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Research Article

Alkaloid Profiling of An Ayurvedic Medicine Using UPLC-ESI-QTOF-MS

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Abstract

Background: Gulguluthikthakam Kashayam is an ayurvedic medicine primarily used for treating inflammatory conditions. It is particularly effective in managing osteoarthritis, various skin diseases, and is considered an excellent remedy for different types of pain. This formulation/medicine comprises 29 herbs and is a complex mixture containing various secondary metabolites in differing concentrations. The identification and characterization of such components pose a significant challenge to researchers.

Hyphenated analytical techniques, such as HPLC-MS, which combine separation methods with mass spectrometry, are valuable tools for identifying these metabolites in complex mixtures. Among them, High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS) stands out as a powerful technique for analyzing complex herbal formulations.

Since alkaloids play a significant role as anti-inflammatory agents in both modern and traditional medicine—and often serve as lead compounds for new drug development—the present study aims to identify the alkaloids present in Gulguluthikthakam Kashayam using Ultra-Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS).

Method: A 300 mL sample of Gulguluthikthakam Kashayam was centrifuged at 4000 rpm for 30 minutes using a REMI 8C centrifuge. The clear supernatant (~200 mL) was carefully decanted and subsequently subjected to analysis using Ultra-Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS).

Result: Ultra-Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (UPLC-QTOF-MS) was employed to identify alkaloids present in Gulguluthikthakam Kashayam. The analysis revealed the presence of several bioactive compounds, including piperine, trans-feruloyl octopamine, vasicinone, coumaperine, tetrahydropiperine, and vasicinolone. The mass fragmentation patterns of these identified compounds are detailed in this paper.

Conclusion: Ultra-Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (UPLC-QTOF-MS) confirmed the presence of six alkaloids with known anti-inflammatory properties in Gulguluthikthakam Kashayam, supporting its traditional use as an anti-inflammatory formulation.

Keywords: Inflammation; Anti-inflammatory; Antioxidant; Immunostimulant; UPLC-ESI -QTOF-MS

Abbreviations

LCMS: Liquid Chromatography-Mass Spectrometry; m/z: Mass to Charge Ratio; RDA: Retro-Diels -Alder Reaction; NSAIDS: Non -Steroidal Anti-inflammatory Drugs; UPLC-ESI-QTOF -MS: Ultra Performance -Electron Spray-Quadrupole-Time of Flight-Mass Spectrometry

Introduction

Indeed, chronic inflammation plays a central role in the development and progression of a wide range of diseases—including depression, diabetes, cancer, heart disease, Alzheimer's, psoriasis, allergy, stroke, asthma, and obesity [1]. So, inflammation is a huge challenge for humans. There are two types of anti-inflammatory drugs. One is plant based and other one is synthetic. The biggest disadvantage of recently available potent synthetic drugs is concerning their toxicity and the reappearance of symptoms after discontinuation of the medicine [2]. Therefore, the screening and development of drugs with anti-inflammatory activity are necessary and there are many efforts to find anti-inflammatory drugs from medicinal plants. Plants have many phyto-constituents helpful in reducing inflammation with fewer side effects. When a decoction of different plant parts (kashayam) is used, there is a good chance of synergism between active components. There are many Ayurveda formulations with anti-inflammatory therapeutic effects with low or no side effects. Gulguluthikthakam kashayam is one of such Ayurvedic formulation to alleviate the inflammation [3]. Gulguluthikthakam kashayam is mainly used for inflammatory conditions so it is good for osteoarthritis, skin diseases and is an excellent medicine for all types of pain.

Gulguluthikthakam Kashayam was prepared from 29 plant parts as per Ashtanga Hridayam. It is the decoction (water extract) includes a wide range of herbs—such as *Cuminum cyminum* L. (Fruit), *Cedrus deodara* (Roxb.exD.Don) G.Don (Wood), *Celastrus paniculatus* Willd. (Seed), *Scindapsus officinalis* (Roxb.) Schott (fruit), *Picrorhiza kurroa* Royle ex Benth. (Root), *Saussura lappa* (Decne.) Sch.-Bip. (Root) *Piper nigrum* L. (Fruit), *Rubia cordifolia* L. (Stem), *Cyperus rotundus* L.(Rhizome), *Curcuma longa* L. (Rhizome), *Adhatoda vasica* Nees (Root), *Tinospora cordifolia* (Willd.) Miers ex Hook.f. & Thoms. (Stem), *Solanum indicum* L. (Root), *Azadirachta indica* (L.) A.Juss. (Stem), *Semecarpus anacardium* L.f. (Seed), *Commiphora mukul* (Hook.ex Stocks) Engl. (Resin), *Plumbago zeylanica* L. (Rhizome), *Piper brachystachyum* Wall.ex Hook.f. (Root), *Embelia ribes* Burm.f. (Fruit), *Acorus calamus* L. (Rhizome), *Trichosanthes dioica* Roxb. (Whole Plant), *Zingiber officinale* Rosc. (Rhizome), *Alpinia calcarata* Willd (Rhizome), *Aconitum heterophyllum* Wall.ex Royle(Tube root), *Holarrhena antidysenterica* (Roth) DC. (Seed), *Anetham graveolens* L. (Fruit), *Cyclea peltate* (Lam.) Hook.f. & Thoms. (Tube root), *Trachyspermum roxburghianum* (DC.) Wolff (Fruit) and *Piper longum* L. (Root). Gulguluthikthakam Kashayam is a complex natural mixture, rich in a diverse array of phytochemicals present in varying concentrations.

Liquid Chromatography–Mass Spectrometry (LC-MS) is a hyphenated analytical technique that combines the separation capabilities of High-Performance Liquid Chromatography (HPLC) with the detection and identification strengths of Mass Spectrometry (MS). This integration allows for the efficient separation of complex mixtures and the precise identification of individual components, making it particularly valuable in the analysis of natural products. Ultra-Performance Liquid Chromatography coupled with Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry (UPLC-ESI-QTOF-MS/MS) is a powerful technique that enhances both separation and detection capabilities. UPLC offers higher resolution and faster analysis times compared to traditional HPLC, while ESI-QTOF-MS/MS provides detailed fragmentation patterns for structural characterization. This combination is particularly effective in metabolomics studies and the profiling of phytochemicals in plant extracts.

Alkaloids, a remarkable class of natural compounds, serve as scaffolds for the development of new drugs. In both traditional and modern medical systems, the biological activities of alkaloids such as anticancer, antibacterial, anti-inflammatory, antimicrobial, antioxidant, AChE inhibitory activity, antimalarial, and antidiabetic activity have been examined [4]. Given the well-documented therapeutic potential of alkaloids in treating inflammatory conditions, this study employs UPLC-QTOF-MS to identify alkaloids in Gulguluthikthakam Kashayam.

Materials and Methods

Gulguluthikthakam kashayam has been procured from the market prepared by The Arya Vaidya pharmacy, Coimbatore. LCMS quality chemicals are used in the analysis.

Preparation of supernatant of kashayam for UPLC-QTOF-MS analysis

Three hundred milliliters of well-shaken kashayam were centrifuged at 4000 rpm (approximately 3000 × g) using a REMI R-8C centrifuge for 30 minutes. Two hundred twenty milliliters of the clear supernatant were carefully decanted and then analyzed using UPLC-ESI-QTOF-MS

UPLC-QTOF -MS

The chromatographic separation and detection of analytes was carried out with ultra-performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (UPLC-QTOF-MS). The acquity UPLC system (Waters) consists of a TUV detector (JI2TUV750A), a column chamber (JI2 CHA730G), a quaternary solvent manager (HI2 QSM632A), and a sample manager FTN (KI2 SDI069G). A reversed -phase BEH C18 column (of dimension 50mm X 2.1mm X 1.7 µm) with a flow rate of 0.3mL min^{-1} was used for chromatographic separation (Waters). The mobile phase was a mixture of 0.1% Formic acid in water (A) and acetonitrile (B) in a gradient elution as follows initial 95% A, 0.1min 95%A, 6.00 min 5%A, 6.5 min 5%A, 9min 95%A and 10 min 95%A. The UPLC system was connected to the quadrupole time of flight mass spectrometer (Waters Xevo G2 QTOF) with electrospray ionization (ESI) interface working in positive and negative ionization modes. The injection volume was 10µL. The scanning m/z range was between 50 and 1000. The desolvation gas flow and the temperature were 900L/h and 350 0C, respectively. The mass spectra were obtained using collision energy ranging from 5 to 30eV.The instrument control and data acquisition was done using MassLynx software (v 4.1).

Result

UPLC-QTOF -MS analysis of supernatant of Gulguluthikthakam kashayam identified 6 alkaloids. All the identified compounds have been validated with the mass fragmentation pattern.

As per the reports all the identified compounds have been found to be anti-inflammatory, anticancer and immunostimulant.

Compound 1, with a retention time of 3.499 minutes, exhibited a molecular ion peak at m/z 218.2122 (100%). A fragment ion at m/z 200.2018 was formed due to the loss of a water molecule (H_2O) from the protonated molecular ion. Further fragmentation resulted in a product ion at m/z 174.1859, which was generated through carbon monoxide (CO) elimination accompanied by ring contraction from the m/z 200.2018 fragment. Subsequently, the loss of an ammonia molecule (NH_3) yielded an ion at m/z 156.1751.

The fragmentation pattern and elemental composition analysis confirmed the molecular formula of Compound 1 as $C_{11}H_{10}N_2O_3$. Based on this data, the compound was identified as Vasicinolone.

Vasicinolone (3,7-dihydroxy-2,3-dihydro-1H-pyrrolo[2,1-b] quinazolin-9-one) is a known alkaloid constituent of *Adhatoda vasica*.

Compound 2, with a retention time of 4.361 minutes, exhibited a protonated molecular ion peak at m/z 290.2694 (100%). A prominent fragment ion at m/z 274 was formed due to the loss of a water molecule (H_2O) from the protonated molecular ion.

The cleavage of the carboxy-amide linkage generated a fragment at m/z 205, which subsequently underwent the successive loss of methanol (CH_3OH) and carbon monoxide (CO) to yield a fragment ion at m/z 145.0248.

Additionally, the elimination of a $C_5H_{10}N$ moiety from the ion at m/z 274 produced a fragment ion at m/z 191. Another significant ion at m/z 242 was formed by the loss of formaldehyde (CH_2O) from the m/z 274 ion.

Based on elemental composition analysis and mass fragmentation patterns, Compound 2 was identified as Tetrahydropiperine (5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpentan-1-one), with the molecular formula $C_{17}H_{23}NO_3$. This compound is an alkaloid found in Piper species.

Compound 3, with a retention time of 4.058 minutes, exhibited a molecular ion peak at m/z 202.2168 (100%) in positive ionization mode. Fragment ions were observed at m/z 185.2084 (28%), 158.1891, and 130.0722.

The ion at m/z 185.2084 was formed due to the loss of a water molecule (H_2O) from the molecular ion, attributed to the hydroxyl group at the C-3 position. This fragment is extensively resonance-stabilized through conjugation with adjacent ring systems.

Subsequent fragmentation produced ions at m/z 158.1891, formed by loss of carbon monoxide (CO) with accompanying ring contraction,and m/z 130.0722, formed by the elimination of a CH_2N moiety from the previous fragment.

The mass fragmentation pattern and elemental analysis confirmed Compound 3 as Vasicinone, with the structure (1S)-1-hydroxy-2,3-dihydropyrrolo[2,1-b]quinazolin-5(1H)-one.

Vasicinone, with a molecular formula $C_{11}H_{10}N_2O_2$, has been reported in Adhatoda vasica and was detected here at a concentration of approximately 3.5%.

Compound 4, with a retention time of 4.883 minutes, exhibited a protonated molecular ion peak at m/z 258.2788 (100%). Fragment ions were observed at m/z 240.2677 (38%) and m/z 211.0569 (2%).

The fragment at m/z 240.2677 was formed through the loss of a water molecule (H_2O) from the protonated molecular ion. Further fragmentation involving cyclization and the elimination of a carbon monoxide (CO) molecule led to the formation of the ion at m/z 211.0569.

Based on elemental analysis, the molecular formula of Compound 4 was determined to be $C_{16}H_{19}NO_2$. The mass fragmentation pattern and structure elucidation confirmed the identity of the compound as Coumaperine.

Coumaperine [(2E,4E)-5-(4-hydroxyphenyl)-1-piperidin-1-ylpenta-2,4-dien-1-one] is a naturally occurring alkaloid found in Piper species.

Compound 5, with a retention time of 5.451 minutes, produced a protonated molecular ion peak ($M+H^+$) at m/z 330.3372 (100%). Significant fragment ions were observed at m/z 312.3265 (33%) and m/z 286.3108 (3%).

The fragment at m/z 312.3265 resulted from the loss of a water molecule (H_2O) from the protonated parent ion. This fragment then underwent elimination of a carbon monoxide (CO) moiety along with ring contraction, forming the ion at m/z 286.3114. Subsequently, amide C–N bond cleavage of this fragment yielded a product ion at m/z 178, corresponding to 177 + H^+.

The observed fragmentation pathway, as outlined in the proposed scheme, is characteristic of trans-Feruloyl octopamine, a compound with the molecular formula $C_{18}H_{19}NO_5$.

This alkaloid has been previously reported from *Tinospora cordifolia*, and the mass spectral data presented here confirm the identification of Compound 5 as trans-Feruloyl octopamine.

Compound 6, with a retention time of 6.148 minutes, exhibited a protonated molecular ion peak at m/z 286.1448 (100%). Notable fragment ions were observed at m/z 201.0554 (50%) and m/z 173.0600 (1%).

The fragment at m/z 201.0554 was formed via the loss of a piperidine moiety, resulting from cleavage of the amide linkage and the formation of an acylium cation. This cation features a conjugated carbonyl group attached to a phenyl ring, and undergoes resonance-stabilized cyclization involving the α-carbon of the carbonyl group and the benzene ring.

Subsequently, this ion loses a formaldehyde group (-CH_2O) to produce a fragment at m/z 171, observed experimentally as m/z 173.0600 due to hydrogen addition (i.e., 171 + H^+ = 172; rounded to 173). The ion at m/z 143 is generated from m/z 173.0600 through the elimination of two carbon monoxide (CO) molecules.

The mass fragmentation pattern, along with elemental analysis, confirmed the identity of Compound 6 as Piperine, with the structure (2E,4E)-5-(2H-1,3-benzodioxol-5-yl)-1-(piperidin-1-yl) penta-2,4-dien-1-one and the molecular formula $C_{17}H_{19}NO_3$.

Piperine is a well-known alkaloid present in *Piper* species and is responsible for their characteristic pungency.

Discussion

UPLC -QTOF -MS is the only technique available to separate and identify the components very efficiently from a complex mixture of natural products. UPLC -QTOF -MS analysis identified 6 alkaloids. All of them explained with the help of the mass fragmentation pattern. The UPLC-ESI-QTOF-MS analysis of *Gulguluthikthakam Kashayam* led to the identification of several bioactive alkaloids, including Vasicinone, Vasicinolone, Coumaperine, Piperine, trans-Feruloyl octopamine, and Tetrahydropiperine. Among these, Vasicinone and Vasicinolone are alkaloids previously reported from the plant *Adhatoda vasica* Nees, while Coumaperine, Piperine, trans-Feruloyl octopamine, and Tetrahydropiperine are known constituents of *Piper* species.

SI No.	Retention Time	Compound name	Type of Compound	Molecular formula	Molecular weight	Molecular ion	Fragments
1.	6.148	Piperine	Alkaloid	$C_{17}H_{19}NO_3$	285.34	286.1448(100%) $M+H^+$	201.0554(50%) and m/z 173.0600 (1%), 143
2.	5.451	Trans feruloylcto-pamine	Alkaloid	$C_{18}H_{19}NO_5$	329.7	330.3372(100%) $M+H^+$	312.3265(33%), 286.3108(3%)
3.	4.058	Vasicinone	Alkaloid	$C_{11}H_{10}N_2O_2$	202.21	202.2168 (100%) M^+	185.2058(28%), 142.0722, 158. 1891
4.	4.883	Coumaperine	Alkaloid	$C_{16}H_{19}NO_2$	257.33	258.2788(100%) $M+H^+$	240.2677(38%) and 211.0569(2%)
5.	4.361	Tetrahydropip-erine	Alkaloid	$C_{17}H_{23}NO_3$	289.4	290.2694(100%) $M+H^+$	272 .2581
6.	3.499	Vasicinolone	Alkaloid	$C_{11}H_{10}N_2O_3$	218.21	218.2122 (100%) M^+	200.2018, 174.1859(100%), 156.1761

Table 1: Compounds identified with the UPLC-Q-TOF-MS.

SI No.	Compound name	Biological activity	Plant source
1.	Transferuloyloctapamine	Anti-oxidant [5]	*Tinospora cordifolia*(Willd)Miers ex Hook.f. and Thoms [10]
2.	Piperine	Anti-inflammatory [6], Im-munomodulatory	*Piper longum* L. [11], *Piper nigrum* L. [12]
3.	Coumaperine	Anti-inflammatory effects [7]	*Piper longum* L. [11]
4.	Tetrahydropiperine	Anti-inflammatory [8]	*Piper longum* L. [11]
5.	Vasicinone	Anti-inflammatory [9]	*Adhatoda vasica* Nees [13]
6.	Vasicinolone	Anti-inflammatory effects [9]	*Adhatoda vasica* Nees [13]

Table 2: Pharmacology of identified compounds.

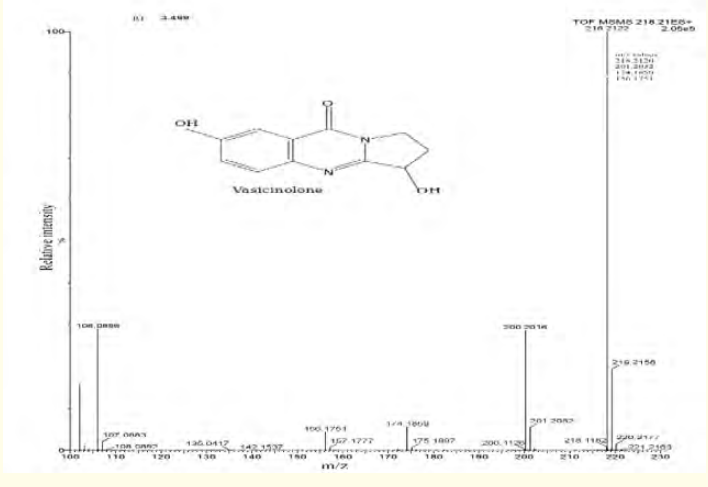


Figure 1: UPLC-ESI-QTOF- Mass spectrum of Vasicinolone. Major fragment peaks are at m/z 201,174 and 156.

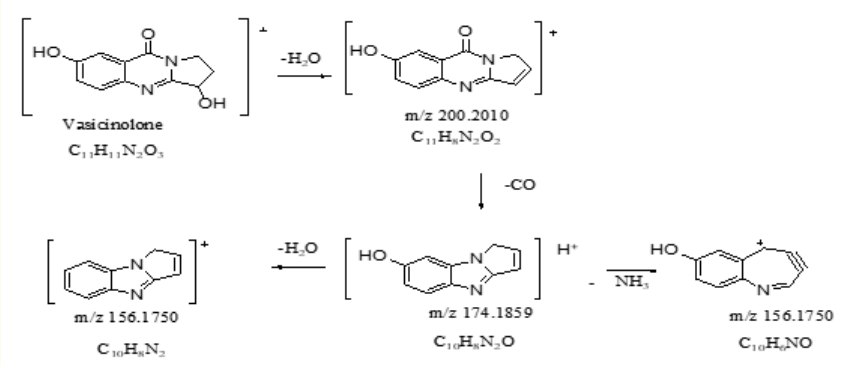


Figure 2: Mass fragments of Vasicinolone. Successive loss of water, CO and water are explained.

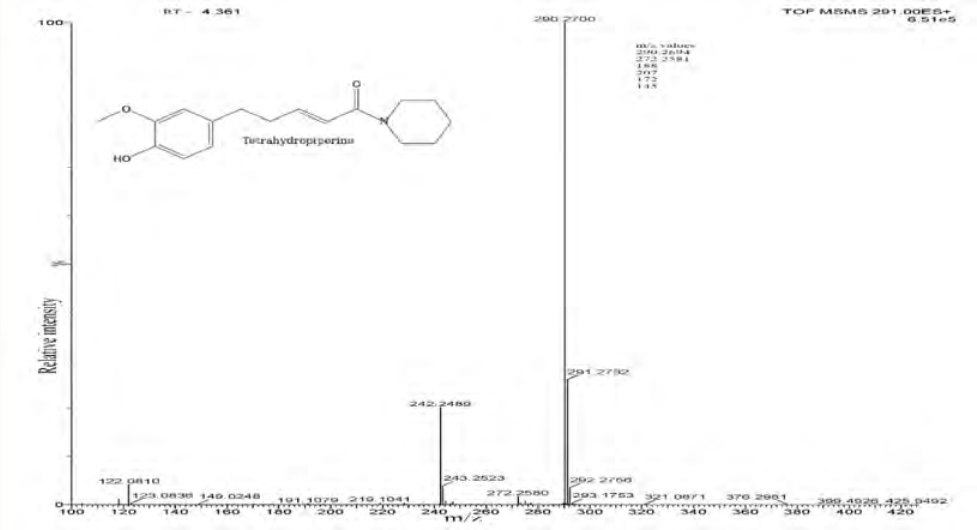
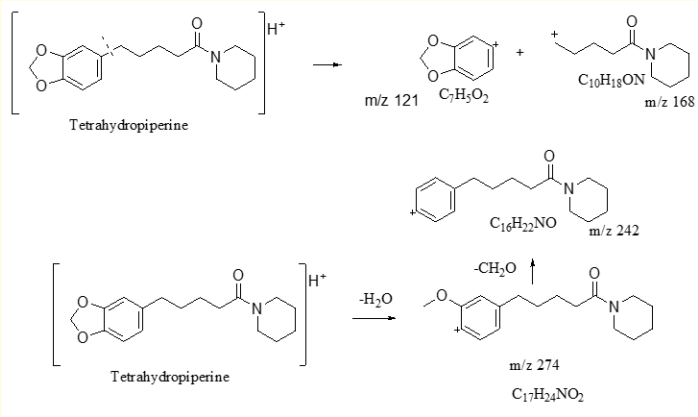


Figure 3: UPLC-ESI-QTOF- Mass spectrum of Tetrahydropiperine. Protonated molecular ion peak formed at m/z 290 and product ions are formed at m/z 242,272, 191 and 122.



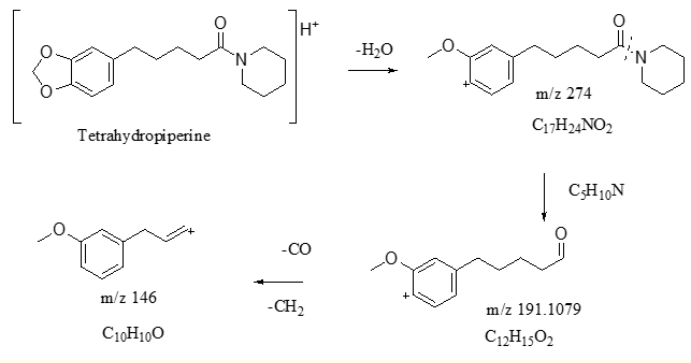


Figure 4: Mass fragments of Tetrahydropiperine. All the mass fragments are explained with diagram.

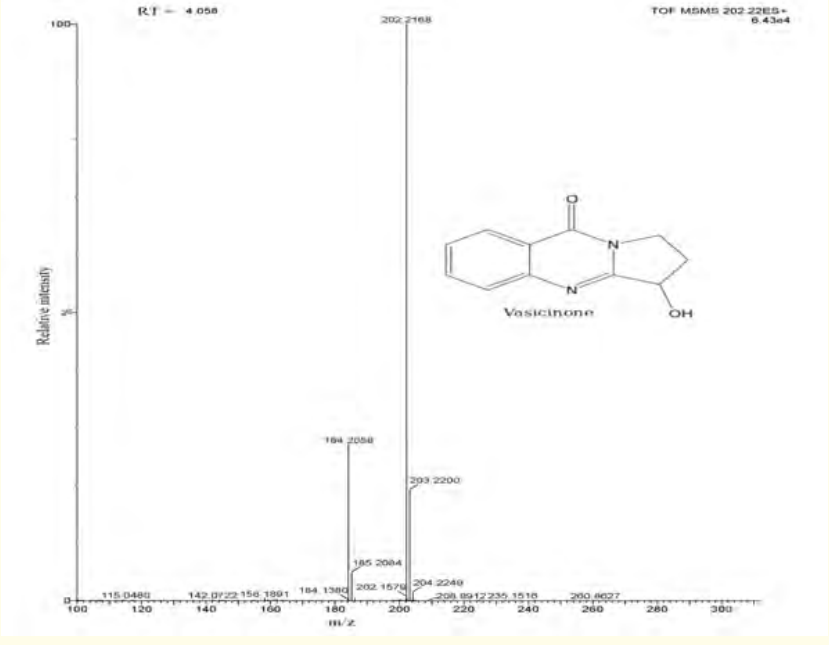


Figure 5: UPLC-ESI-QTOF- Mass spectrum of Vasicinone. Molecular ion peak at m/z 202 and fragment ions are at m/z 184, 157 and 130.

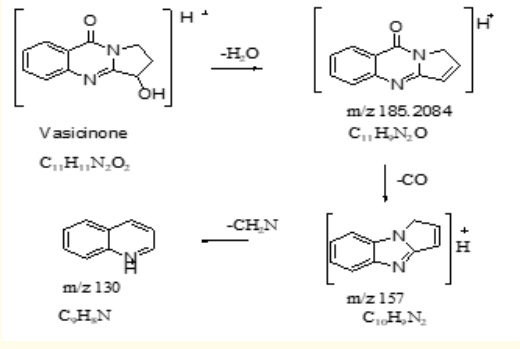


Figure 6: Mass fragments of Vasicinone. The mass peak at m/z 185 is due to the loss of water molecules from the molecular ion. The peak at m/z 157 results from the loss of CO from fragment with the m/z 185.

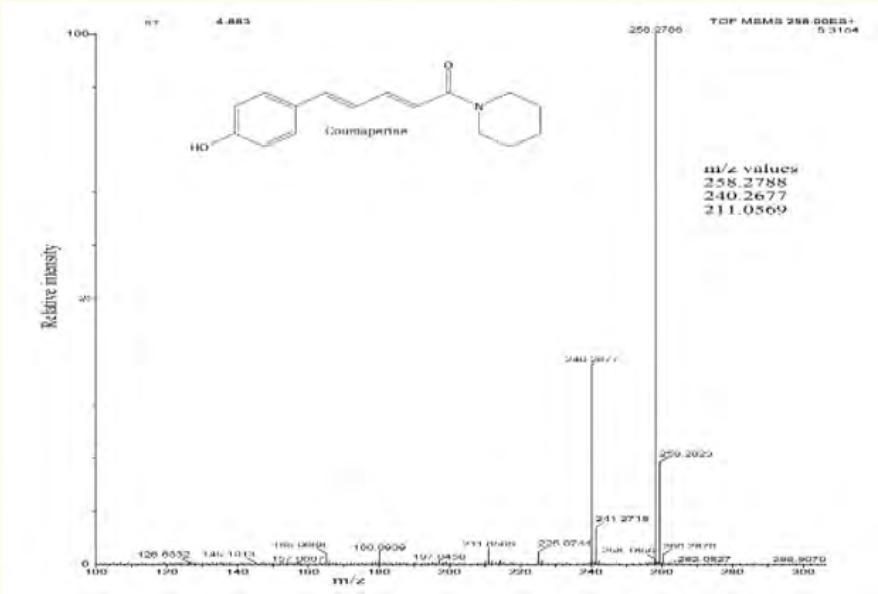


Figure 7: UPLC-ESI-QTOF- Mass spectrum of Coumaperine. Molecular ion peak at m/z 256 and product ions are at m/z 240 and 211.

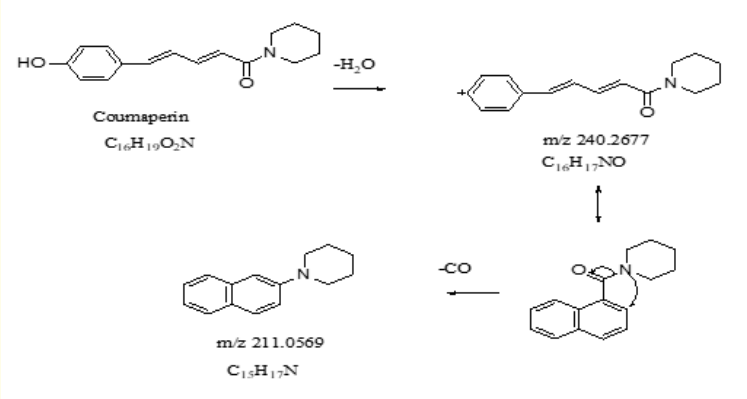


Figure 8: Mass fragments of Coumaperine. The fragments formed at m/z 240.2677 with the loss of water molecule from the protonated molecule. This molecule is undergoing cyclization and the elimination of the CO molecule results in the peak at m/ z 211. 0569.

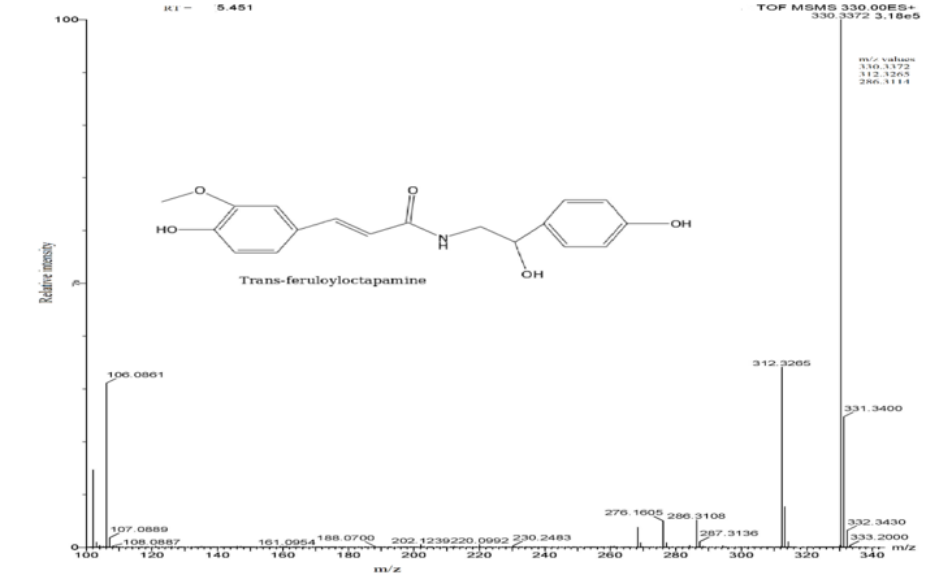


Figure 9: UPLC-ESI-QTOF- Mass spectrum of Trans feruloyl octopamine. Molecular ion peak at m/z 330 and product ion peaks are at m/z 312,286 and 106.

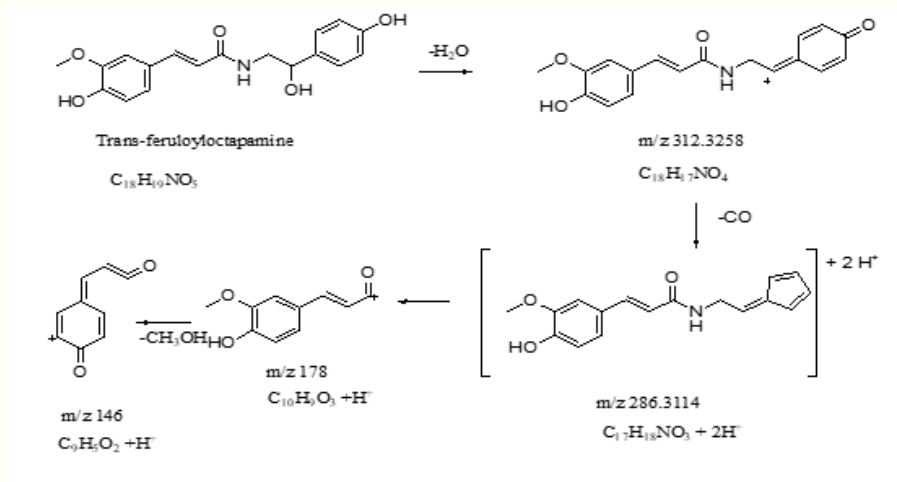


Figure 10: Mass fragments of Trans feruloyl octopamine. The mass peak at m/z 312.3265 formed by the loss of water from the protonated molecule, which then eliminates a CO moiety with ring contraction to form m/z 286.3114. This fragment ion then undergoes the amide C-N fission to give the ion at m/z 178 (177+H+).

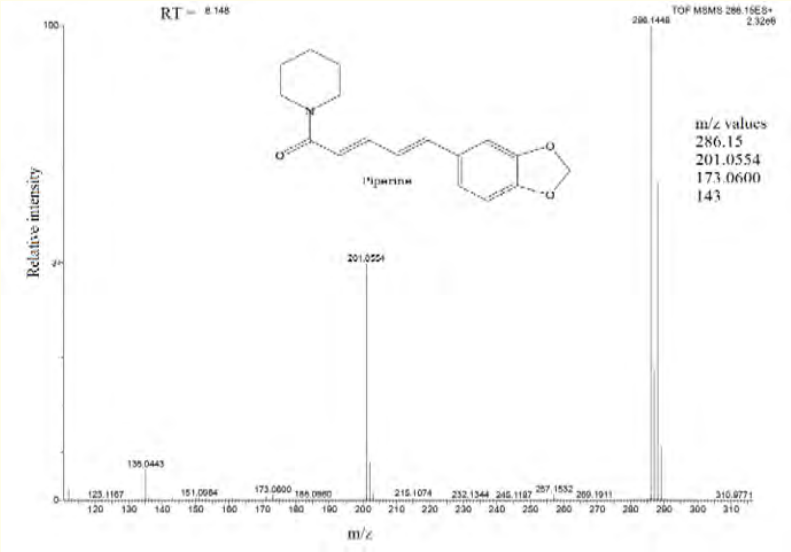


Figure 11: UPLC-ESI-QTOF- Mass spectrum of Piperine. Molecular ion peak is formed at m/z 286 and product ion peaks are at m/z 201,173 and 143.

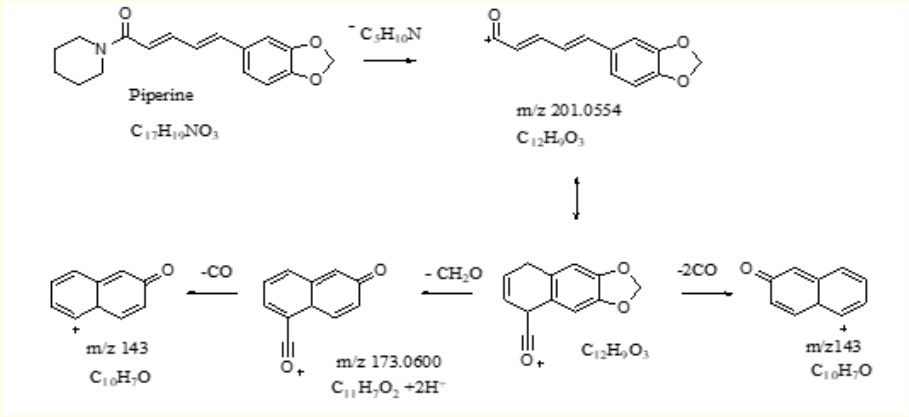


Figure 12: Mass fragments of Piperine. The fragment with m/z 201.0554 formed by the loss of piperidine molecule by the cleavage of the amide linkage and the formation of the acylium cation. This moiety has a conjugated carbonyl system attached to a phenyl ring, and by resonance activation cyclization with the α carbon of the carbonyl and the benzene moiety occurs. This ion loses a -CH2O to give the ion at m/z 171(171+2=173). The mass peak at m/z 143 formed from the fragment at m/z 173.0600 by the removal of a CO molecules.

Conclusion

The present study aims to use UPLC-QTOF-ESI-MS analysis to identify substances that might be responsible for the anti-inflammatory property of Gulguluthikthakam Kashayam. When synergism is considered, polyherbal preparation offer some benefits not available with single herb preparation. It is evident that better therapeutic effect can be reached with multi-constituent formulation. The realization that most chronic diseases are multi-genic, multi-targeted approach is required to ameliorate or cure the chronic diseases. According to the literature, all these identified

compounds possess anti-inflammatory properties. Based on the present findings, it can be concluded that *Gulguluthikthakam Kashayam* is a potent Ayurvedic formulation enriched with anti-inflammatory phytoconstituents, and may be effective in alleviating inflammation-associated disorders.

Bibliography

1. Di Matteo V and Esposito E. "Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis". *Current Drug Targets-CNS and Neurological Disorders* 2 (2003): 95-107.

2. Namboothiri D G., *et al*. "Anti Inflammatory, Anti Oxidant and Anti Microbial Activity of a New Herbal Eye Drop". *International Journal of Research in Ayurveda and Pharmacy* 6.2 (2015): 256-260.

3. Girija PV., *et al*. "Terpenoids in Gulguluthikthakam kashayam: an Ayurvedic formulation". *Journal of Pharmacognosy and Phytochemistry* 11.2 (2022): 33-45.

4. Babita Aryal., *et al*. "Potential Therapeutic Applications of Plant-Derived Alkaloids against Inflammatory and Neurodegenerative Diseases". *Evidence-Based Complementary and Alternative Medicine* (2022): 18.

5. Jing Li., *et al*. "N-trans-Feruloyloctopamine Wakes Up BBC3, DDIT3, CDKN1A, and NOXA Signals to Accelerate HCC Cell Apoptosis". *Hindawi Analytical Cellular Pathology* (2021): 9.

6. Lee E B., *et al*. "Central nervous system depressant and anti -inflammatory activity of Piperine". *Archives of Pharmacology Research* 7 (1984): 127-132.

7. Nandakumar Natarajan., *et al*. "Synthesis of coumaperine derivatives: Their NF-κB inhibitory effect, inhibition of cell migration and their cytotoxic activity". *European Journal of Medicinal Chemistry* (2016): S0223523416309096.

8. Inder Pal Singh and Alka Choudhary. "Peperine and derivatives: Trends in structure-activity relationships". *Current topics in Medicinal Chemistry* 15 (2015).

9. Singh B and Sharma RA. "Anti-inflammatory and antimicrobial properties of pyrroloquinazoline alkaloids from Adhatoda vasica Nees". *Phytomedicine* 20.5 (2013): 441-445.

10. Varsha K and Arun Kumar KV. "Rubia cordifolia and Tinospora cordifolia A magic remedy for inflammation". *Wpmr* 4.8 (2018): 317-322.

11. Maitreyi Zaveri., *et al*. "Chemistry and pharmacology of piper longum L". *International Journal of Pharmaceutical Science s Review and Researches* (2010).

12. Aswad M., *et al*. "Nature is the best source of anti-inflammatory drugs; indexing natural products for their anti-inflammatory". *Inflammatory Research* 67.1 (2018): 67-75.

13. Shi-Biao Wu., *et al*. "Ytotoxic Triterpenoids and steroids from the Bark of Melia azedarach". *Planta Medica* 77 (2011): 922-928.