2	25.00 min	0.00 %	100.00 %	0.300 mL/min	1200.00 bar
3	30.00 min	0.00 %	100.00 %	0.300 mL/min	1200.00 bar
4	31.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar
5	35.00 min	95.00 %	5.00 %	0.300 mL/min	1200.00 bar

RESULTS: The results of HR-LC MS were tabulated in **Table 5, 6, 7** and **8**. The Compound name, RT, Mass with m/z and chemical formula, chromatogram observed in two of the solvents A and B were tabulated.

TABLE 5: NAME OF THE METABOLITES WITH MOLECULAR FORMULA OBTAINED FROM HR-LCMS USING Q-TOF METHOD. R_F-RETENTION FACTOR

Compound Label	**RT**	**Mass**	**Name**	**Formula**	**m/z**
Compound1	0.883	-	-	-	299.0427
Cpd2:D-Tryptophan	2.503	204.0879	D-Tryptophan	C11H12N2O2	205.0951
Cpd3:Antipyrine	3.29	188.0963	Antipyrine	C11H12N2O	189.1043
Cpd4:Antipyrine	3.61	188.0958	Antipyrine	C11H12N2O	189.1036
Cpd5:Peganine	4.001	188.0945	Peganine	C11H12N2O	189.1019
Cpd6:Peganine	4.373	188.0938	Peganine	C11H12N2O	189.1011
Cpd7:2-Methoxy-3-(1-methylpropyl) pyrazine	4.712	166.1116	2-Methoxy-3-(1- methylpropyl) pyrazine	C9H14N2O	189.1008
Cpd8:2-Methoxy-3-(1-methylpropyl) pyrazine	5.028	166.1115	2-Methoxy-3-(1- methylpropyl) pyrazine	C9H14N2O	189.1007
Cpd9:Fluacrypyrim	5.25	426.1396	Fluacrypyrim	C20H21F3N2O5	427.1469

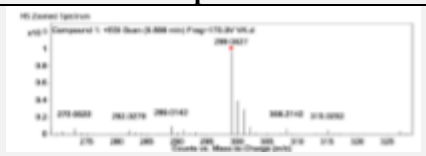
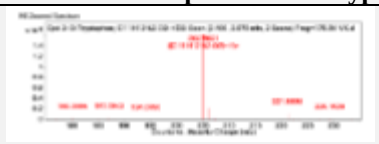
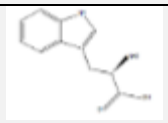
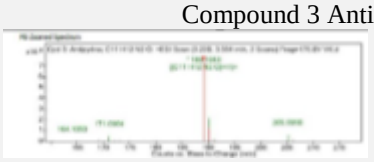
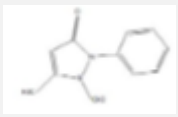
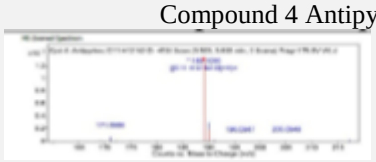
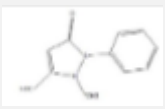
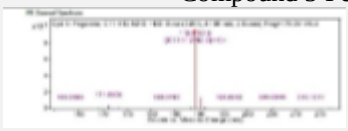
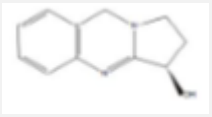
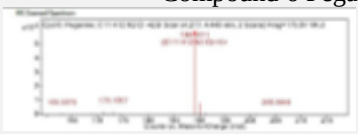
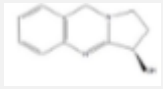
Cpd10:2-Methoxy-3-(1-methylpropyl) pyrazine	5.327	166.1114	2-Methoxy-3-(1- methylpropyl) pyrazine	C9H14N2O	189.1006
Cpd11:2-Methoxy-3-(1-methylpropyl) pyrazine	5.624	166.1113	2-Methoxy-3-(1- methylpropyl) pyrazine	C9H14N2O	189.1005
Cpd12:2-Methoxy-3-(1-methylpropyl) pyrazine	5.928	166.1113	2-Methoxy-3-(1- methylpropyl) pyrazine	C9H14N2O	189.1005
Compound13	5.928	-	-	-	470.189
Cpd14:Fluacrypyrim	6.191	426.1397	Fluacrypyrim	C20H21F3N2O5	427.1471
Cpd15:3-(4-Hydroxy-3-methoxyphenyl)-1,2-propanediol 2-O-(galloyl-glucoside)	6.919	512.1517	3-(4-Hydroxy-3-methoxyphenyl)-1,2-propanediol2-O-(galloyl-glucoside)	C23H28O13	535.1409
Cpd16:Zanthodioline	7.385	305.123	Zanthodioline	C16H19NO5	306.1302
Cpd17:Cynometrine	7.48	285.1478	Cynometrine	C16H19N3O2	308.1369
Compound18	7.52	-	-	-	292.1788
Cpd19:Ergine	7.689	267.1364	Ergine	C16H17N3O	290.1257
Compound20	7.864	-	-	-	292.1785

TABLE 6: THE CHROMATOGRAM OF VK HR-LCMS WITH SOLVENT A AND SOLVENT B

TABLE 7: NAME OF THE METABOLITES WITH MOLECULAR FORMULA OBTAINED FROM HR-LCMS USING Q-TOF METHOD. RF-RETENTION FACTOR

Compound Label	RT	Mass	Name	Formula	m/z
Cpd1:2,4-Dichloro-3-oxoadipate	0.86	227.9573	2,4-Dichloro-3-oxoadipate	C6H6Cl2O5	272.9558
Compound2	0.884	-	-	-	386.9355
Cpd3:Isoacteoside	1.152	624.208	Isoacteoside	C29H36O15	683.2221
Compound4	1.383	-	-	-	903.2629
Cpd 5:5-Hydroxyferulate	3.595	210.0509	5-Hydroxyferulate	C10H10O5	255.0492
Cpd6:(-)-Epicatechin3'-O-glucuronide	3.685	466.1076	(-)-Epicatechin3'-O-glucuronide	C21H22O12	511.1059
Cpd7:L-Djenkolicacid	3.711	254.0399	L-Djenkolicacid	C7H14N2O4S2	253.0325
Cpd 8:5-Hydroxyferulate	3.888	210.0504	5-Hydroxyferulate	C10H10O5	255.0486
Cpd9:PhrymarolinI	6.461	488.1287	PhrymarolinI	C24H24O11	533.1271
Cpd10:Kaempferol3-rhamnoside7-xyloside	6.541	564.1451	Kaempferol3-rhamnoside7-xyloside	C26H28O14	563.1381
Cpd11:Trovafloxacin	6.74	416.1068	Trovafloxacin	C20H15F3N4O3	461.1051
Cpd12:PhrymarolinI	6.794	488.129	PhrymarolinI	C24H24O11	533.1272
Cpd13:Kaempferol3-rhamnoside7-xyloside	6.809	564.1443	Kaempferol3-rhamnoside7-xyloside	C26H28O14	563.1372
Compound14	6.961	-	-	-	1103.2396
Compound15	6.963	-	-	-	1067.2644
Cpd16:PhrymarolinI	7.147	488.1291	PhrymarolinI	C24H24O11	533.1273
Cpd17: KuwanonZ	7.484	594.1536	KuwanonZ	C34H26O10	593.1464
Cpd18: PhrymarolinI	7.526	488.1289	PhrymarolinI	C24H24O11	533.1272
Cpd19: Sulindac	7.827	356.0868	Sulindac	C20H17FO3S	401.085
Cpd20:Jatrophone	23.496	312.1729	Jatrophone	C20H24O3	311.166

TABLE 8: ZOOMED SPECTRUM, COMPOUND STRUCTURE OF THE 20 IDENTIFIED COMPOUNDS ACCORDANCE WITH NIST LIBRARY (VK)

Compound 1	Compound 2 D- Tryptophan
	 
Compound 3 Antipyrine	Compound 4 Antipyrine
 	 
Compound 5 Peganine	Compound 6 Peganine
 	 
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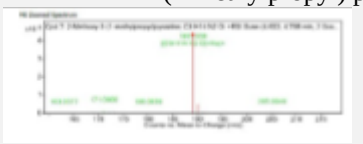
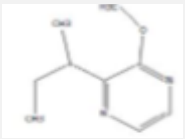
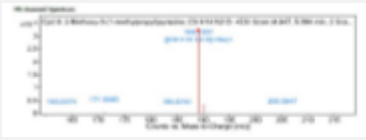
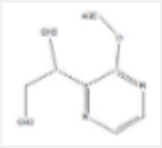
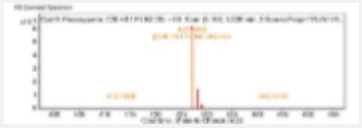
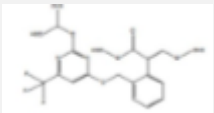
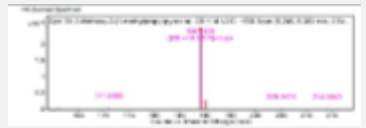
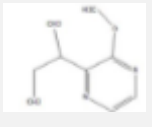
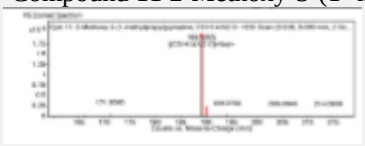
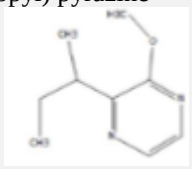
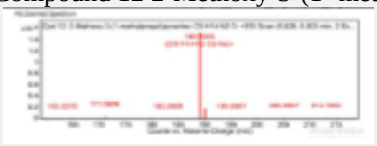
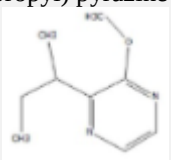
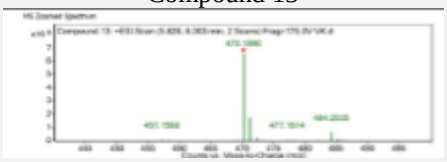
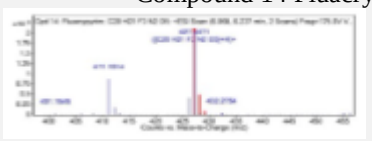
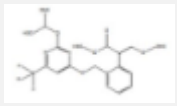
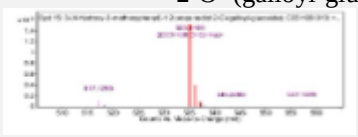
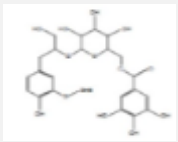
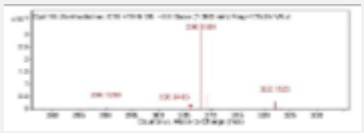
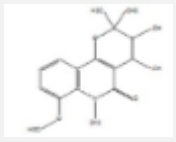
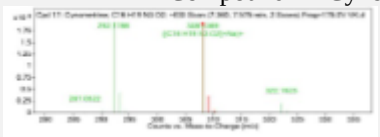
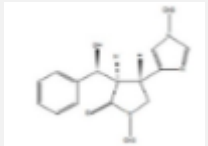
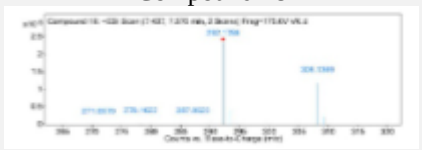
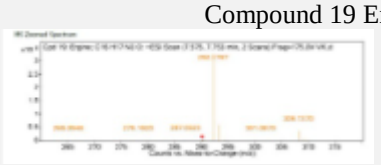
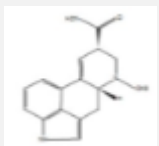
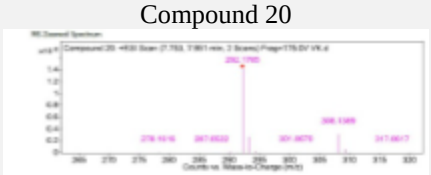
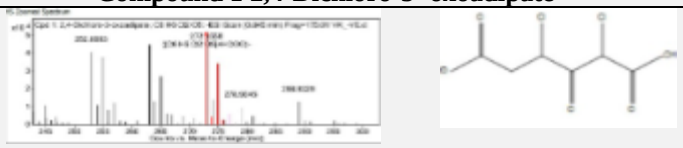
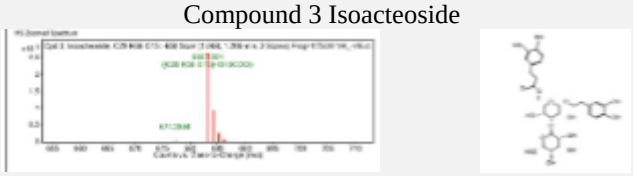
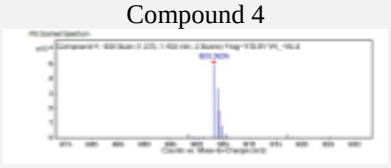
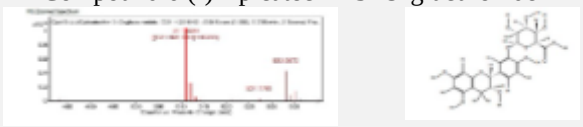
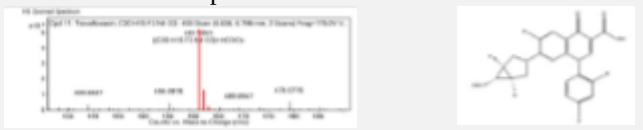
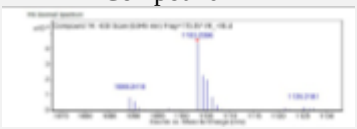
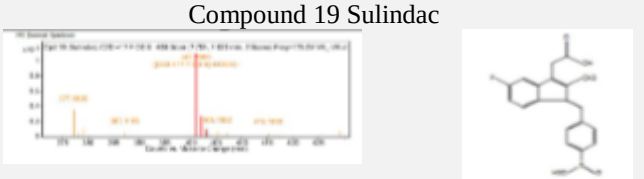
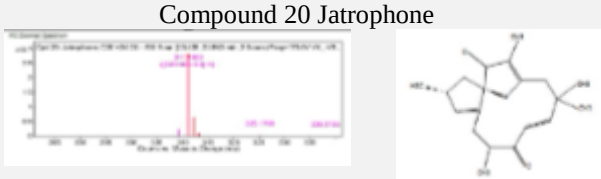
 	 
Compound 9 Fluacrypyrim	Compound 10 2-Methoxy-3-(1- methylpropyl) pyrazine
 	 
Compound 11 2-Methoxy-3-(1- methylpropyl) pyrazine	Compound 12 2-Methoxy-3-(1- methylpropyl) pyrazine
 	 
Compound 13	Compound 14 Fluacrypyrim
	 
Compound 15 3-(4-Hydroxy-3- methoxyphenyl)-1,2- propanediol 2-O- (galloyl-glucoside)	Compound 16 Zanthodioline
 	 
Compound 17 Cynometrine	Compound 18
 	
Compound 19 Ergine	Compound 20
 	

TABLE 9: ZOOMED SPECTRUM, COMPOUND STRUCTURE OF THE 20 IDENTIFIED COMPOUNDS ACCORDANCE WITH NIST LIBRARY (VK_VE)

Compound 1 2,4-Dichloro-3- oxoadipate	Compound 2
	
Compound 3 Isoacteoside	Compound 4
	
Compound 5 5-Hydroxyferulate	Compound 6 (-)-Epicatechin 3'-O-glucuronide
	
Compound 7 L-Djenkolic acid	Compound 8 5-Hydroxyferulate
	
Compound 9 Phrymarolin I	Compound 10 Kaempferol 3- rhamnoside 7-xyloside
	
Compound 11 Trovafloxacin	Compound 12 Phrymarolin I
	
Compound 13 Kaempferol 3- rhamnoside 7-xyloside	Compound 14
	
Compound 15	Compound 16 Phrymarolin I
	
Compound 17 Kuwanon Z	Compound 18 Phrymarolin I
	
Compound 19 Sulindac	Compound 20 Jatrophone
	

On, further literary search of the identified compounds in PubMed, PubChem, Google scholar, the chemical nature and therapeutic use relevant to hypertension are documented in the **Table 10.**

TABLE 10: NAME OF THE METABOLITES IDENTIFIED, CLASS AND THEIR RELATED ANTI-HYPERTENSIVE ACTIVITIES

Compound	Chemical Nature	Therapeutic Use
Peganine	Vasicine - Quinazoline alkaloid	Anti-hypertensive, antidepressant [4]
Jatrophone	Macrocyclic diterpenoid	Concentration-dependent inhibition of noradrenaline (NA)-induced concentrations, activating K+ channels, inhibited Ca2+-induced contractions [5], Vasorelaxant by inhibition of PKC-dependent mechanisms [6]
Kuwanon Z	Flavans	Targets both the spike S1 RBD and the ACE2 receptor [7], Modulates the renin–angiotensin system (RAS) [8]
Isoacteoside	Hydrocinnamic acid	Inhibit ACE activation in SHR and protect organ damage against hypertension [9], Antihypertensive activity - it reduces the number of cardiac contractions and MABP [10]
5-Hydroxyferulate	Monocarboxylic acid anion	Ferulic acids prevented gain in body weight induced by the high-fat diet and improved hyperglycemia, hypercholesterolemia and hypertriglyceridemia [11], Short acting B2 adrenoreceptor, strong antioxidant action [12]
(-)-Epicatechin 3'-O-glucuronide	Flavonoids and a beta-D-glucosiduronic acid	Protects the cells against signs of oxidative stress elicited by oxidized LDL, angiotensin II or TNF-α [13]
Kaempferol 3-rhamnoside 7- xyloside	Flavanoid 7 O glucosides	Inhibition of angiotensin-converting enzyme activity [14]
Phrymarolin I	Benzodioxoles, conjugate base of a quinapril	FAAH inhibitors [15]
Zanthodioline	Organonitro heterocyclic compound	Antioxidant, thrombolytic and antimicrobial activity [16]
3-(4-Hydroxy-3-methoxyphenyl)-1,2-propanediol 2-O-(galloyl- glucoside)	Galloyl-glucoside	Antioxidant, antidiabetic, antibacterial, anti-inflammatory, antiproliferative activities [17]
2-Methoxy-3-(1-methylpropyl) pyrazine	Azaheterocycle	Sedative-hypnotic used in the treatment of insomnia, improving both the latency phase and the maintenance phase of sleep, Compound of pyrazine used to treat Hypertension [18]
Sulindac	Arylalkonic acid	COX - 2 inhibitor, Anti-inflammatory [19, 20]
Antipyrine	NSAIDS with pyrazolone nucleus	Analgesic, antipyretic and anti-inflammatory drug [21], adrenergic blocking agent used to treat mild to moderate hypertension [22]
D-Tryptophan	D-aminoacids	Reduces BP in hypertensive rats [23], Blunts tissue ACE activity, reduces matrix metalloproteinase-2 activity and improves coronary flow reserve [24]
Trovafloxacin	Fluronaphthyridone	Anti-microbial plant metabolite [25], Thrombolytic, Cardioprotective, Hypotensive
2,4-Dichloro-3-oxoadipate	Oxo dicarboxylate	Endothelin-1 receptors, improving endothelial function and decreasing atherosclerotic plaque [26]
Cynometrine	Imidazole alkaloids	Diuretic, sympatholytic, Antioxidant [27]
Fluacrypyrim	Pyrimidine	Effective inhibitor of STAT3, Protective role in hematopoietic damage and modulation of apoptotic activities in HSCs [28]
Ergine	D-lysergic acid amide	Antagonist action on neurotransmitters, adrenergic, dopaminergic and Serotonergic receptors [29]
L-Djenkolic acid	Dithioacetal, a L-cysteine derivative and a non-proteinogenic L-alpha-amino acid.	Selenium-containing compound that inhibits the bacterial enzyme, β-lactamase [30]

DISCUSSION: The high-resolution liquid chromatography-mass spectrometry (HR-LCMS) profiling of the Siddha herbal formulation Vaasathi Kashayam has successfully identified a total of 40 metabolites, of which 21 were specifically examined for their anti-hypertensive and antioxidant activities through a comprehensive review of literature on databases such as PubMed, Google Scholar, and PubChem. This thorough investigation underscores the therapeutic potential of Vaasathi Kashayam in managing hypertension, a significant global health concern. Peganine or

vasicine is an alkaloid of the quinazoline family and one of the prominent metabolites in Eucalyptus globulus. Prior studies have indicated vasicine to show potent hypertensive and antidepressant activities [4]. The presence of two activities in this metabolite indicates an interesting dynamic in the management of hypertension that is both cardiovascular and mental health focused. Jatrophone, a macrocyclic diterpenoid, concentrates and inhibits norepinephrine induced contractions through a different mode of inhibition. In this case, this metabolite also has vasodilating activity by activating potassium channels and inhibiting calcium induced contraction [5, 6].

The presence of all these activities may considerably lower vascular resistance, which is characteristic of hypertension. Kuwanon Z, a component identified as a flavan has proven to become more relevant because it could bind with both spike S1 RBD and ACE2. This is crucial because it can help to regulate the renin angiotensin systems (RAS), which is an important mechanism that regulates blood pressure [7, 8]. In view of this potential, Kuwanon Z may improve endothelial function by modulating such systems and thereby assist in the control of blood pressure. Isoacteoside is another important metabolite with origin from hydrocinnamic acid that has been reported to block the activation of ACE thereby preventing the organ damage associated with hypertension. Studies show that Isoacteoside lowers the systolic contractions of the heart and mean arterial blood pressure (MABP) further cementing its potency in the protection of the cardiovascular system [9, 10].

The ability of this compound to not only lower blood pressure but also the target organ damage related to hypertension is quite relevant in clinical therapeutics. The metabolite 5-Hydroxyferulate, a monocarboxylic acid anion, further increases the effectiveness of the formulation by ameliorating high fat diet induced obesity and improving metabolic disorders including hyperglycemia, hypercholesterolemia and hypertriglyceridemia [11]. Besides, it has strong anti-oxidative capacity and therefore, plays a protective role against oxidative stress [12], which is another major contributor of the hypertensive pathogenesis. Same effect was obtained in the past with (-)-Epicatechin 3'-O-glucuronide, a flavonoid, on protecting against oxidative damage induced by oxidized low density lipoproteins (LDL), angiotensin II, or TNF-alpha [13]. This protective effect against oxidative stress is crucial, as such stress is known to exacerbate hypertensive conditions. The presence of Kaempferol 3-rhamnoside 7-xyloside, another flavonoid, is noteworthy for its ability to inhibit angiotensin-converting enzyme (ACE) activity, a central target in anti-hypertensive therapies [14]. This inhibition reduces angiotensin II levels, facilitating vasodilation and improved blood pressure regulation. Phrymarolin I, as a FAAH inhibitor and a derivative of quinapril, likely promotes vasodilation *via* enhanced endocannabinoid signalling, which may reduce blood pressure. Its role in modulating stress responses could also alleviate anxiety-related insomnia [15].

Zanthodioline, an organonitro heterocyclic compound, exhibits antioxidant and anti-inflammatory properties that can protect vascular health [16]. The galloyl-glucoside compound 3-(4-Hydroxy-3-methoxyphenyl)-1,2-propanediol 2-O-(galloyl-glucoside) offers broad bioactivity, including significant antioxidant and anti-inflammatory effects, which can enhance endothelial function and contribute to better hypertension management, alongside its potential benefits for sleep quality [17]. 2-Methoxy-3-(1-methylpropyl) pyrazine directly targets insomnia by improving sleep latency and maintenance, while also serving as a therapeutic agent for hypertension, likely through its influence on neurotransmitter systems [18].

Sulindac, a COX-2 inhibitor, mitigates inflammation, which is a known contributor to hypertension. Its anti-inflammatory effects may also indirectly support better sleep quality by alleviating discomfort associated with chronic pain [19, 20]. Lastly, Antipyrine, as an NSAID with adrenergic-blocking properties, serves dual roles in treating mild to moderate hypertension and providing analgesic effects that can aid in sleep quality [21, 22]. Several metabolites also contribute to the diuretic effects of Vaasathi Kashayam, such as D-Tryptophan and Cynometrine. D-Tryptophan not only reduces blood pressure in hypertensive models but also blunts tissue ACE activity and improves coronary flow reserve [23, 24]. Similarly, Cynometrine is recognized for its diuretic and sympatholytic

properties, aiding in blood pressure reduction through enhanced fluid excretion and sympathetic nervous system modulation causing decrease in vascular resistance and heart rate [27]. The antioxidant properties of various metabolites, including 3-(4-Hydroxy-3-methoxyphenyl)-1,2-propanediol 2-O-(galloyl-glucoside) and Trovafloxacin, further contribute to mitigating oxidative stress and promoting cardiovascular health. 2,4-Dichloro-3-oxoadipate, an oxo dicarboxylate, has been shown to interact with endothelin-1 receptors, which play a crucial role in vascular function. By improving endothelial function and reducing atherosclerotic plaque formation, this compound can potentially decrease vascular resistance and lower blood pressure [26]. Fluacrypyrim, a pyrimidine compound, is an effective inhibitor of STAT3, which is involved in inflammatory pathways and vascular remodelling. By modulating apoptotic activities in hematopoietic stem cells (HSCs) and reducing inflammation, Fluacrypyrim may help prevent pathological changes in the vasculature that contribute to hypertension [28]. Ergine, a D-lysergic acid amide, acts as an antagonist on adrenergic, dopaminergic, and serotonergic receptors. This multi-receptor antagonism can lead to reduced sympathetic tone and lower heart rate, both of which are beneficial for managing hypertension [29].

CONCLUSION: In conclusion, HR-LCMS profiling of Vaasathi Kashayam shows a rich mix of metabolites that together have a strong impact on lowering blood pressure and fighting oxidative damage. These compounds work in different ways such as adjusting the renin-angiotensin system, blocking ACE activity helping in vasoconstriction, and shielding against oxidative stress. The interplay of these varied biochemical processes highlights Vaasathi Kashayam's potential as a multi-faceted treatment option to manage high blood pressure. This calls for more clinical studies to understand the mechanism of action of this drug.

ACKNOWLEDGMENTS: I would like to thank my guide, Dr. T. Komalavalli MD (S) Ph.D., for her guidance and support during this research. I'm thankful to Dr. B. Malarvizhi Head of the Institution, for creating an inspiring place to learn. I appreciate the faculty of the department for their encouragement and insights that helped my work a lot. I'm grateful to the SAIF, IIT Bombay for their expertise and help with the HR-LCMS analysis. I would like to extend my sincere thanks to Dr.Kingsly, Head of the Department, Department of PG Gunapadam (Pharmacology), GSMC, Tirunelveli for his expertise and meticulous assistance in plant identification. I owe special thanks to Dr. K. P. S. Raja Abdul Hameethu BSMS, for his computational assistance, which played a key role in this project's success. To my juniors and friends, thanks for being there and supporting me on this journey. I'm grateful to my family for their constant encouragement and love, which have kept me going every step of the way.

CONFLICT OF INTEREST: None

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How to cite this article:
Preyadarsheni K and Komalavalli T: HR LC-MS for the management of systemic hypertension (Raththa Kothippu NOI). Int J Pharm Sci & Res 2025; 16(3): 741-51. doi: 10.13040/IJPSR.0975-8232.16(3).741-51.