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Original Article

Characterization and Chromatographic Evaluation of *Shatapushpadi Lepa*

Sushmita Sanjay Gengane[1], Anil Koralli[1,*], Pranjali Abhay Patil[2], Shreya Balasaheb Nikam[1]

[1]Department of Panchakarma, KLE Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi, Karnataka, INDIA.
[2]Department of Shalakya Tantra, KLE Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi, Karnataka, INDIA.

ABSTRACT

Background: *Shatapushpadi Lepa* is a traditional external application specified in Ayurveda. The composition that is advised for Vata-dominant inflammatory joint diseases. To determine quality, and therapeutic effectiveness, such a conventional formulation must be scientifically validated. **Purpose:** The present study is aimed to evaluate the quality of *shatapushpadi lepa* in inflammatory joint diseases like Rheumatoid arthritis through physiochemical, phytochemical profiling and Chromatographic analysis. **Objectives:** To evaluate the quality of *Shatapushpadi Lepa* through comprehensive assessment of physiochemical, phytochemical profiling and Chromatographic analysis. **Materials and Methods:** The raw drugs were obtained from a GMP-certified Ayurveda pharmacy and authentication and analysis was done in an AYUSH-approved laboratory. *Shatapushpadi Lepa* was prepared in accordance with classical reference Organoleptic and physicochemical characteristics such as ash values, extractive values, moisture content, and pH were evaluated in accordance with API standards. Aqueous and alcoholic extracts were used to carry out phytochemical screening. Toluene was used for the Thin Layer Chromatography (TLC) study. The mobile phase consists of ethyl acetate and formic acid (3:6:1). To detect bioactive compounds, GC-MS analysis was performed on a Shimadzu GCMS-QP 2010SE. **Results:** All raw drugs and the finished formulation complied with in-house quality requirements. The formulation had low ash values, a high moisture content (66.20%), and a slightly acidic pH (5.226), indicating its stability and appropriateness for topical application. Phytochemical research indicated the presence of flavonoids, tannins, glycosides, and reducing sugars, all of which have anti-inflammatory and antioxidant properties. TLC examination found many well-resolved spots with a high R_f value of 0.63, indicating a variety of phytoconstituents. GC-MS analysis revealed bioactive substances such triterpenes, apiol, carvone, longifolene, α-terpineol, and pentacosane that have anti-inflammatory, analgesic, antioxidant, and penetration-enhancing activities. **Conclusion:** This study illustrates the quality control for Ayurvedic products, ensuring their reliability and therapeutic value. The compounds which are present in formulation shows anti-inflammatory, analgesic, and antioxidant effects, as indicated by pharmacological tests such as phytochemical, physiochemical, TLC, and GCMS analysis. Future study should focus on bioavailability and clinical effectiveness to further substantiate the therapeutic potential of this formulation.

Keywords: Amavata, Anti-inflammatory, Ayurveda, GC-MS, Rheumatoid Arthritis, *Shatapushpadi Lepa.*

Correspondence:
Dr. Anil Koralli
Department of Panchakarma, KLE Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi-590003, Karnataka, INDIA.
Email: anilkoralli@gmail.com

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INTRODUCTION

Ayurveda emphasises the importance of both internal and external therapies. The concept behind preparation of lepa is to make a fine paste using wet herbs, on other hand dry herbs are grounded into fine powder and mix with an appropriate liquid media, such as Swarasa (fresh juice), kwatha (decoction), Sneha (oil), gomutra (cow urine), or water depending on the disease condition and desired therapeutic requirement (Angadi, 2016; Wagh *et al*., 2018). The use of liquid media as the base of lepa in the preparation increases its potency (Chanekar *et al*., 2024). *Shatapushpadi Lepa* is mentioned in Gadanigraha as external application which is beneficial in inflammatory arthritis such as Amavata (Rheumatoid Arthritis) (Tripathi, 1969) According to the clinical presentation Amavata appears similar to Rheumatoid Arthritis (RA) in terms of symptoms such as joint pain, swelling, stiffness, fever, and overall debility. A wide range of disorders known as inflammatory arthritis are defined by inflammation of the joints, which cause pain and tissue damage. Pro-inflammatory cytokines including Tumour Necrosis Factor (TNF), Interlukin-4

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(IL-4), Interlukin-6 (IL-6), serve as important mediators in the inflammatory process (Pisetsky *et al*., 2012).

The medicinal efficacy of Ayurvedic formulations depends on the quality and consistency of the ingredients used (Nhawkar *et al*., 2014). Purpose of the study is to examines the physiochemical, phytochemical, TLC analysis and chemical composition of the Ayurvedic herbal preparation *Shatapushpadi Lepa* using GC-MS to discover bioactive chemical compound and its therapeutic role in inflammatory arthritis like Amavata (Rheumatoid Arthritis). These findings highlight the importance of combining traditional knowledge with current scientific analytical techniques to potentially increase the use of herbal medicine in healthcare.

MATERIALS AND METHODS

Materials

Ingredients of Shatapushpadi Lepa

Ingredients of *Shatapushpadi Lepa* are Shatapushpa, Shatapushpa *(Anethum sowa)*, Erandamoola *(Ricinus communis)*, Devadaru *(Cedrus deodara)*, Shunthi *(Zingiber officinale)*, Saindhava (rock salt) and Kanji (fermented liquid). Quantity of each ingredient is mentioned in Table 1.

Sample Collection

All the raw drugs used in *Shatapushpadi Lepa* including Kanji were procured from GMP- Certified KLE Society's Ayurveda Pharmacy, Khasbag, Belagavi, Karnataka.

Place of Preparation of *Shatapushpadi lepa*: *Shatapushpadi Lepa* was prepared in GMP- Certified KLE Society's Ayurveda Pharmacy, Khasbag, Belagavi, Karnataka.

Methods

Place of Authentication and Analysis

The authentication and analysis of raw drugs and analysis of finished drugs, *Shatapushpadi lepa* and kanji was done at Central Research Facility, AYUSH-Approved Drug Testing Laboratory for ASU drugs, KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi.

Gas Chromatography and Mass Spectrometry (GC-MS) analysis was conducted at Amritha Labs (Nisarga Privet Limited), Shivamogga, Karnataka.

Preparation of *Shatapushpadi Lepa*

Shatapushpadi Leoa was prepared as per the classical reference of Gadanigraha. Specified equal parts of Shatapushpa, Shatapushpa *(Anthenum sowa),* Erandamoola *(Ricinus communis)*, Devadaru (*Cedrus deodara*), Shunthi (*Zingiber officinalis*) and Saindhava (rock salt) all the raw ingredients were taken, and physical impurities were removed and then dried completely. The powder was prepared by using a centrifugal impact pulveriser. Then, it was sieved with a mesh size of 120 mm. A fine powder was obtained and treated to UV light in UV chamber for 24 hr before being stored in an airtight container. For the preparation of *Shatapushpadi lepa*, the mixture of all the fine powders of drugs resulted in a smooth-textured brown powder with an aromatic odour is mixed with quantity sufficient kanji (fermented liquid) to get semisolid consistency of lepa.

Organoleptic and Physiochemical Analysis

Organoleptic and physiochemical analysis were carried out for all the raw drugs used in *shatapushpadi lepa* formulation, as well as for the finished product *shatapushpadi lepa* and kanji.

Phytochemical Analysis

Phytochemical analysis was carried out for *Shatapushpadi lepa* and kanji.

Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) of alcoholic extract of *Shatapushpadi lepa* was carried out in mobile phase- Toluene: Ethyl acetate: Formic acid in ratio 3:6:1. It is observed under short wave and long wave.

Method of analysis

Organoleptic, physiochemical, phytochemical and TLC analysis tests listed were carried out in accordance with API standard guidelines (API, 2008).

Gas Chromatography and Mass Spectrometry (GC-MS) Analysis

Instrument for GC-MS

GCMS-QP 2010SE Gas Chromatography Shimadzu model.

Sample Preparation

0.1 mL sample was diluted with 10 mL of petroleum ether and 1 mL extract was injected and the above instrument is used. After agitation of the sample for 10 sec, the sample was taken for GC-MS analysis.

GC-MS Protocol

0.1mL sample of lepa was diluted with 10 mL of petroleum ether and a non-polar extract was prepared, filtered through a syringe filter (Nylon 13 mm 0.2 µm) and injected into GC-MS model. The GC-MS model column consists of DB-4MS (30 mm × 0.25 mm diameter × 0.25 mm thickness). Analysis was performed by injecting 1 µL of sample with an injected temperature of 280.00°C and split ratio of 1:10. Helium gas (99.999%) was used as a carrier gas with a flow rate of 19.5 mL/min. The analysis was performed in EI (electron impact) mode with 70eV of ionisation energy. The injected temperature was maintained at 280°C. The compound is identified by GC-MS Library (NIST and WILEY). The ion source temperature was set to 200°C and interface temperature was

pointed at 300°C. The solvent cut time was 1.40 min, minimising solvent interference and the detector gain mode was set relative to tuning result with a gain of 0.95 kV.

Oven temperature

Temperature Ramp Profile

The Gas Chromatography analysis was performed using oven temperature. Initially, the temperature was 80°C for 2 min, then increased at rate of 10°C/min to 280°C and held for 10 min. Subsequently, the temperature was raised by 20°C/min to 330°C and maintained for a final hold time of 5 min.

Mass Spectrometry detection was carried out in scan mode with mass range of m/z 35-500. The scan speed was 1666, and the event time was 0.30 sec.

These conditions are set and allowed for the precise separation and identification of analysts.

Ethical Statement

The study did not involve human or animal subjects; therefore, ethical clearance was not required for the laboratory-based analysis of the herbal formulation.

RESULTS

Results of Organoleptic and Physiochemical Analysis

The observation of organoleptic and physiochemical parameters of raw drugs used in Shatapushpa lepa. The observation of organoleptic and physiochemical parameters of saindhava (rock salt). *Shatapushpadi Lepa* exhibits organoleptic standards like dark brown colour, pungent taste and characteristic odour. The values are within permissible limits. Saindhava represents, slightly alkaline pH 6.98% and shows the presence of sodium (Na^+) and Potassium (K^+). The physiochemical analysis is of kanji is presented in Table 2 shows low pH 1.006, the total solids content 1.986% and greater specific gravity 2.74. The physiochemical analysis of *Shatapushpadi Lepa* is presented in Table 3 which shows high moisture content 66.2023%, low ash value 1.00%, acid-insoluble ash 0.884%, water soluble extract 13.465%, alcohol soluble extract 12.311% and slightly acidic pH 5.226.

Result of Phytochemical analysis

Phytochemical screening of *Shatapushpadi Lepa* Table 4 shows the presence of carbohydrates and monosaccharides in water extract. Flavonoid and reducing sugar were detected in both water and alcoholic extracts, while tannins and cardiac glycosides were found only in alcoholic extract. Phytochemical screening of Kanji presence of carbohydrates.

- Result of Thin Layer Chromatography Analysis of *Shatapushpadi Lepa*.
- Ref. No. CRF/FG/139/2025-26.
- TLC: (Alcohol Extract).
- Mobile Phase.
- Toluene: Ethyl acetate: Formic Acid.
- Short Wave: 0.32, 0.58, 0.63, 0.86.
- Long Wave: 0.36,0.63,0.69,0.72,0.90.
- Ratio -3:6:1.

TLC of *Shatapushpadi Lepa* showed presence of numerous well-defined spots on TLC chromatogram. Short-wave UV light exhibited spots at R_f values of 0.32, 0.58, 0.63, and 0.86, and long-wave UV light revealed spots at 0.36, 0.63, 0.69, 0.72, and

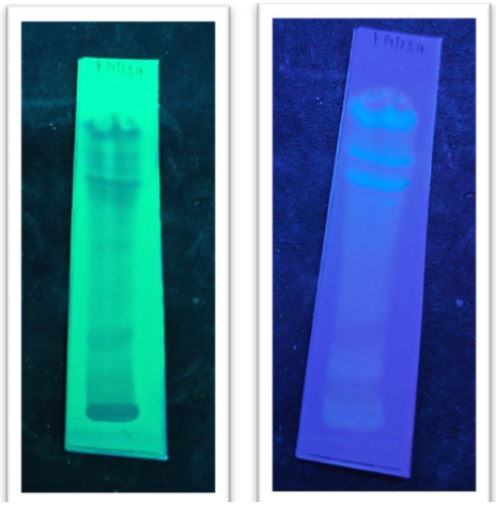

Figure 1: TLC film of *Shatapushpadi Lepa*.

Table 1: Ingredients of *Shatapushpadi Lepa*.

Drug	Botanical Name	Family Name	Parts Used	Parts	Authentication Reg. No.
Shatapushpa	*Anethum sowa*	*Apiaceae*	Seeds	1 Part	BMK/CRF/185/2025-26
Devadaru	*Cedrus deodara*	*Pinaceae*	Bark	1 Part	BMK/CRF/182/2025-26
Shunthi	*Zingiber officinale*	*Scitaminae*	Rhizome	1 Part	BMK/CRF/160/2025-26
Eranda	*Ricinus communis*	*Euphorbiaceae*	Root	1 Part	BMK/CRF/526/2024-2025
Saindhava	-	-	-	1 Part	BMK/CRF/186//2025-26
Kanji	-	-	-	Q.S.	-

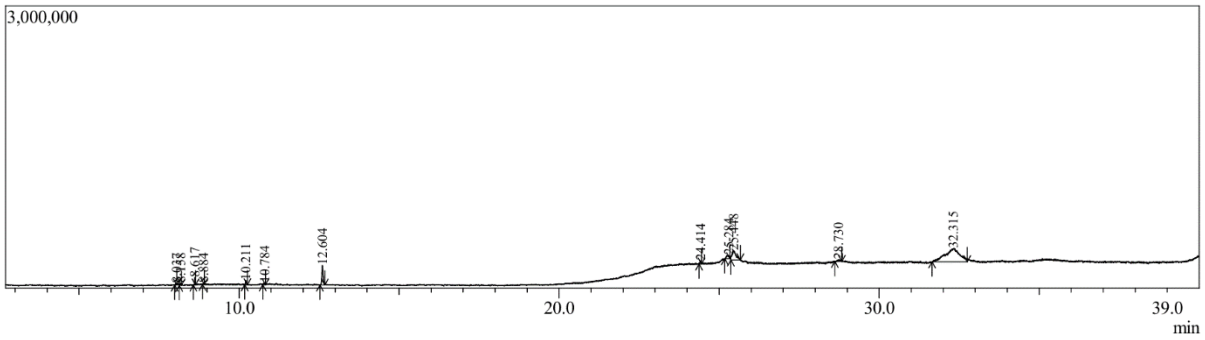

Figure 2: Chromatographic presentation Compounds of *Shatapushpadi Lepa*.

Table 2: Organoleptic and Physiochemical Analysis of Kanji.

	Kanji
Ref. No.	**CRF/RM/110/2025-26**
Organoleptic Standards	
Form	Liquide
Colour	Translucent
Odour	Characteristic
Physico-Chemical Standards	
pH (10% Solution)	1.006
Specific Gravity	2.74
Total Solids	1.986%

0.90. The R_f value of 0.63 is seen frequently. TLC film shown in Figure 1.

Result of GC-MS Analysis

According to the relative contents, the primary chemicals found were Tricyclo(20.8.0.0(7,16)) triacontane, 1(22),7(16)-diepoxy-, 1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-one, Apiol -, (-)-Carvone -, Glutaric acid -, Longifolene, 2- Methylhexacosane -, Phenol, 2-methyl-5-(1-methylethyl)-, Benzenemethanol, alpha.,. alpha.,4-trimethyl- OR p-Cymen-8-ol, α-Terpineol, Pentacosane. These compounds are known for its anti-inflammatory, analgesic and antioxidant properties. The *Shatapushpadi Lepa* GC-MS profile features detailed information, including retention time, possible compound, molecular formulas, molecular weight, and percentage peak area are mentioned in Table 5 and Figure 2.

Statistical Analysis

Statistical analysis was not done, as this study do not require statistical analysis.

DISCUSSION

Preliminary analysis is required for the verification and standardisation of Ayurvedic herbal formulations using modern analytical parameters. It is an integrative approach which validates quality the Ayurvedic herbal formulations.

Discussion on Physicochemical Analysis

The foreign matter content of the raw drugs and finished drug confirms proper procurement and processing, while low ash value (1.000%) and acid-insoluble ash value (0.884%) suggest minimal contamination which reflect the purity and identity if the raw drugs in the lepa powder. (Dhavale *et al.*, 2025). *Shatapushpadi Lepa* had a high moisture content 66.20%, which provide excellent spreadability, adherence and prolonged skin contact. shows the presence of organic acids that aid in the uptake of active component and sufficient fermentation is confirmed by the acidic pH 1.006 of Kanji (Table 2). Its pH of 5.226 which is slightly acidic, is in harmony with the physiology of the skin, improving transdermal absorption (Table 3) (Kumar *et al.*, 2023). Saindhava shows the presence of Sodium and potassium which helps to reduce stiffness in inflammatory joint diseases (Aswani *et al.*, 2018).

Discussion on Phytochemical Analysis

Carbohydrates and Monosaccharides (Table 4) exsert anti-inflammatory and anti-arthritic activities (Kilcoyne *et al.*, 2007). Carbohydrates (Table 4) are also present in Kanji which again enhance the anti-inflammatory and anti-arthritic effect of the formulation. Reducing Sugar possess anti-oxidative property and helps reducing oxidative stress in cell (Khatri *et al.*, 2020). The formulation contains flavonoids (Table 4) which exhibit strong anti-inflammatory and analgesic effect when applied topically, principally via suppressing inflammatory mediators, lowering oxidative stress, and modifying peripheral pain pathway (Al-Khayri *et al.*, 2022). According to several studies, flavonoids modulate pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 and inhibit key inflammatory enzymes and signalling pathways, including NF-κB (Al-Khayri *et al.*, 2022; Ekambaram *et al.*, 2022). Flavonoids play a role in reducing oxidative stress and inflammation in autoimmune disease like rheumatoid arthritis (Jomova *et al.*, 2025). They also exhibit anti-inflammatory activity in inflammatory arthritis (Mahmoud *et al.*, 2022). Cardiac Glycosides (CGs) (Table 4) are natural compounds occurring in plants. It has immunomodulatory effects by inhibiting pro inflammatory cytokines like NF-κB, resulting modulations of cytokine and chemokines level and

changes immune cell ratios could be potentially useful in treating autoimmune and inflammatory diseases (Škubník *et al.*, 2021). Tannins (Table 4) have significant pharmacogenetic value in the treatment of anti-inflammation. Its inhibitory effect on COX and LOX enzymes and their ability to scavenge reactive oxygen species, reduce oedema, oxidative stress, and swelling of joints (Kaushik, 2025).

Discussion on Thin Layer Chromatographic Analysis

Multiple well resolved spots under short and long wave UV light were observed in the TLC analysis Figure 1 of the alcoholic extract of *Shatapushpadi Lepa*, suggesting the presence of diverse phytoconstituents. While the recurring R_f value of 0.63 indicates a significant bioactive component contributing to therapeutic efficacy (Dhavale *et al.*, 2025).

Discussion On GC-MS Analysis of *Shatapushpadi Lepa* Tricyclo[20.8.0.0(7,16))tricontane

It is chemical compound belongs to the group of Triterpenes, it is a cyclic hydrocarbon that have Anti-oxidant, antibacterial properties seen at retention time 32.31 with relative peak area of 67.01% (Table 5, Figure 3) as Triterpenes known to have Anti-inflammatory, anticancer, anti-viral activity (Arsana *et al.*, 2024). As Rheumatoid arthritis is a chronic autoimmune inflammatory disease and triterpenes may act by inhibition of pro-inflammatory cytokines like TNF- alpha, IL- beta, IL-6 etc. which are mainly involved in RA pathogenesis. Triterpenes also possess anti-oxidant properties and may help reducing oxidative stress and enhancing anti-oxidant enzymes (Faustino *et al.*, 2023). Some Triterpenes reduce Vascular Endothelial Growth Factor (VEGF), limiting blood vessel growth in inflamed joints, as in Rheumatoid arthritis synovium shows excessive angiogenesis which worsens inflammation (Faustino *et al.*, 2023).

1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-on -

Leucocyte infiltration into synovium is the main pathogenesis seen in RA, and causes chronic inflammation. 1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-on Table 5 it is also called as Shogaol. Reduce leucocyte aggregation and oedema formation and may limit immune cell accumulation in inflamed joints, thereby reducing synovial hyperplasia. It also contains antioxidant properties helps reducing local oxidative stress, thus its local application may help reducing symptoms of joint inflammation. It has anti-inflammatory properties by

Table 3: Organoleptic and Physiochemical Analysis of *Shatapushpadi Lepa*.

	Shatapushpadi Lepa
Ref. No.	**CRF/RM/134/2025-26**
Organoleptic Standards	
Form	Lepa
Colour	Dark Brown
Taste	Punjent
Odour	Characteristic
Physico-Chemical Standards	
Moisture Content	66.2023%
Ash Value	1.000%
Acid Insoluble Ash	0.884%
Water Soluble Extractive	13.465%
Alcohol Soluble Extractive	12.311%
pH	5.226

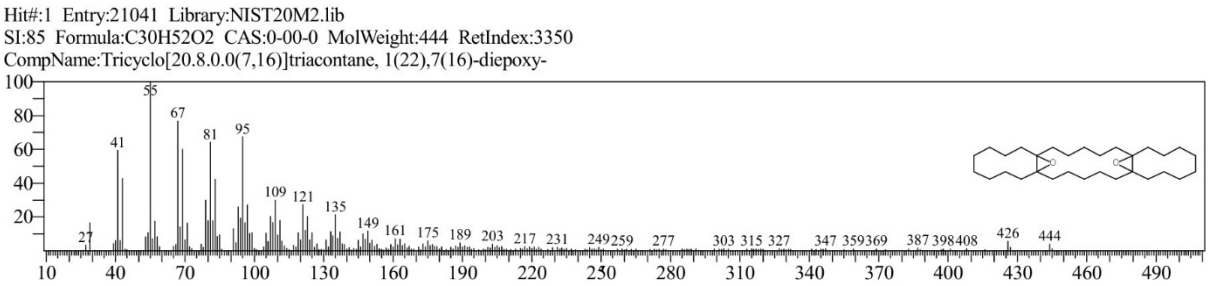

Figure 3: Tricyclo[20.8.0.0(7,16)]tricontane.

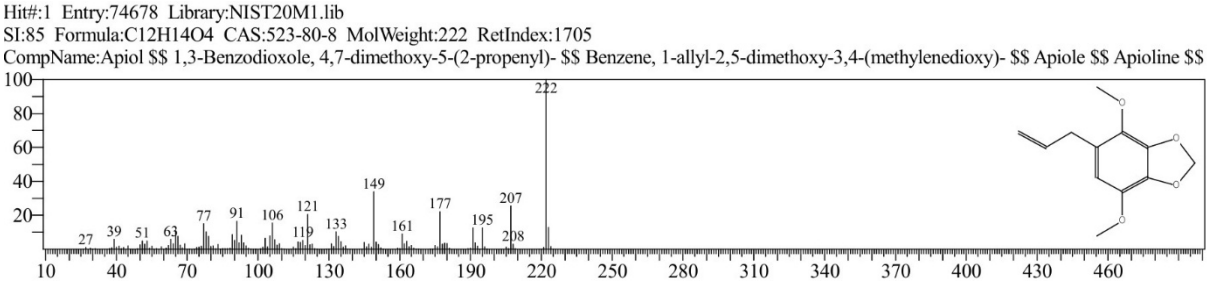

Figure 4: Apiol.

Table 4: Phytochemical Analysis of *Shatapushpadi lepa* and Kanji.

	Shatapushpadi Lepa		Kanji
Ref. No.	**CRF/RM/134/2025-26**		**CRF/RM/1/264/2025-26**
Tests	**Water**	**Alcohol**	**Water**
Carbohydrates	Positive	Negative	Positive
Reducing Sugar	Positive	Positive	Negative
Monosaccharides	Positive	Negative	Negative
Pentose Sugar	Negative	Negative	Negative
Non-Reducing Sugar	Negative	Negative	Negative
Hexose Sugar	Negative	Negative	Negative
Proteins	Negative	Negative	Negative
Amino Acids	Negative	Negative	Negative
Steroids	Negative	Negative	Negative
Flavonoids	Positive	Positive	Negative
Alkaloids	Negative	Negative	Negative
Tannins	Negative	Positive	Negative
Cardiac Glycosides	Negative	Positive	Negative
Anthraquinone Glycosides	Negative	Negative	Negative
Saponins Glycosides	Negative	Negative	Negative

reducing the level pro-inflammatory cytokine like IL-6, IL-4, TNF- alpha, etc. in the body (Bischoff-Kont, *et al.*, 2021). RA involves dysregulation of signalling pathways such as MAPKs which regulate cytokine production, cell proliferation, etc. 1-(4-hydroxy-3-methoxyphenyl) dec-4-en-3-on may exert broad immunomodulatory effects and may help in synovia cell survival, chronic inflammation (Bischoff *et al.*, 2021).

Apiol

Apiol Table 5, Figure 4 is one of the benzenoid compounds has anti-inflammatory action by activation of TLR4 or NF-KB Signaling pathway which is central to LPS induced macrophages activation. By suppressing TLR4 expression apiol reduces initial recognition of LPS, thereby limiting downstream inflammatory signaling (Tabassum *et al.*, 2021).

Apiol decreases the expression of iNOS and COX-2 which are key enzymes responsible for excessive NO and prostaglandin production. As COX2 Derived prostaglandins sensitize peripheral nociceptors and can contribute to inflammatory pain Inhibition of COX-2 and NO levels further limit neurologic inflammation and pain (Tabassum *et al.*, 2021).

Apiol induces heme oxygenase-1 expression which is an anti-inflammatory and cytoprotective enzyme.

Carvone

Carvone Table 5 and Figure 5 is present with relative peak area of 1.54%, studies suggests that carvone scavenges free radicals, inhibits lipid perioxidation, and reduces oxidative stress. Also, through local application it may suppress the release of pro-inflammatory mediators like TNF- alpha, IL-6, prostaglandins etc. which are key cytokines involved in RA pathogenesis. All this collectively may result into reduced vascular permeability, edema and inflammatory cellular infiltration. Also, antioxidant action of Carvone may reduce reactive oxygen species that causes limiting oxidative damage to synovial tissue and cartilage (Pina *et al.*, 2022). By concentrating its action carvone may help in alleviating pain, swelling and stiffness when applied locally in Rheumatoid arthritis.

Glutaric acid

Mitochondrial dysfunction and oxidative stress are recognized contributors to the pathogenesis of rheumatoid arthritis and they can later precede to cell death. Glutaric acid (Table 5) increases reactive oxygen species can reduce the oxidative damage seen in Rheumatoid arthritis, it also helps to identify metabolic and mitochondrial targets whose modulation may help amplifying inflammatory cytokine signaling eg. TNF- alpha, IL-6 etc. and this modulation may yield anti-inflammatory action in inflammatory arthritis (Li *et al.*, 2021).

Longifolene

Longifolene Table 5 is a bioactive sesquiterpene found in essential oils that demonstrates significant anti-inflammatory and analgesic effects when applied topically. It works primarily by suppressing inflammatory mediators, reducing joint swelling, and potentially providing a rubefacient (warming/counterirritant) effect (Yang *et al.*, 2021).

2- Methylhexacosane

2- Methylhexacosane Table 5 contains anti-cancer activity, also long chain branched hydrocarbon, being rich in lipids when applied topically it may help to enhance penetration through skin into deeper tissues and may provide mild topical symptomatic relief (Ryu *et al.*, 2020).

Phenol, 2-methyl-5-(1-methylethyl)-

Phenol, 2-methyl-5-(1-methylethyl) Table 5 is also called as Carvacrol which has been used to cure pain caused by inflammation and mechanical hypernociception. Rheumatoid Arthritis (RA) is the inflammation of the joints, and carvacrol has been proven by different studies that it can reduce inflammation in fibroblast-like synoviocytes (Imran *et al.*, 2020).

Benzenemethanol, alpha.,. alpha.,4-trimethyl- OR p-Cymen-8-ol

Numerous studies have demonstrated the pharmacological properties of the monoterpenes p-cymene, Table 5 including antioxidant and anti-inflammatory activities. Because p-cymene is lipophilic, it enters the skin and underlying tissues after topical

Table 5: The retention values, the types of possible compound, peak height, their Functional group and medicinal roles of each compound of GC-MS profile of *Shatapushpadi Lepa*.

Sl. No.	Retention Time (min)	Name of the compound	Mol. Formula	Mol. Weight	% Peak Area	Possible medicinal role
1.	32.315	Tricyclo[20.8.0.0(7,16) triacontane, 1(22),7(16)-diepoxy-	$C_{30}H_{52}O_2$	444	67.01	Anti-inflammatory, anticancer, and anti-viral activity (Arsana *et al.*, 2024) (Faustino *et al.*, 2023)
2.	24.414	1-(4-Hydroxy-3-methoxyphenyl) dec-4-en-3-one	$C_{17}H_{24}O_3$	276	1.13	Anti-inflammatory, Analgesic (Bischoff *et al.*, 2021)
3.	12.604	Apiol	$C_{12}H_{14}O_4$	222	9.53	Cytoprotective, Anti-inflammatory (Tabassum *et al.*, 2021)
4.	8.617	(−)-Carvone	$C_{10}H_{14}O$	150	1.54	Anti-inflammatory, antioxidant (Pina *et al.*, 2022)
5.	28.730	Glutaric acid, tridec-2-yn-1-yl dec-4-enyl ester	$C_{28}H_{48}O_4$	448	1.76	anti-inflammatory (Li *et al.*, 2021)
6.	10.211	Longifolene	$C_{15}H_{24}$	204	1.15	Anti-inflammatory (Yang *et al.*, 2021)
7.	25.284	2-Methylhexacosane	$C_{27}H_{56}$	380	3.10	anti-cancer, local inflammatory, an Analgesic (Ryu *et al.*, 2020).
8.	8.884	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	150	0.28	Immunomodulatory, Anti-inflammatory, Analgesic (Imran *et al.*, 2020)
9.	8.037	Benzenemethanol, α, α,4-trimethyl- p-Cymen-8-ol	$C_{10}H_{14}O$	150	0.46	Anti-inflammatory, Analgesic, immunomodulatory (Balahbib *et al.*, 2021)
10.	8.158	α-Terpineol	$C_{10}H_{18}O$	154	0.53	Enhancing Skin Permeation, Anti-inflammatory (Sapra, 2008) (Zhang *et al.*, 2023)
11.	25.448	Pentacosane	$C_{25}H_{52}$	352	12.73	anti-bacterial (Wang *et al.*, 2023)

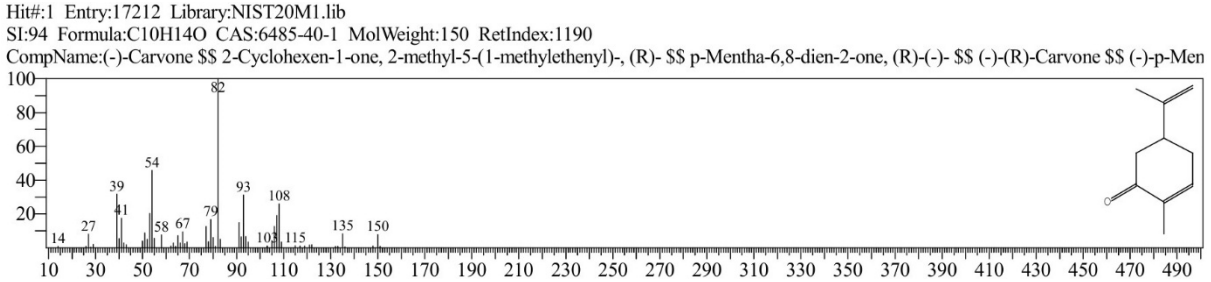

Figure 5: (-)-Carvone.

administration over the afflicted joint. It may work therapeutically through several complementary methods, including an analgesic, antinociceptive, immunomodulatory, vasorelaxant, and neuroprotective agent. (Balahbib *et al*., 2021).

α-Terpineol

In enhancing Skin Permeation, α-Terpineol Table 5 serves as a chemical penetration enhancer, acting by increasing the fluidity of the stratum corneum lipids and disrupting the intracellular lipid bilayers to improve the absorption of therapeutic agents into lower skin layers (Sapra, 2008).

When formulated into advanced systems like nanoemulgels or hydrogels, α-terpineol demonstrates sustained drug release and increased cellular uptake, making it a viable, non-invasive treatment option for local inflammation. Studies indicate it can significantly reduce oedema and inflammation (such as in TPA-induced skin inflammation) (Zhang *et al*., 2023).

Pentacosane

Pentacosane Table 5 consists of anti-bacterial activity, when applied locally it disrupts bacterial cell membranes due to its lipophilic nature, reduces microbial adhesion on skin and enhances penetration of antibacterial phytochemicals (Wang *et al*., 2023).

CONCLUSION

The present study features a comprehensive scientific evaluation of the Ayurvedic formulation *Shatapushpadi Lepa* by organoleptic, physicochemical, phytochemical, TLC and GC-MS studies. The results indicating that the composition conquers with standard quality parameters, demonstrating its quality, purity, potential for topical application and therapeutic effectiveness. Physicochemical components such as ash value, extractive value, and slightly acidic pH enhance the compatibility of the formulation with skin physiology and its potential for effective dermal absorption. Phytochemical screening indicating the presence of significant bioactive components including flavonoid, tannins, cardiac glycosides, reducing sugar, carbohydrates and monosaccharides which are recognised for their anti-inflammatory, antioxidant and analgesic activities. Thin Layer Chromatography shown several well-resolved spots, demonstrating the presence of various phytoconstituents and verifying the uniformity of the formulation. GC-MS analysis further discovered various biologically active chemical such as terpenoids, phenolic compounds that contribute to anti-inflammatory, immunomodulatory, antioxidant and penetration enhancement effects. These bioactive elements support the traditional usage of *Shatapushpadi Lepa* in the management of Amavata (Rheumatoid Arthritis), inflammatory arthritis and other vata-dominating inflammatory joint diseases. The study scientifically validates by the means of quality of *Shatapushpadi Lepa*. The study highlights the importance of integrating classical Ayurvedic knowledge with modern analytical techniques for the standardization and quality assurance of Ayurvedic herbal formulations. Further pharmacological and clinical studies are recommended to explore its bioavailability, safety and therapeutic efficacy in the management if inflammatory arthritis.

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ABBREVIATIONS

GMP: Good Manufacturing Practice; **NA:** Not applicable; **GC-MS:** Gas Chromatography-Mass Spectrometry; **TLC:** Thin Layer Chromatography; **ASU:** Ayurveda, Siddha, and Unani; **UV:** Ultraviolet; **API:** Ayurvedic Pharmacopoeia of India; **EI:** Electron Impact; **NIST:** National Institute of Standards and Technology; **RA:** Rheumatoid Arthritis; **TNF:** Tumor Necrosis Factor; **IL:** Interleukin; **COX:** Cyclooxygenase; **LOX:** Lipoxygenase; **NF-κB:** Nuclear factor kappa-light-chain-enhancer of activated B cells; **MAPKs:** Mitogen-activated protein kinases; **iNOS:** Inducible nitric oxide synthase; **NO:** Nitric Oxide; **TPA:** 12-O-tetradecanoylphorbol-13-acetate; **VEGF:** Vascular endothelial growth factor; **LPS:** Lipopolysaccharide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Dr. Anil Koralli: Research concept and design, collection of data, data analysis and interpretation, writing article, critical revision of the article, final approval of article. Dr. Sushmita Sanjay Gengane: Collection of data, data analysis and interpretation, writing article, critical revision of the article. Dr. Pranjali Abhay Patil: Data analysis and interpretation, writing article, critical revision of the article. Dr. Shreya Balasaheb Nikam: Data analysis and interpretation, writing article, critical revision of the article.

SUMMARY

Shatapushpadi Lepa is a traditional Ayurvedic topical preparation used to treat inflammatory joint conditions such as Amavata (Rheumatoid arthritis). The objective of this study was to assess its quality using physicochemical, phytochemical, TLC, and GC-MS studies. The results revealed that the formulation provides conventional quality requirements, including minimal ash levels and a slightly acidic pH suited for skin application. Phytochemical screening revealed the existence of medicinal

compounds such as flavonoids, tannins, and glycosides. TLC and GC-MS investigations identified many phytoconstituents, including triterpenes, apiol, and carvone, which contribute to its pharmacological action. Overall, the study scientifically supports *Shatapushpadi Lepa's* anti-inflammatory, analgesic, and antioxidant properties, which support its traditional usage.

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