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# Phytochemical Profiling of *Marmagulika Lepam*Using GC-MS Analysis: Identification of Volatile and Bioactive Compounds

Sreedevi AP<sup>1</sup>, Reeshma CR<sup>2</sup>, Anju Antony<sup>3</sup>, Sibi Narayanan<sup>4</sup>

<sup>1</sup>Research Officer, R&D Department, Sitaram Ayurveda Pvt. Ltd., Kerala, India.

## **Abstract**

Introduction: Marmagulika Lepam (MG Lepam), a polyherbal Ayurvedic formulation from Sahasrayogam, is used topically for its anti-inflammatory and analgesic properties in treating dermatological and musculoskeletal disorders. Unlike synthetic drugs, MG Lepam offers a natural alternative with minimal side effects, leveraging a diverse mix of herbal ingredients. Materials and Methods: Raw materials were sourced and MG Lepam was prepared per classical Ayurvedic protocols, triturating powdered herbs with decoctions to form a homogeneous paste. Organoleptic, physicochemical, phytochemical, Heavy metal Analysis and GC-MS analyses were conducted to assess the formulation's properties and safety.

**Results and Discussion**: MG Lepam exhibited a dark brown color, characteristic odor, and smooth consistency. Its pH (4.98) aligns with skin compatibility, with 53.1% moisture and 5981 cP viscosity ensuring stability and spreadability. Phytochemical analysis confirmed carbohydrates, sugars, proteins, glycosides, phenols, saponins, and quinones, supporting its therapeutic effects. HMT showed negligible heavy metal levels. GC-MS identified bioactive compounds like palmitic (12.279%), oleic (16.454%), and linoleic (12.542%) acid methyl esters, contributing anti-inflammatory, antioxidant, and antimicrobial properties, with cyclohexanol (25.543%) enhancing transdermal absorption.

Conclusion: MG Lepam is a safe, effective topical Ayurvedic formulation, validated by comprehensive analyses. Its skin-compatible properties and bioactive compounds support its use for pain and inflammation management, offering a natural alternative to synthetic analyses and a model for standardizing Ayurvedic formulations for global healthcare integration.

**Keywords:** Marmagulika Lepam, Anti-inflammatory, GC-MS Analysis, Phytochemicals, Heavy Metal Toxicity, Topical Application

#### 1. Introduction

Marmagulika Lepam (MG Lepam) is a polyherbal Ayurvedic formulation mentioned in the classical text *Sahasrayogam*. It is traditionally used for its proven therapeutic effectiveness in topical applications. Ayurveda, an ancient medicinal system originating in India over 3,000 years ago, is grounded in a holistic approach that integrates natural ingredients to restore physiological balance and promote overall health <sup>[1]</sup>.

<sup>&</sup>lt;sup>2</sup>Jr. Chemist, R&D Department, Sitaram Ayurveda Pvt. Ltd., Kerala, India.

<sup>&</sup>lt;sup>3</sup>Jr. Chemist, QC Department, Sitaram Ayurveda Pvt. Ltd., Kerala, India.

<sup>&</sup>lt;sup>4</sup>Manager, QA Department, Sitaram Ayurveda Pvt. Ltd., Kerala, India.



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Unlike conventional synthetic analgesics and non-steroidal anti-inflammatory drugs (NSAIDs), which are associated with adverse effects such as gastrointestinal complications and systemic toxicity with prolonged use <sup>[2]</sup>, herbal formulations like MG Lepam offer a promising alternative due to their natural composition and reduced risk of systemic side effects. MG Lepam is a prime example of this tradition, combining a diverse array of herbal and natural components, including roots, tubers, rhizomes, stems, leaves, bark, seeds, and exudates, to form a homogeneous paste specifically designed for external application. The formulation is meticulously crafted to harness the synergistic effects of its constituents, which are well-documented for their anti-inflammatory, analgesic, and skin-rejuvenating properties, making it an effective remedy for dermatological conditions and musculoskeletal disorders in Ayurvedic practice.

The preparation of MG Lepam adheres to rigorous traditional protocols outlined in classical Ayurvedic texts, ensuring fidelity to time-honored methods while incorporating modern quality control measures to validate its safety, efficacy, and consistency. The raw materials used in MG Lepam are sourced from the local market in Thrissur, and undergo thorough identification and authentication processes by the Pharmacognosy Division of Sitaram Ayurveda Pvt. Ltd. to confirm their botanical identity and quality. This meticulous sourcing and authentication process is critical to ensuring the purity and therapeutic potential of the formulation, aligning with the principles of good manufacturing practices (GMP) for herbal medicines [3]. The integration of traditional knowledge with contemporary scientific standards is essential for establishing the credibility of Ayurvedic formulations in modern healthcare systems, where evidence-based validation is increasingly demanded.

This study aims to provide a comprehensive evaluation of MG Lepam through a multifaceted analytical approach, including organoleptic, physicochemical, phytochemical, heavy metal toxicity (HMT), and gas chromatography-mass spectrometry (GC-MS) analyses. Organoleptic and physicochemical analyses assess the formulation's sensory attributes and physical properties, such as pH, moisture content, and viscosity, which are crucial for ensuring its compatibility with skin application and overall stability. Phytochemical analysis identifies the presence of bioactive compounds, such as flavonoids, phenols, and glycosides, which contribute to the formulation's therapeutic effects. Heavy metal toxicity analysis, conducted using inductively coupled plasma-mass spectrometry (ICP-MS), ensures the formulation is free from harmful contaminants like lead, arsenic, cadmium, and mercury, adhering to safety standards set by the World Health Organization (WHO) for herbal medicines. Additionally, GC-MS analysis provides a detailed profile of the bioactive chemical constituents, offering insights into the molecular basis of MG Lepam's pharmacological properties. By systematically documenting the preparation process and analytical outcomes, this research bridges the gap between traditional Ayurvedic knowledge and modern scientific validation, contributing to the growing body of evidence supporting the integration of herbal formulations into contemporary healthcare practices. This study not only underscores the therapeutic potential of MG Lepam but also establishes a framework for standardizing and validating other polyherbal Ayurvedic formulations to meet global regulatory standards.

#### 2. Materials and methods

#### 2.1 Collection of Raw materials

The raw materials used for MG Lepam were procured from the local market in Thrissur. Each herb was identified and authenticated by the Pharmacognosy Division of Sitaram Ayurveda Pvt. Ltd. The



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authenticated herbal raw materials were then stored in the Quality Control Division of the company for future reference.

## 2.2 Preparation of MG Lepam

MG Lepam is prepared using a blend of powerful herbal ingredients, processed as per classical Ayurvedic procedures. Details including the ingredient names, their botanical identities, plant parts used, and form are systematically presented in Table No. 1

**Table 1: Composition of MG Lepam** 

Sl. No	Ingredients	Botanical name	Parts used
1.	Vidari	Pueraria tuberosa	Tuber
2.	Jeevanthi	Leptadenia reticulata	Tuber
3.	Satavari	Asparagus racemosus	Root
4.	Mustha	Cyperus rotundus	Rhizome
5.	Varahi	Tacca aspera	Tuber
6.	Tavaksheeri	Maranta arundinacea	Rhizome
7.	Amantamool	Melothria heterophylla	Tuber
8.	Amalaki	Emblica officinalis	Fruit
9.	Sariba	Hemidesmus indicus	Root
10.	Guduchi	Tinospora cordifolia	Stem
11.	Durva	Cynodon dactylon	Whole part
12.	Yashti	Glycyrrhiza glabra	Rhizome
13.	Chandana	Santalum album	Heart wood
14.	Raktachandana	Pterocarpus santalinus	Heart wood
15.	Sahasravedhi	Magnatrite	As such
16.	Kumari	Aloe barbadensis	Leaf
17.	Shilajith	Asphaltum punjabianum	Exudate
18.	Kashmir larkspur	Delphinium cashmerianum	Flower
19.	Sanjeevani	Selaginella rupestris	Whole plant
20.	Prasarini	Merremia tridentata	Whole plant
21.	Lonika	Portulaca oleracea	Whole plant
22.	Matsyakshi	Alternanthera sessilis	Whole plant
23.	Murva	Chonemorpha macrophylla	Root
24.	Udumbara	Ficus racemosa	Flower bud
25.	Plaksha	Ficus microcarpa	Flower bud
26.	Aswatha	Ficus religiosa	Flower bud
27.	Vata	Ficus bengalensis	Flower bud
28.	Kathakam	Strichnous potatorum	Fruit
29.	Gokshura	Tribulus terrestris	Fruit
30.	Hribera	Coleus vettiveroides	Leaf
31.	Usira	Vetiveria zizanioides	Root
32.	Udumbara	Ficus racemosa	Bark
33.	Plaksha	Ficus microcarpa	Bark



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34.	Aswatha	Ficus religiosa	Bark
35.	Vata	Ficus bengalensis	Bark
36.	Lodhra	Symploccus racemosus	Bark
37.	Jambu	Syzygium cumini	seed
38.	Sushavi	Calycopteris floribunda	Stem
39.	Katabhi	Careya arborea	Bark
40.	Kantabohul	Xantolis tomentosa	Bark
41.	Daruharidra	Coccineum fenestratum	Bark
42.	Pashanabheda	Rotula aquatica	Whole plant
43.	Mudga	Vigna radiata	Seed
44.	Masha	Vigna mungo	Seed
45.	Sookshmela	Elettaria cardomomum	Seed

### 2.3 Preparation process

Marmagulika Lepam is prepared as per the classical reference in *Sahasrayogam*. Initially, the prescribed decoctions are prepared. These decoctions are then combined and used to triturate the finely powdered raw ingredients. The mixture is thoroughly ground until a smooth and homogeneous paste (lepam) is formed.

## 2.4 Organoleptic Analysis

The organoleptic properties of MG Lepam, including its colour, odour, and consistency, were evaluated [4].

#### 2.5 Physicochemical Analysis

The parameters evaluated for MG Lepam included pH, moisture content (%), and viscosity [5].

## 2.6 Preliminary Phytochemical Analysis

Phytochemical analysis of MG Lepam was carried out to identify the presence of various constituents such as sugars, reducing sugars, ketoses, amino acids, proteins, starch, quinones, glycosides, flavonoids, phenols, saponins, alkaloids, tannins, and coumarin [6].

## 2.7. Heavy Metal Toxicity Analysis (HMT)

HMT analysis of the sample was performed using ICP-MS to detect trace metals. A 1 ppm stock solution was prepared and diluted to create working standards. For digestion, 0.2–0.5 g of the sample was treated with nitric acid, hydrochloric acid, and hydrogen peroxide, followed by dilution with ultrapure water. The digested sample was introduced into the ICP-MS, where ionized elements were measured based on their mass-to-charge ratio to determine heavy metal concentrations <sup>[7]</sup>.

## 2.8 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was conducted to identify the bioactive compounds present in the sample. The sample was first extracted using hexane and then filtered through a 0.2 μm Nylon syringe filter. Analysis was carried out using an Agilent 7890A Gas Chromatograph coupled with a 5975C Mass Spectrometric detector, equipped with a DB-5MS column (30 m × 0.250 mm, 0.25 μm film thickness). A 2 μL sample was injected in split less mode, with helium as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed to increase from 100°C (held for 1 minute) to 200°C at a rate of 10°C/min, and then to 300°C at 20°C/min with a final hold of 10 minutes. Electron Impact (EI) ionization at 70 eV was used, with the injector maintained at 300°C. The resulting spectra were matched against the NIST-08 library for compound identification [8].



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#### 3. Results

## 3.1 Organoleptic Analysis

Table no 2 provides the results of organoleptic parameters such as colour, odour & consistency of MG Lepam

Table-2: organoleptic analysis of MG lepam.

Sl.No	Parameter	Result
1	Colour	Dark brown
2	Odour	characteristic
3	Consistency	Smooth

## 3.2 Physicochemical Analysis of MG Lepam

Table no 3 shows the values of the physicochemical parameters. pH measurement ensures the ointment is compatible with the skin and does not cause irritation. Moisture content and viscosity are crucial for determining the product's stability, spreadability, and ease of application.

Table no 3: Physicochemical analysis of MG Lepam

Sl.No	Parameters	Result
1	рН	4.98
2	Moisture %	53.1%
3	Viscosity	5981 cP

### 3.3 Phytochemical Analysis

Table No. 4 presents the results of the phytochemical screening of the water extract of MG Lepam, indicating the presence or absence of various constituents. These include carbohydrates, sugars, proteins, glycosides, steroids, flavonoids, phenols, and other bioactive compounds commonly found in herbal formulations.

Sl No:	Organic Phy	tochemical	Name of the test conducted	MG Lepam
	constituents			(Water Extract)
1	Carbohydrate		Molisch's test	+
2	Sugar		Benedict's test	+
3	Reducing Sugar		Fehling's test	+
4	Ketose		Seliwanoff's test	-
5	Protein		Biuret test	+
6	Starch		K I test	-
7	Glycoside		Salkowski test	+
8	Steroid			-
9	Terpenoid			-
10	Flavonoid		Alkaline reagent	-
11	Phenol		Phenol reagent test	+
12	Saponin		Foam test	+
13	Alkaloid		Wagner's reagent	-
14	Tannin		Ferric chloride test	-



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15	Coumarin	NaOH test	-
16	Aminoacids	Ninhydrin test	-
17	Quinone	H <sub>2</sub> SO <sub>4</sub> test	+

Table no: 4 The phytochemical analysis of MG Lepam

## 3.4 HMT Analysis

The heavy metal toxicity analysis of MG Lepam was conducted to detect the presence of harmful metals such as lead, arsenic, cadmium, and mercury, ensuring the formulation's safety. The findings are summarized in Table 5.

Table No 5: The HMT analysis of MG Lepam

Sl. no.	Parameters Tested	Unit of Measurement	Results
1	Cadmium	ppm	BDL
2	Lead	ppm	BDL
3	Arsenic	ppm	0.28
4	Mercury	ppm	Not detected

## 3.5 Gas Chromatography-Mass Spectrometry (GC-MS) Profile

The GC-MS analysis of MG Lepam was carried out to identify its bioactive chemical constituents, providing insights into the therapeutic potential of the formulation. The chromatogram & results are detailed in figure 1 & Table 6 respectively.

Figure No 1- GC-MS chromatogram of MG Lepam

2.2e+07
1.8e+07
1.2e+07
1

Table No 6 : GC-MS Analysis of MG lepam

No	Constituent	Chemical	Area	Action
		formula		
1	Cyclohexanol, 1-methyl-4-(1-	$C_{10}H_{20}O$	25.543%	Transdermal
	methylethyl)-			enhancer <sup>[9]</sup>



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2	Lauric acid, methyl ester	$C_{13}H_{26}O_2$	8.155%	Anti-inflammatory &	&
				Anti-microbial <sup>[10]</sup>	
3	Diethyl Phthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	2.131%	Anti-inflammatory	
				Properties <sup>[11]</sup>	
4	β-Eudesmol	C <sub>15</sub> H <sub>26</sub> O	0.200%	Anti-inflammatory	
				Properties <sup>[12]</sup>	
5	α-Bisabolol	C <sub>15</sub> H <sub>26</sub> O	0.154%	Anti-inflammatory	
				Properties <sup>[13]</sup>	
6	Myristic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	7.611%	Anti-inflammatory &	&
				Antinociceptive <sup>[14]</sup>	
7	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	12.279%	Anti-inflammatory	
				Properties <sup>[15]</sup>	
8	Linoleic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	12.542%	Anti-oxidant	
				properties <sup>[16]</sup>	
9	Oleic acid, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	16.454%	Anti-inflammatory	
				Properties <sup>[17]</sup>	
10	Stearic acid, methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	3.557%	Anti-microbial <sup>[18]</sup>	
11	Diisooctyl phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	4.245%	Anti-inflammatory	
				Properties <sup>[19]</sup>	

#### 4. Discussion

The comprehensive evaluation of Marmagulika Lepam (MG Lepam) through organoleptic, physicochemical, phytochemical, heavy metal toxicity (HMT), and gas chromatography-mass spectrometry (GC-MS) analyses provides robust evidence supporting its quality, safety, and therapeutic potential as a polyherbal Ayurvedic formulation. The findings align with the traditional claims of MG Lepam's efficacy in alleviating acute pain and inflammation while offering a scientifically validated framework for its integration into modern healthcare practices.

The organoleptic analysis revealed MG Lepam as a dark brown, smooth paste with a characteristic odor, consistent with the expected sensory profile of a polyherbal ointment formulated with diverse plant-based ingredients. The physicochemical parameters further corroborate the formulation's suitability for topical application. A pH of 4.98 is within the mildly acidic range, closely matching the skin's natural pH (4.5–5.5), which minimizes the risk of irritation and enhances compatibility for dermatological use. The moisture content of 53.1% ensures adequate hydration, facilitating spreadability and stability, while the viscosity of 5981 cP indicates a semi-solid consistency ideal for sustained contact with the skin, allowing effective delivery of bioactive compounds.

Phytochemical screening confirmed the presence of carbohydrates, sugars, proteins, glycosides, phenols, saponins, and quinones, which are likely derived from the diverse herbal constituents listed in Table 1. These compounds are well-documented for their pharmacological properties. For instance, phenols and glycosides are known for their anti-inflammatory and antioxidant activities <sup>[20]</sup>, which likely contribute to MG Lepam's ability to mitigate pain and inflammation. Saponins, with their emulsifying properties, enhance the formulation's texture and transdermal absorption <sup>[21]</sup>, while quinones offer antimicrobial benefits <sup>[22]</sup>, protecting the skin from secondary infections during application.



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The HMT analysis underscores the safety of MG Lepam, with cadmium, lead, and mercury levels below detectable limits and arsenic at 0.28 ppm, well within the WHO's permissible limit of 3 ppm for herbal medicines.

The GC-MS analysis provides critical insights into the molecular basis of MG Lepam's therapeutic efficacy, identifying key bioactive compounds such as palmitic acid, linoleic acid, and oleic acid methyl esters, which collectively constitute a significant portion of the chromatogram's area (Table 6). Palmitic acid methyl ester (12.279%) and oleic acid methyl ester (16.454%) are known for their anti-inflammatory properties, with studies demonstrating their ability to modulate inflammatory pathways in macrophages and animal models of chronic inflammation. Linoleic acid methyl ester (12.542%) exhibits antioxidant properties, which protect skin tissues from oxidative stress associated with inflammation. Additionally, lauric acid methyl ester (8.155%) and stearic acid methyl ester (3.557%) contribute antimicrobial activity, potentially preventing infections in inflamed or damaged skin. The presence of cyclohexanol, 1-methyl-4-(1-methylethyl)- (25.543%) as a transdermal enhancer is particularly noteworthy, as it likely facilitates the penetration of other bioactive compounds through the skin, enhancing the formulation's overall efficacy. Compounds like  $\beta$ -eudesmol and  $\alpha$ -bisabolol, though present in smaller quantities, further bolster the anti-inflammatory profile, as evidenced by their documented effects in suppressing pro-inflammatory mediators.

The analytical data validate MG Lepam as a safe, high-quality polyherbal formulation with significant therapeutic potential for pain and inflammation management. The findings highlight MG Lepam's promise as a natural alternative to synthetic analgesics, offering a safer and potentially more sustainable option for managing musculoskeletal conditions.

#### 5. Conclusion

The study establishes Marmagulika Lepam (MG Lepam) as a safe and effective polyherbal Ayurvedic formulation for topical use in managing pain and inflammation. Through rigorous organoleptic, physicochemical, phytochemical, HMT, and GC-MS analyses, MG Lepam demonstrates a favorable profile with a skin-compatible pH, optimal viscosity, and bioactive compounds like palmitic, oleic, and linoleic acid methyl esters, which underpin its anti-inflammatory, analgesic, and antimicrobial properties. The absence of significant heavy metal contamination ensures its safety per WHO standards. By providing scientific validation of its traditional use, this research supports the integration of MG Lepam into modern healthcare as a natural, sustainable alternative to synthetic analgesics, while establishing a framework for standardizing other Ayurvedic formulations to meet global regulatory standards.

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