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Original Article

Metabolite Profiling of *Chiruvilwadi Kashaya* basti Emulsion through GC-MS Analysis: A Scientific Validation of an Ayurvedic Formulation in the Management of Bhagandara (Fistula in Ano)

Sreechand S R[1,*], Pradeep S Shinde[1], Raj Joshi[1], Arya M S[2], Anjali Sudhakar [3]

[1]Department of Shalya Tantra, KLE B.M.K Ayurveda Mahavidyalaya, Belagavi, Karnataka, INDIA.
[2]Department of Shalyatantra, Sri Shivayogeeshwar Rural Ayurveda Medical College, Inchal, Belagavi, Karnataka, INDIA.
[3]Department of Kriyasharira, KLE B.M.K Ayurveda Mahavidyalaya, Belagavi, Karnataka, INDIA.

ABSTRACT

Background: *Chiruvilwadi Kashaya* basti emulsion is a polyherbal Ayurvedic formulation used in the management of inflammatory and anorectal disorders, especially indicated for *bhagandara* (fistula in ano). Scientific characterisation of phytochemical constituents enables therapeutic understanding and quality control standardisation. **Objectives:** To investigate the phytochemical composition of *Chiruvilwadi Kashaya* basti emulsion using Gas Chromatography-Mass Spectrometry (GC-MS) and identify compounds with potential pharmacological significance in the management of fistula in ano. **Materials and Methods:** The methanolic extract of *Chiruvilwadi Kashaya* basti emulsion was analysed using an Agilent 7890A Gas Chromatography system coupled with an Agilent 5975C Mass Selective Detector. Separation was achieved on a DB-5MS capillary column using helium as the carrier gas. Identification of compounds was performed by comparing mass spectral data with the NIST spectral library. **Results:** GC-MS analysis revealed eleven phytochemical constituents. The major compounds identified were 5-hydroxymethylfurfural, furfural, 2-formyl-5-methylfuran, and o-fluorobenzyl chloride. Other constituents included acetic acid, hepta-2,4-dienoic acid methyl ester, levoglucosenone, asarone, palmitic acid methyl ester, methyl 13-octadecenoate, and stearic acid methyl ester. Literature evidence indicates that many of these compounds possess antioxidant, anti-inflammatory, antimicrobial, analgesic and tissue-protective activities. **Conclusion:** The GC-MS profile of *Chiruvilwadi Kashaya* basti emulsion demonstrated the predominance of bioactive furan derivatives and fatty acid esters. These identified compounds provide scientific support for its therapeutic application in fistula in ano through potential anti-inflammatory and tissue repair activities. The findings may contribute to the standardization, quality control, and further pharmacological evaluation of *Chiruvilwadi Kashaya* basti emulsion.

Keywords: *Chiruvilwadi Kashaya* basti, Fistula in ano, GC-MS, Phytochemical profiling.

Correspondence:
Dr. Pradeep Shindhe
Hod, Professor , Department of Shalya Tantra, KLE B.M.K. Ayurveda Mahavidyalaya, Belagavi, Karnataka, INDIA.
Email: pshindhe@gmail.com

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INTRODUCTION

Chiruvilwadi Kashaya emulsion is an Ayurvedic polyherbal formulation used for basti chikitsa (medicated enema) which contains honey, *saindhava* (rock salt), pippalyadi anuvasana taila, *triphala choorna* and *Chiruvilwadi Kashaya* (Table 1). It exhibits anti-inflammatory and analgesic activities beneficial in the management of anorectal disorders and also helps in improving digestive fire (Bijeshmon *et al*., 2023). The pharmacological efficacy of Ayurvedic formulations is largely attributed to the presence of diverse phytoconstituents that act individually or in combination to produce therapeutic benefits. Scientific characterization of these bioactive composites is essential for understanding the molecular basis of their therapeutic actions and for establishing quality control standards.

Fistula-in-ano is a prevalent anorectal inflammatory condition characterized by a high rate of recurrence and therapeutic challenges shows 50% of recurrence rate even after surgery (Li *et al*., 2016). *Chiruvilwadi Kashaya* has been traditionally indicated for its management and has demonstrated anti-inflammatory activity in previous studies (Varsha *et al*., 2022). The addition of honey, *Saindhava* Lavana, *Triphala choorna*, and Pippalyadi Anuvasana Taila is expected to augment the therapeutic potential of the formulation through complementary and combined effects.

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Nevertheless, scientific evidence regarding the combined action and overall efficacy of Chiruvilwadi emulsion remains limited.

Gas Chromatography-Mass Spectrometry (GC-MS) is a prevailing analytical technique widely used for the identification and characterization of various phytochemicals present in herbal formulations. GC separates individual bioactive constituents based on their volatility and affinity for the stationary phase, while MS provides structural information through fragmentation patterns, enabling accurate compound identification by comparison with spectral libraries.

Hence in the present study, GC-MS analysis was performed to identify the bioactive constituents present in *Chiruvilwadi Kashaya* emulsion. It will help to substantiate the synchronising therapeutic action in the management of fistula in ano. The GC-MS profile also serves as a valuable phytochemical pattern for standardization, quality assurance, and future pharmacological investigations of the formulation.

MATERIALS AND METHODS

Ethical Statement

The present study was an investigation involving phytochemical profiling and evaluation of a classical Ayurvedic formulation. No human participants or experimental animals were involved; therefore, institutional ethical approval was not required.

Preparation of *Chiruvilwadi Kashaya* emulsion

Chiruvilwadi Kashaya Emulsion is a polyherbo-mineral homogeneous formulation, prepared by sequentially mixing 80 mL of honey, 8 g of *Saindhava* Lavana, 80 mL of Pippalyadi Anuvasana Taila, 15 g of *Triphala choorna*, and 300 mL of *Chiruvilwadi Kashaya*. The ingredients were added one after another in the above-mentioned order and triturated continuously until a uniform and homogeneous emulsion was obtained.

Preparation of *Chiruvilwadi Kashaya* emulsion extract

The obtained *Chiruvilwadi Kashaya* emulsion was subjected to phytochemical extraction using methanol as the extraction solvent. The extract was filtered through a sterile 13 mm nylon syringe filter (0.20 µm pore size) directly into GC-MS autosampler vials to remove suspended particulate matter and ensure analytical purity prior to chromatographic analysis.

GC-MS Analysis

Phytochemical characterization of the methanolic extract of *Chiruvilwadi Kashaya* emulsion was performed using an Agilent Technologies 7890A Gas Chromatography system coupled with an Agilent 5975C Mass Selective Detector (MSD) equipped with a Triple-Axis Detector. The analysis was conducted under optimized chromatographic and mass spectrometric conditions.

Chromatographic Conditions

Separation of volatile and semi-volatile constituents was achieved using a DB-5MS fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium (99.9995% purity) was employed as the carrier gas at a constant flow rate of 1.0 mL/min.

A 2 µL aliquot of the filtered extract was injected in splitless mode. The injector temperature was maintained at 300 °C throughout the analytical run.

Oven Temperature Program

The oven temperature was programmed as follows:

- Initial temperature: 40 °C, held for 5 min.
- Increased at 10 °C/min to 150 °C and held for 5 min.
- Further increased at 10 °C/min to 300 °C.
- Final temperature maintained for 10 min.

The temperature program was optimized to achieve efficient separation of phytochemical constituents present in the extract.

Mass Spectrometric Conditions

Mass spectral detection was performed using Electron Impact (EI) ionization at an ionization energy of 70 eV. Mass spectra were acquired over an appropriate Mass-to-charge (m/z) range to facilitate compound identification.

Identification of Phytoconstituents

The individual compounds were identified by comparing their mass spectral fragmentation patterns with those available in the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST 08). Compound identity was assigned based on spectral matching scores and retention characteristics. The relative abundance of each identified compound was calculated from the corresponding peak area percentage obtained from the Total Ion Chromatogram (TIC).

Data Analysis

The chromatographic peaks obtained from the GC-MS analysis were integrated automatically using the Agilent Chem Station software. Relative percentage composition of the identified compounds was determined based on peak area normalization.

Table 1: List of ingredients used in *Chiruvilwadi Kashaya* emulsion.

Sl. No.	Ingredient	Quantity
1.	Honey	60 mL
2.	*Saindhava* Lavana	8 g
3.	Pippalyadi Anuvasana Taila	60 mL
4.	Triphala choorna	15 g
5.	*Chiruvilwadi Kashaya*	300 mL

The identified phytoconstituents were subsequently evaluated through literature review to determine their reported pharmacological activities, including anti-inflammatory, analgesic, antioxidant, antimicrobial, and wound-healing properties.

Statistical Analysis

As the study was exploratory and aimed at qualitative phytochemical profiling, no inferential statistical analysis was performed.

RESULTS

The GC-MS analysis of *Chiruvilwadi Kashaya* emulsion revealed the presence of 11 pharmacologically important compounds, including acetic acid, furfural, 5-Hydroxymethylfurfural (5-HMF), asarone, and various fatty acid methyl esters. Among these, 5-hydroxymethylfurfural was identified as the predominant constituent, accounting for 62.19% of the total peak area, followed by furfural (13.15%) and 2-formyl-5-methylfuran (10.45%). The detailed description of the identified constituents and its area % is shown in Table 2 and Figure 1.

Above obtained 11 compounds have been reported in the literature to possess antioxidant, anti-inflammatory, antimicrobial, analgesic, and wound-healing properties.

DISCUSSION

The GC-MS analysis revealed the presence of several bioactive phytoconstituents that may collectively contribute to the analgesic, anti-inflammatory, antioxidant, and wound-healing potential of the formulation.

Acetic acid (RT 6.84 min; 2.21%) was identified as one of the initial compounds. Acetic acid has been extensively used in wound care owing to its antimicrobial activity, particularly against *Pseudomonas aeruginosa*, and its ability to reduce local inflammation while supporting tissue repair and wound healing (Nagoba *et al*., 2013; Gruber *et al*.,1975).

Furfural (RT 7.08 min; 13.15%) was detected as a major constituent, it acts as a cyclic aldehyde that exerts moderate anti-inflammatory effects by scavenging free radicals. It helps mitigate secondary tissue damage by suppressing cellular oxidative stress cascades that normally amplify inflammation. Furfural and its derivatives have been reported to possess antioxidant and anti-inflammatory activities through free radical scavenging and modulation of inflammatory mediators, thereby contributing to protection against oxidative tissue damage and inflammatory responses (Zhang *et al*., 2021; Luo *et al*., 2021).

Levoglucosenone (RT 13.05 min; 0.77%) is an oxygenated furan derivative. Although direct pharmacological evidence remains limited, structurally related furan compounds have demonstrated antioxidant and anti-inflammatory properties that may support the biological activity of the formulation (Zhang *et al*., 2021; Luo *et al*., 2021). It is also widely used as a chiral scaffold in medicinal chemistry to synthesize novel, targeted anti-inflammatory drugs.

Hepta-2,4-dienoic acid, methyl ester (RT 13.65 min; 1.87%) belongs to the fatty acid methyl ester group. Fatty acid methyl esters have been reported to exhibit anti-inflammatory, antioxidant, and membrane-stabilizing activities, which may facilitate tissue regeneration and wound-healing processes (Aparna *et al*., 2012; Silva *et al*., 2018). It downregulates inflammatory responses by structurally interfering with the Lipoxygenase (LOX) and Cyclooxygenase (COX) enzyme pathways.

o-Fluorobenzyl chloride (RT 14.08 min; 7.60%) was detected in moderate concentration; however, there is currently insufficient evidence regarding its direct contribution to analgesic, anti-inflammatory, or wound-healing activities. Its fluorinated structure is highly valued for engineering advanced synthetic Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) with enhanced bio-availability.

Table 2: Phytochemical Profiling of *Chiruvilwadi Kashaya* Vasti via GC-MS Analysis.

Sl. No.	RT (min)	Molecules	Area %
1.	6.84	Acetic acid	2.21%
2.	7.08	Furfural	13.15%
3.	13.05	Levoglucosenone	0.77%
4.	13.65	Hepta-2,4-dienoic acid, methyl ester	1.87%
5.	14.08	o-Fluorobenzyl chloride	7.60%
6.	15.60	5-Hydroxymethylfurfural	62.19%
7.	22.54	Asarone	0.88%
8.	27.62	Palmitic acid, methyl ester	0.28%
9.	29.14	2-Formyl-5-methylfuran	10.45%
10.	29.61	Methyl 13-octadecenoate	0.48%
11.	29.88	Stearic acid, methyl ester	0.13%

RT=Retention Time; values expressed as percentage peak area.

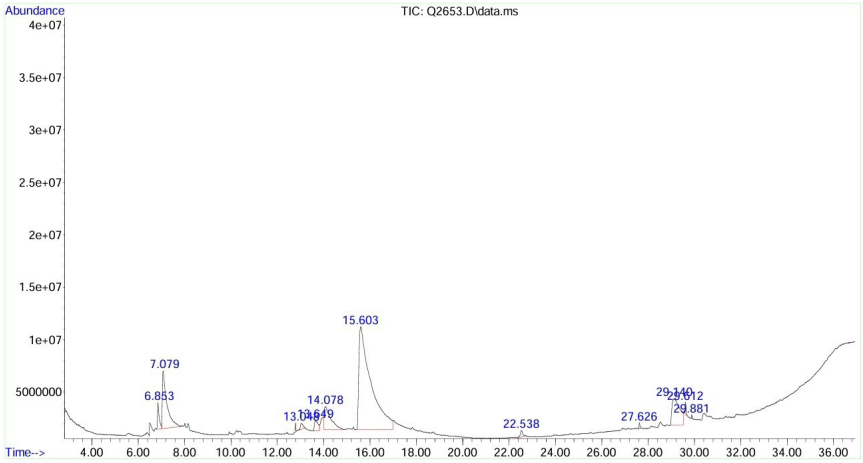


Figure 1: The GC-MS chromatogram of *Chiruvilwadi Kashaya* Vasti identified Total 11 phytochemical constituents with distinct retention times and peak areas.

Table 3: Analgesic, Anti-inflammatory, and Wound-Healing Activities of Phytoconstituents Identified in *Chiruvilwadi Kashaya* emulsion.

Compound	Analgesic Activity	Anti-inflammatory Activity	Wound Healing Activity
Acetic acid	Indirect pain reduction by controlling wound infection and inflammation.	Demonstrates anti-inflammatory and antimicrobial effects in infected wounds.	Widely used in wound management, especially infected wounds; does not significantly impair healing.
Furfural	-	Reported antioxidant and anti-inflammatory potential in experimental studies.	May contribute indirectly through antioxidant activity; evidence limited.
Levoglucosenone	-	Reported as a bioactive furan derivative with potential anti-inflammatory effects.	-
Hepta-2,4-dienoic acid methyl ester	-	Fatty acid ester derivatives are reported to possess anti-inflammatory activity.	Possible membrane repair effects.
5-Hydroxymethylfurfural (5-HMF)	May reduce inflammatory pain through cytokine suppression.	Strong anti-inflammatory activity by inhibiting MAPK, NF-κB, mTOR, and NLRP3 inflammasome pathways.	Promotes tissue protection and repair through reduction of oxidative stress and inflammatory mediators.
Asarone	Reported analgesic and neuroprotective activity in several experimental studies.	Significant anti-inflammatory effects through suppression of inflammatory mediators.	Supports tissue repair through antioxidant and anti-inflammatory mechanisms.
Palmitic acid methyl ester	Mild analgesic activity reported in GC-MS-based phytochemical studies.	Exhibits anti-inflammatory activity by modulating inflammatory mediators.	May support epithelial regeneration and membrane stabilization.
2-Formyl-5-methylfuran	--	Furan derivatives are reported to possess antioxidant and anti-inflammatory properties.	Possible indirect wound-healing role via antioxidant action.
Methyl 13-octadecenoate	Fatty acid methyl esters may contribute to analgesic activity.	Known anti-inflammatory activity reported for unsaturated fatty acid esters.	Supports tissue repair and cellular membrane regeneration.
Stearic acid methyl ester	Mild analgesic activity reported in phytochemical studies.	Demonstrates anti-inflammatory effects in several plant extracts.	Contributes to skin barrier restoration and wound repair.

MAPK=Mitogen-Activated Protein Kinase; NF-κB=Nuclear Factor Kappa B; mTOR= Mammalian Target Of Rapamycin; NLRP3=NOD-like Receptors.

The predominant constituent identified was 5-Hydroxymethylfurfural (5-HMF) (RT 15.60 min; 62.19%). Previous studies have demonstrated that 5-HMF exhibits potent anti-inflammatory, antioxidant, and cytoprotective activities through inhibition of NF-κB activation, suppression of pro-inflammatory cytokine production, attenuation of oxidative stress, and modulation of inflammatory signalling pathways. These mechanisms contribute significantly to tissue protection, pain reduction, and wound healing (Kim *et al*., 2011; Pagare *et al*., 2024; Uchida *et al*., 2020; Zhang *et al*., 2021).

Asarone (RT 22.54 min; 0.88%) is a well-established bioactive phytoconstituent possessing anti-inflammatory, analgesic, antioxidant, and neuroprotective properties. Experimental investigations have shown that asarone suppresses inflammatory mediator release and oxidative stress, thereby promoting tissue repair and reducing inflammatory damage (Limón *et al*., 2009). It functions as a potent neuroprotective agent that addresses both central and peripheral pain mechanisms. It actively suppresses the NLRP3 inflammasome pathway and limits pro-inflammatory microglial activation to reduce swelling and pain (Liu *et al*., 2024; Radwan *et a*., 2024).

Palmitic acid methyl ester (RT 27.62 min; 0.28%), Methyl 13-octadecenoate (RT 29.61 min; 0.48%), and Stearic acid methyl ester (RT 29.88 min; 0.13%) are fatty acid methyl esters that may contribute to anti-inflammatory and tissue-regenerative effects through membrane stabilization and modulation. It prevents IκB phosphorylation to block NF-κB activation, successfully reducing acute carrageenan-induced tissue edema.

2-Formyl-5-methylfuran (RT 29.14 min; 10.45%), another furan derivative identified in appreciable concentration, may further contribute antioxidant and anti-inflammatory activities similar to other biologically active furan compounds (Zhang *et al*., 2021; Luo *et al*., 2021). Exerts immunomodulatory and cellular protective effects across vascular and mucosal tissues. It protects tissues by lowering the expression of oxidative stress biomarkers that normally drive chronic inflammatory cycles. Table 3 showing an overall briefing of wound healing and anti-inflammatory activity of phytoconstituents in this formulation.

CONCLUSION

The GC-MS analysis of *Chiruvilwadi Kashaya* Emulsion revealed the presence of several bioactive compounds, with 5-Hydroxymethylfurfural, Furfural, 2-Formyl-5-methylfuran, Acetic acid, Asarone, and fatty acid methyl esters being the major constituents. These compounds have been reported to possess antioxidant, anti-inflammatory, antimicrobial, analgesic, and wound-healing properties. The combined presence of these constituents may contribute to the therapeutic potential of the formulation in the management of fistula-in-ano. The findings provide preliminary scientific support for its traditional use; however, further pharmacological and clinical studies are needed to validate its efficacy.

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ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; **MSD:** Mass Selective Detector; **EI:** Electron Impact; **NIST:** National Institute of Standards and Technology; **RT:** Retention Time; **TIC:** Total Ion Chromatogram; **5-HMF:** 5-Hydroxymethylfurfural; **LOX:** Lipoxygenase; **COX:** Cyclooxygenase; **NSAIDs:** Non-steroidal Anti-inflammatory Drugs; **MAPK:** Mitogen-activated Protein Kinase; **NF-κB:** Nuclear Factor Kappa B; **mTOR:** Mammalian Target of Rapamycin; **NLRP3:** NOD-like Receptor Protein 3; **m/z:** Mass-to-charge Ratio.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Dr. Sreechand S R: Conceived and designed the study, conducted the experimental work, performed data acquisition and analysis, and prepared the original draft of the manuscript. **Dr. Pradeep S Shindhe:** Contributed to study supervision, methodological guidance, and critical revision of the manuscript for important intellectual content. **Dr. Raj Joshi**: Assisted in data interpretation, literature review. **Dr Arya M S and Dr Anjali Sudhakar:** Done manuscript editing and review. All authors read and approved the final version of the manuscript.

SUMMARY

Chiruvilwadi Kashaya basti emulsion is a classical ayurvedic formulation traditionally used in the management of *Bhagandara* (fistula in ano). In the present study, GC-MS analysis of its methanolic extract identified eleven phytochemical constituents, with 5-hydroxymethylfurfural, furfural, and 2-formyl-5-methylfuran as the predominant compounds. Many identified constituents have been reported to possess antioxidant, anti-inflammatory, anti-microbial, analgesic and tissue protective activities, which may collectively contribute to the therapeutic effects of formulation. The GC-MS profile generated in this study provides a scientific basis for the traditional use of *Chiruvilwadi Kashaya basti* emulsion and serves as a valuable reference for its quality control and standardization. These findings also support the need for further pharmacological and clinical studies to establish its efficacy and mechanism of action in the management of fistula in ano.

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