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Characterisation of *Shirashulahara Lepa* – A Non-Codified Formulation

Research Article

Sreedevi V P[1], Rajeshwari V Kamat[2*]

1. Final Year PG Scholar, 2. Professor, Department of Rasashastra and Bhaishajya Kalpana, KAHER's Shri BMK Ayurveda Mahavidyalaya, Shahapur. Belagavi. Karnataka. India.

Abstract

The *Shirashulahara lepa* is used in folklore medicine practiced as preventive and curative healthcare need in Kerala. The present study was aimed to validate the traditional medicinal knowledge through pharmacognostical standardization. Aims and objective: The objective is to create a Non-codified formulation called *Shirashulahara lepa* using two different approaches, and then analyse its properties and composition. Methods: The preparation methods of *Shirashulahara lepa* differ based on the base ingredient used. The first method involves utilizing *Narikela taila* (Coconut oil) while the second method involves the use of Beeswax. The Preparation of medicine has been carried out and the Physico-chemical parameters of finished lepa such as p^H, Spreadability, Rancidity etc were performed as per API guidelines. Observations: Detailed TLC fingerprint profile of methanolic extracts of the formulation and GC-MS of the selected Method has also been performed. Further in-vivo and clinical studies are also suggested. Result: The results obtained from the first method employing *Narikela taila* were superior.

Keywords: Pharmacognosy, API, Standardization, *Shirashulahara lepa*, Physico-chemical characters, GC-MS.

Introduction

India has glorious tradition of arts and science of healing. According to the World Health Organization (2008), folklore is defined as the knowledge, skills, and practices based on the beliefs and experiences of a society.(1) The origin of Indian medicines are shrouded in myths. It can certainly be valuable source of clues to the usefulness of plants and the kinds of relationships that human communities have had with them. In view of worldwide increasing interest in plant based medicine; safety, efficacy and quality control of such medicines are becoming important for both public and health authorities. The developing countries are trying to promote and revive their respective native systems, and in turn, make their use in health care. Even in the developed nations, attempts are afoot to investigate the efficacy and usefulness of such systems(2). Regulatory bodies have laid down the standardization procedures and specifications for Ayurvedic preparations. In India, the department of AYUSH, Government of India, launched a central scheme to develop a standard operating procedures for the manufacturing process to develop pharmacopeial standards for Ayurvedic preparations.(3) We have a vast store of oral medical knowledge available in the form of home remedies and local health traditions, the people who living in remote areas are untouched by modern civilization uses plants for their basic health care needs.

The *Shirashulahara lepa* is one of such in folklore medicine practiced as preventive and curative healthcare need in Kerala which cure the headache (*Shirashula*). The ingredients included in the formulation are *Sallaki* (*Boswellia serrata.* Linn*), Karpura* (*Cinnamomum camphora.* Linn*)* and *Narikela Taila* (*Cocos nucifera.* Linn*)*. It is used as mild paste. Due to urbanization and fast changing trends in the life style of the younger generation, they do not want to follow the footsteps of their ancestors. Indian traditions continue to provide health care needs in vast rural masses and it need to be protected from being lost forever. Thus proper identification, standardization of the drug and systematic documentation is essential. Standardization guidelines to be followed for herbal products provided by World Health Organization and Ayurvedic pharmacopoeia of India have been considered.(4,5) Characterization plays a crucial role in Ayurveda preparations as it enables the evaluation of their quality by examining the concentration of chemical or bioactive markers. Hence physico-chemical studies of a particular drug by various parameters help in Characterisation and validation. Contemporary bioanalytical methods such as GC-MS, TLC (Thin Layer Chromatography) and physicochemical parameters are employed to fulfil the aforementioned goals The primary objective of the current research was to examine the physical and chemical characteristics of the formulation and determine the most effective method through analysis.

*** Corresponding Author:**
Rajeshwari V Kamat
Professor,
Department of Rasashastra and Bhaishajya Kalpana,
KAHER's Shri BMK Ayurveda Mahavidyalaya,
Shahapur. Belagavi. Karnataka – 590003. India.
Email Id: drrajeshwarikamat@gmail.com

Materials and Methods

The formulation consist of three ingredients viz, *Sallaki* (*Boswellia serrata.* Linn*)*, *Karpura* (*Cinnamomum camphora.* Linn*)*, and *Narikela taila* (*Cocos nucifera.* Linn*)* and water. All the ingredients were collected from GMP certified pharmacy and authentified in Central research facility of Shri BMK Ayurveda Mahavidyalaya.Belgaum. before the study were carried out. The *sallaki* (*Boswellia serrata.* Linn*)*, and *Karpura* (*Cinnamomum camphora.* Linn*)* were powdered in pulverizer and sieved through 300 mesh size. All the ingredients weighed separately and mixed together in specified proportions in a geometrical manner to get uniform mixer and stored under controlled conditions. The formulation is given in Table 1.

Table 1: Composition of the formulation *Shirashulahara lepa*

	Method 1	Method 2
Ingredients	*Sallaki*	*Sallaki*
	Karpura	*Karpura*
	Narikela taila	Bees wax
	Jala	*Jala*
Ratio	Equal part	Equal part

Pharmaceutical part

Preparation of *Shirashulahara lepa*

Method 1: *Shirashulahara Lepa* (SL)

The equal quantity of *sallaki* (*Boswellia serrata.* Linn*)*, and *Karpura (Cinnamomum camphora.* Linn) has been mixed homogeneously in mortar using pestle. Then the *taila* and water was added into it and made it into a *lepa* (paste) form. Here the *Narikela taila* acts as base.

Method 2: *Shirashulahara Lepa* with Bee wax (SL with Bee wax)

The Bees wax is heated in mild fire till the Bees wax completely melts. After melting it transferred to *Khalwa yantra* (Mortar and pestle) and then the homogeneous mixture of *Sallaki* (*Boswellia serrata.* Linn*)*, and *Karpura (Cinnamomum camphora.* Linn) is added and triturated homogeneously till it get a *lepa* (Paste) consistency.

Figure 1: Method 1 *(Shirashulahara Lepa)*

Figure 2: Method 2 (*Shirashulahara lepa* with Bee wax)

Analytical part

Evaluation Parameters of Cream

1. **Determination of pH**(6): The pH of the cream can be measured by a standard digital pH meter at room temperature by diluting the formulation in a water in a beaker.
2. **Homogeneity**(7): Homogeneity of cream is tested by visual appearance and by touch.
3. **Spreadability**(8): 10gm of sample is taken between two glass slides and a weight of 100gm is applied on the slides for 5 minutes.
4. **Rancidity (Kries test)**(9): Shake 5g of sample vigorously with 0.1% of Phloroglucinol solution in diethyl ether and add 5ml of conc. Hydrochloric acid. Pink colour indicates Rancidity.
5. **Extrudability**(10): Extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5 cm of ribbon of ointment in 10 second**.**
6. **Viscosity**(11): Viscosity is determined by using Brookfield Viscometer in spindle 100rpm.
7. **Microbial Load Test- Qualitative**(12): 0.1gm sample in two sterile petri plate before incubation and pour 15-20ml of Soyabean Casein Digest Agar (SCDA) having temperature of about 45^0 C. Mix the contents of petri plates by gently swirling the plate to proper mixing of sample. Allow the plate to solidify at room temperature and keep in incubator for incubation at 30^0 C to 35^0 C for 5 days in inverted position.

- **Test for Escherichia coli:** After incubation of Sample shake and transfer 0.1ml to 10ml of Macconkeys nbroth and incubate at 42^{0}C to 44^{0}C for 24 to 48 hrs. Take loopful sample from Macconkeys broth and stick on Macconkeys agar plate and incubate at 30^{0}C to 35^{0}C for 18 to 72 hrs. Growth of pink, non-mucoid colonies, indicates the presence of Escherchia coli. If there is no growth of such type of colonies, or the identification test are negative, it indicates absence of E. coli and the sample passes the test.
- **Test for Staphylococcus aureus:** After incubation, take loopful sample and strick on Mannitol salt agar plate and incubate at 30^{0}C to 35^{0}C for 18 to 72 hrs. Yellow or White colonies with yellow zone indicates the possibility of presence of Staphylococcus aureus. If there is no growth of such type of colonies, or the identification test are negative, it indicates absence of S. aureus and the sample passes the test.
- **Test for Pseudomonas aeruginosa:** After incubation, take loopful sample and strick on Cetrimide agar plate and incubate at 30^{0}C to 35^{0}C for 18 to 72 hrs. A greenish colony indicates the possibility of presences of Pseudomonas aeruginosa.
- **Test for Salmonella aboney:** After incubation, transfer 0.1gm of sample to 10ml of Rappaport Vassilias Salmonella Enrichment (RVSE) broth and incubate at 30^{0}C to 35^{0}C for 24 to 48 hrs. Take loopful sample from RVSE broth and strick on Xylose Lysine Deoxycholate Agar plate and incubate at 30^{0}C to 35^{0}C for 24 to 48 hrs. Well developed, red colonies with or without black centers indicate the presences of Salmonella aboney.

8. **Microbial Limit Test - Quantitative (13):**

- Total Bacterial Count: 10gm of sample is added to 100ml of buffered sodium chloride peptone solution.

1gm of sample has been aseptically transfer to petridish and addition of 20ml of sterilized SA medium. Then the plate swirled to mixing and allow to solidify for 1hr. Plates are incubated in inverted position in incubator at 35-37^{0}C for 5 days.

- Total Fungal Count: The media used is SD instead of SA in Bacterial count, and incubated at 20-25^{0}C for 3 days. Rest all procedure is same as Bacterial count.

Observations and Results

The formulations was evaluated for organoleptic characters (**Table 2**), Physico-chemical parameters (**Table 3**) and Microbial assay for both the formulations (**Table 4 & 5**).

Table 2: Organoleptic characters of the Formulations

Test	Method 1 (*Shirashulahara Lepa*)	Method 2 (SL with Bee wax)
Colour	Whitish cream	Whitish cream
Odour	Camphor smell	Camphor smell
Form	Semi-solid	Semi-solid

Table 3: Physico-chemical characters of Formulations

Test	Method 1 (*Shirashulahara Lepa*)	Method 2 (SL with Bee wax)
p^H	5.1	4.16
Homogeneity	Pass	Pass
Spreadability	60mm	45mm
Rancidity	Negative	Negative
Extrudability	Good	Good
Viscosity	836cp	130cp

Table 4: Microbial load of *Shirashulahara lepa*: Test for specified microorganisms (Qualitative)

Microorganisms	Method 1 (*Shirashulahara Lepa*)		Method 2 (*Shirashulahara lepa* with Bees wax)	
	Limit	**Result**	**Limit**	**Result**
Escherichia coli	Absent/ 100ml	Absent	Absent/ 100ml	Absent
Staphylococcus aureus	Absent/ 100ml	Absent	Absent/ 100ml	Absent
Pseudomonas aeruginosa	Absent/ 100ml	Absent	Absent/ 100ml	Absent
Salmonella aboney	Absent/ 100ml	Absent	Absent/ 100ml	Absent

Table 5: Microbial load of *Shirashulahara lepa*: Microbial limit test (Quantitative)

	Method 1 (*Shirashulahara Lepa*)		Method 2 (*Shirashulahara Lepa* with Bees wax)	
Count	**Limit**	**Result**	**Limit**	**Result**
Total Bacterial count	30-300 cfu/ml	11cfu/ ml	30-300 cfu/ml	13cfu/ml
Total fungal count	10-100 cfu/ml	03 cfu/ ml	10-100 cfu/ml	01cfu/ml

TLC (Thin layer chromatography)(14)

Thin layer chromatography and High performance thin layer chromatography were performed for the *Shirashulahara lepa*. Solvent system was prepared by taking Toluene: Ethyl acetate: Hexane: Formic acid in the proportion of 8:2:0.5:0.3 respectively. The spots obtained from both the extracts were examined under ultra violet light of wavelength 254nm and 366nm.The resolution factor (Rf) was calculated by using the formula Rf = Distance travelled by solute/Distance travelled by solvent.

Table 6: Short and Long wave values of the sample (SL)

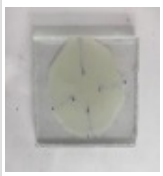
		Short wave		Long wave	
Sample	**Track**	**Number of spots**	**Rf**	**Number of spots**	**Rf**
SL	Toluene: Ethyl acetate: Hexane: Formic acid **Ratio - [8:2:0.5:0.3]**	4	0.29, 0.40, 0.52, 0.59	0	Nil
SL with beeswax	Absent	0	Absent	0	Nil

Figure 3: The Figures of physico-chemical evaluations of the *Shirashulahara Lepa*

a) p^H

a) Rancidity

c) Viscosity



d) Spreadability

Figure 4: TLC Fingerprints of n-Hexane extracts of **Method 1: *Shirashulahara lepa*** at Longwave

Figure 5: TLC Fingerprints of n-Hexane extracts of **Method 1: *Shirashulahara lepa*** at Shortwave

Figure 6: TLC Fingerprints of n-Hexane extracts of **Method 2: *Shirashulahara lepa* with bees wax** at Longwave

Figure 7: TLC Fingerprints of n-Hexane extracts of **Method 2: *Shirashulahara lepa* with bees wax** at Shortwave

(Solvent system: Toluene: Ethyl acetate: Hexane: Formic acid Ratio - [8:2:0.5:0.3])

The analysis of the physicochemical properties of *lepa* revealed a pH range of 5.1 to 6.8, indicating its safety for applying on the skin since it falls within the typical pH range of 4.0 to 7.014 for skin. Moreover, it does not cause any skin irritation. The first method of application demonstrated better spreadability than second, allowing it to quickly cover a larger surface area on the affected part. On the other hand, the second sample (*Shirashulahara Lepa* with beeswax) exhibited lower viscosity, which can be attributed to the thick nature of beeswax. As a result, when compared to the second method, the *Shirashulahara lepa* displayed higher viscosity, making it suitable for external application due to its consistent texture. The formulation containing beeswax does not show any visible bands when subjected to TLC analysis under both long and short waves. This lack of bands could be attributed to the absence of certain components in the formulation. Additionally, the formulation has lower spreadability and viscosity, which can impact the absorption rate. Considering the favourable results observed thus far, the formulation was further analysed using GC-MS to obtain more detailed information.

GC-MS (Gas Chromatography – Mass Spectrometry)

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. The GC-MS profile of *Shirashulahara Lepa* is represented in **Figure 7.**

Figure 7: Depicts the GC-MS Profile of *Shirashulahara Lepa*

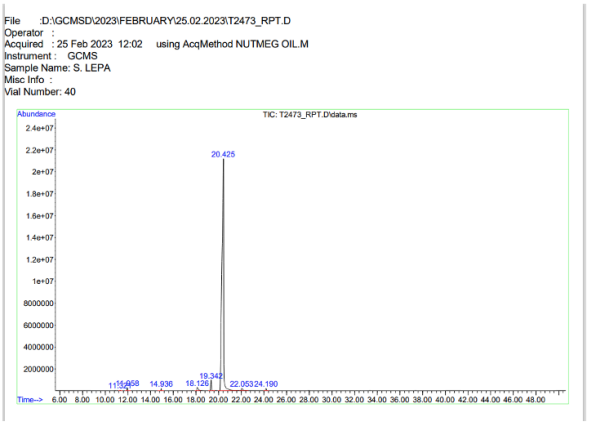


Table 7: Indicates the retentions time, types of possible compound, their molecular formula, Peak height and their medicinal roles of each compound as shown in the GC MS profile of *Shirashulahara Lepa*.

Sl no	Retention time	Compound name	Molecular Formula	Peak height	Possible medical roles
1	11.323 min	α-Pinene	C10H16	57072	α-Pinene is highly bioavailable with 60%, an anti-inflammatory (15)
2	11.960 min	Camphene	C10H16	269082	Significant role in reducing inflammation. (16)
3	14.936	D-Limonene		213549	Shows action in inflammation.(17)
4	18.126	Bicyclo[2.2.1]heptan-2-ol, 1,5,5-trimethyl	C10H18O	294477	-
5	19.342	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	C10H16O	979190	-
6	20.425	Isoborneol	C10H18O	20442100	Present in Camphor, actions similar to Camphor.(18)
7	22.053	Borneol	C10H18O	199990	It has Anti-inflammatory as well as Analgesic properties.(19)
8	24.190	Isobornyl formate	C11H18O2	208524	Its having analgesic effects.(20)

Discussion

The selected *Shirashulahara lepa* sample underwent GC-MS analysis, which unveiled the existence of eight phytoconstituents exhibiting dual properties of analgesic as well as anti-inflammatory. α-pinene exhibits potential as a therapeutic agent for addressing different inflammatory conditions due to its ability to inhibit MAPKs and the NF-κB pathway. Camphene, D-Limonene, Isoborneol, Borneol, and Isobornyl formate also demonstrate anti-inflammatory and analgesic properties. According to classics, *Sallaki (Boswellia serrata.* Linn*)* is having *Katu Rasa* (Pungent taste) and *shulaghna* (Analgesic) property(21), *Karpura* (*Cinnamomum camphora.* Linn*)* is *sita virya* (Cold potency)(22) that will give cooling effect and *Narikela taila* (Coconut oil) is *vatapittasamaka* (Reduce *Vata* and *Pitta dosha*) and *Dahahara* (Reduce Burning sensation).(23) Many research papers show the Analgesic and Anti-inflammatory effect of resin of *sallaki* due to its nervine tonic property and *guru* (Heavy) *snigdha guna* (Slimy) and *ushna virya* (Hot potency) and *vataharatwa*.(Reduce *vata dosha*).(24) Camphor is evaluated in researches and concluded that external application of camphor oil produces a feeling of coolness which related to stimulation of nerve endings sensitive to cold and also activates some

channels causing warm sensation, excitation, and desensitization of sensory nerves and thus relieves pain in the applied area.(25) The antipyretic, analgesic and anti-inflammatory effect of coconut oil reduces the stress and burning sensation of the applied area.(26) The results of physicochemical parameters were found to be significant and encouraging towards the goal for characterising this *Shirashulahara lepa*. The results of the present study revealed that its physico-chemical characters and fingerprints in TLC profiles and GC-MS can be utilized as marker parameters for identity and monitoring the quality of the drug.

Conclusion

The current endeavour involved the preparation of *Shirashulahara lepa* using two different methods: Method 1 and Method 2, which incorporates beeswax. Various parameters were assessed, and based on the results, it can be concluded that the *lepa* prepared through first method outperformed the *lepa* prepared through Method 2. Differences between the two formulations can be observed through physicochemical analysis, specifically in terms of Thin Layer Chromatography (TLC), spreadability, and viscosity. Based on the findings, it can be inferred that the *Shirashulahara lepa* formulation prepared using Method 1, which is currently being utilized, can be considered as the superior choice. Further areas of study for this topic could include conducting a clinical trial and exploring dosage modification as potential avenues for research.

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