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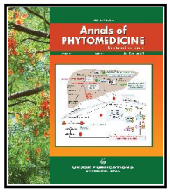

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Development of standard operating procedures and pharmaceutical study of herbal lipid preparation

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Abstract

In Ayurveda, the lipid preparation (Sneha kalpana) is mentioned since Samhita period and it has own importance. Lipid preparation (Shatavari ghrut: SG) is one of the formulation suggested for the management of male infertility. SG contains cow ghee (Goghrut), paste of *Asparagus racemosus* Willd. roots, cow milk and water. There are some rules mentioned in Ayurveda classics for lipid preparations. According to that one of the rules is 'Ksheere dviratram' which means whenever lipid (Sneha) is prepared by using milk for 2 days. Analytical study showed that SG prepared by using 2 days rule, has proper extraction of active principles which is shown in HPTLC analysis. HPTLC technique helps to create an account of active phytoconstituents present in prepared SG. Jacketed vessel gives good yield and there are no pharmaceutical inaccuracies in method of SG preparation. As per the testing protocol, this study might produce preparatory standards for SG.

1. Introduction

Lipid preparation (Sneha kalpana) is one of the broadly utilized and favored dosage forms of Ayurvedic system of medicine. Lipid formulation is invented to get especially lipophilic action of the cell of the epithelium overlying the whole-body surfaces externally and internally. This action is only possible by the formulation which is prepared by using medias made up of lipids, protein and water at a very particular temperature for certain duration till the accomplishment of tests. Here, the main principle is to transfer active constituent of herbs in lipid and water, depending upon its solubility. Cow ghee is considered as the best by virtue of its unique properties (Fulkar *et al*., 2014). It readily incorporates the properties of the constituents in drugs which are associated with it and does not leave its own natural qualities when in contact with other drugs (Ashwini *et al*., 2017). In Bhaishjya Ratnawali, they also mentioned some rules of lipid preparations (Snehpak) in details (Shastri, 2002). The duration of processing time depends entirely on the nature and constituents of the herbs used with lipid (Bekal and Seema, 2009). Lipids prepared with milk are expressed as having to be prepared for 2 days (Ksheere dviratram). Here, selected lipid formulation, SG having milk as one of the content (Thakare and Dobade, 2020). Therefore, it is an attempt to study and standardize SG according to the given rule. Ayurveda complies around 13 different references of SG (Pal *et al*., 2016). Selected formulation is mentioned in Yogratnakar vajikaran adhikar (Shastri, 2002; Ansari *et al*., 2020; Dash *et al*., 2022).

SG is classical Ayurvedic formulation usually prescribed in the treatment of infertility (Patil *et al*., 2016) which is major progressively around the world concern. *A. racemosus*. It is a shrub and climbing plant that grows in low forest areas, everywhere in India. The meaning of the word Shatavari itself is "a woman who possess 100 children". It is one of the rejuvenative drugs in Ayurveda classics (Wani *et al*., 2011). *A. racemosus* rejuvenate the body and also maintain vat dosha and pitta dosha. It is used in gastric complaints. Besides this, it also acts on male reproductive system. Hence, the attempt has been made to prepare SG and standardize it to ensure its quality and safety (Jain, 2014; Mishra *et al*., 2010 ; Nagaiah, 2022).

2. Materials and Methods

2.1 Pharmaceutical research

The pharmaceutical research includes the entire procedure of medicine preparation, from drug collection to final product. It is divided into the following sections:

2.1.1 Raw material collection

For the manufracture of SG, required crude drugs were purchased from the local market and wet *A. racemosus* roots were collected from Pune, Maharashtra, India.

2.1.2 Authentication of raw drugs

All raw drugs authentication was done at the Aagarkar Institute Pune, Maharashtra, India.

2.1.3 Preparation of SG

Medicine preparations and analysis of them were carried out at the pharmaceutical unit of MGACHRC and other required analysis was carried out in the Vasu laboratory, Vadodara, Gujarat, India.

2.1.4 Instruments

Jacketed vessel, weighing machine, mortal and pastel, stainless steel vessels, cotton cloth, *etc*.

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2.1.5 Materials

Table 1: Shows the ingredients, part and quantity used for the preparation of SG

S. No.	Name of drug	Latin name	Part use	Quantity
1	Shatavari	*A. racemosus*	Root	125 g
2	Cow ghee (Goghrut)	-	-	1 liter
3	Cow milk (Godugdha)	-	-	10 liter
4	Water	-	-	4 liter

2.1.6 Preparation method

Table 1 shows the ingredients, part and quantity used for the preparation of SG. Roots of wet *A. racemosus* were collected in specified amount and a paste was prepared. Then, specified amount of cow ghee was placed in jacketed vessel and heated on low temperature. After that, above paste was mixed to lukewarm cow ghee. Then, specified amount of boiled cow milk and water were poured in to the above mixture. After adding water, it was heated to moderate temperature. The mixture was reduced to certain extent each day over a period of two days. Paste of wet *A. racemosus* and milk were converted into condensed milk (mava) like consistency gradually. After 2 days, the paste gets separated from cow ghee, and then a confirmatory test was done. On 3rd day, (Ksheere dviratram-2 nights rest period), SG was heated gently and sifted through clean cloth in its mild warm stage. Then, it was stored in airtight glass container.

All 3 batches were prepared by the same method and the prepared formulation was then subjected to analytical testing.

Wet *Asperagus Racemosus* roots

Paste of *A.racemosus*roots

Cow ghee (Goghrut)

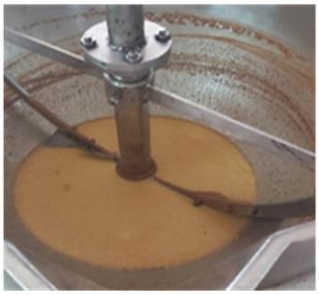
Cow milk (Godugdha)

Heating of cow ghee

Adding of paste

Adding of milk

Adding of water



Stages of lipid preparation (Shatavari Ghrut : SG)

Mava like appearance

Separation of SG

Filtration of SG

No cracking sound observed

Temperature

Prepared SG

Figure 1: Showing images of preparation method of SG.

3. Results

3.1 Pharmaceutical study

During preparation of SG, temperature was recorded after every 60 min and on second day during preparation of SG, temperature was recorded after every 60 min and on second day, during last 2 h temperature was recorded after every 30 min. Colour changes and other observations are recorded which is shown into Table 2. Total duration for preparation, obtained yield, loss of SG during preparation, for all three batches are mentioned in Table 3.

Table 2: Showing changes observed in the SG (Day 1-3)

Day	Time	Temperature	Observation	Colour
Day-1	11.00 am	40°C	Odour of cow ghee	Golden yellow
	11.05 am	40°C	Added paste of *A. racemosus* roots	Brownish yellow
	11.10 am	45°C	Added milk	Whitish yellow
	11.15 am	45°C	Added water	Whitish yellow
	12.00 pm	50°C	Odour of cow ghee reduced	Whitish yellow
	01.00 pm	50°C	Paste is mixed properly	Off white
	02.00 pm	50°C	Slight white creamy part observed	Off white
	03.00 pm	55°C	Boiling started	Off white
	04.00 pm	55°C	Boiling	Off white
Day-2	10.00 am	40°C	White creamy part observed	Beige colour
	11.00 am	55°C	Boiling started	Beige colour
	12.00 pm	58°C	Thickening of the mixture started	Brownish yellow
	01.00 pm	58°C	Slight bubbles started appearing	Brownish yellow
	02.00 pm	60°C	Bubbles disappeared	Brown
	02.30 pm	55°C	Mava like consistency of paste	Brown
	03.00 pm	55°C	Cow ghee absorbed by paste	Dark brown
	03.30 pm	55°C	Started separation of paste from cow ghee	Golden yellow
	04.00 pm	55°C	Paste separated completely	Golden yellow
	04.20 pm	55°C	Odour, colour of SG observeddisappearance of foam	Golden yellow
Day-3	10.00 am	40°C	Odour, colour of SG observedNo cracking sound when put on fire	Golden yellow
	11.00 am		Filter through cotton cloth	Golden yellow

In this way, same observations were found for all 3 batches, as we had maintained same method of preparation, time duration (10-13 h) and temperature (40^0C-55^0C).

Table 3: Showing pharmaceutical work of SG

Batch	Processing days	Cow ghee	Paste of *A. racemosus*	Cow milk	Water	Duration	Outcome	Difference	% of loss
Batch 1	2 days	1liter	125 g	10 liter	4 liter	11 h, 20 min	650 ml	350 ml	35 %
Batch 2	2 days	1liter	125 g	10 liter	4 liter	11 h, 10 min	660 ml	340 ml	34 %
Batch 3	2 days	1liter	125 g	10 liter	4 liter	11 h, 25 min	640 ml	360 ml	36 %

3.2 Analytical study

Analytical study of all three batches of SG was done. In the organoleptic evaluation of SG, all three samples were found to be yellow colour with pungent taste and had characteristic odor of *A. racemosus*. In physicochemical analysis, moisture content, pH, specific gravity, viscosity, acid value, saponification value, refractive index, iodine value, peroxide value, rancidity test were carried out and average value was calculated. The results of the organoleptic and physicochemical analysis are tabulated in Table 4 and Table 5. In instrumental analysis, HPTLC was done. Calculate quantification of Shatavarin IV by HPTLC which is tabulated in Table 6. Microbial analysis of all the three batches of SG showed total plate count as <10 cfu/g, yeast and molds counts and total coliform were reported as absent. The pathogenic bacteria, *Salmonella* sp, *Escherichia coli, Aureus, Vibrio cholera* were found to be absent in the test samples of formulation (Table 7). Qualitative phytochemical analysis of SG showed the presence of phytoconstituents like steroids, flavonoids, alkaloids, saponins, glycosides, tannins, amino acids, proteins and sugar. The results are presented in Table 8.

3.2.1 Organoleptic characteristics

Table 4: Showing organoleptic characteristics

S.No.	Parameters	Batch 1	Batch 2	Batch 3
1	Colour	Yellow	Yellow	Yellow
2	Odour	Characteristic	Characteristic	Characteristic
3	Taste	Pungent	Pungent	Pungent

3.2.2 Physicochemical analysis

Table 5: Physicochemical analysis

S.No.	Parameters	Batch 1	Batch 2	Batch 3	Average
1	Moisture content	0.62 %	0.61 %	0.62 %	0.61 %
2	pH	6.24	6.21	6.32	6.25
3	Specific gravity	0.908	0.910	0.907	0.908
4	Viscosity by oswald	17.65 cp	17.47 cp	17.83 cp	17.52 cp
5	Acid value	0.77	0.81	0.83	0.80
6	Saponification value	233.84	231.28	232.65	232.59
7	Refractive index	1.466	1.466	1.467	1.466
8	Iodine value	2.030	2.029	2.031	2.03
9	Peroxide value	6	7	8	6
10	Rancidity test	Negative	Negative	Negative	Negative

3.2.3 Instrumental analysis

Table 6: Instrumental analysis

S.No.	Shatavarin IV by HPTLC	Result
1	Batch 1	0.015 %
2	Batch 2	0.015 %
3	Batch 3	0.014 %
	Average	0.015 %

3.2.4 Microbial analysis

Table 7: Microbial analysis

S.No.	Parameters	Batch 1	Batch 2	Batch 3
1	Total microbial plate count	11 cfu/g	10 cfu/g	10 cfu/g
2	Total yeast and mould count	Absent	Absent	Absent
3	Total coliform	Absent	Absent	Absent
4	*Salmonella* sp.	Absent	Absent	Absent
5	*Escherichia coli*	Absent	Absent	Absent
6	*S. aureus*	Absent	Absent	Absent
7	Vibro cholera	Absent	Absent	Absent

3.2.5 Phytochemical screening

The presence of several primary and secondary metabolites was assessed in SG by a variety of phytochemical tests conducted in accordance with established protocols.

Table 8: Phytochemical analysis

S. No.	Parameters	Test name	Batch 1	Batch 2	Batch 3
1	Steroids	Salkowski test	+	+	+
2	Flavonoids	Ferric chloride reagent test	+	+	+
3	Alkaloids	Dragendorff test	+	+	+
4	Saponins	foam test	+	+	+
5	Glycosides	keller-kiliani test	+	+	+
6	Tannins	Ferric chloride test	+	+	+
7	Amino acids	Ninhydrin test	+	+	+
8	Proteins	Millons test	+	+	+
9	Sugar	Molisch test, Barfoeds test	+	+	+

4. Discussion

All the ingredients of SG were procured from local market. The standard ghee preparation was carried out as per API. According to Bhaishajya ratnavali text, the lipid preparation along with milk requires two days. After two days, prepared mixture was reduced and converted into mava like consistency. Later on, cows ghee was separated from paste and attains disappearance of foam (Phena shanti). Finally, on 3rd day SG was heated gently and filtered through clean cloth by squeezing it in warm stage to attain maximum yield. All the three batches are prepared by same method. Obtained volume of the SG was 650 ml (average weight), the loss during preparation was 350 ml (average loss), because of 2 days processing period and the absorption of cow ghee by paste of *A. racemosus*. The color of SG was golden yellow in colour (Bhokardankar *et al*., 2019).

Evaluation of the lipid standard parameters was the goal of the analytical investigation. The organoleptic character shows yellow colour of SG which is due the presence of cow ghee. Odor was strong and taste was pungent in nature. The physicochemical parameters shows about 0.61% of moisture content which indicates the less chance of deteriorate. The average pH of all three SG was 6.25 which was acidic in nature (Dutta and Sengupta, 2018).

The expected modification of the specific gravity in lipid (Sneha) due to the presence of dissolved substances makes it a significant parameter for the analysis of medicated lipid preparations. This is valuable for acquiring molecular information through a non-invasive approach. The data indicates that the average specific gravity of SG is 0.908, revealing the presence of active constituents. Viscosity, denoting the resistance of a fluid (whether liquid or gas) to changes in shape or movement of adjacent portions, reflects its resistance to flow. The average viscosity of SG is recorded at 17.52 cp.

The rancidity of the compound is attributed to the presence of free fatty acids. A higher concentration of free fatty acids leads to increased rancidity in ghee, while a stable number of fatty acids mitigates rancidity. The edibility of a fat is inversely correlated with its acid number. The acid value of SG is 0.80, indicating the presence of free acids and serving as an indicator of the rancid state. Rancidity induces the liberation of free acids. Medicated ghee with a high saponification value exhibits enhanced absorption and allows for the determination of molecular size, which is inversely proportional to the molecular weight of fat. A high saponification value suggests the presence of low molecular weight fatty acids in a simple form, while a low saponification value implies complex molecular structures. This value tends to be elevated in fats containing short-chain fatty acids.

The amount of alkali required to saponify a given quantity of fat depends on the presence of COOH groups. Therefore, fats containing short-chain fatty acids will absorb more -COOH groups per gram than those with long-chain fatty acids, resulting in a higher demand for alkali and, consequently, a higher saponification number. The average saponification value of SG is 232.59, indicating increased

stability. Refractive index measurement is valuable for both qualitative and quantitative analyses, as well as structural studies, representing an inherent property of a substance. It is instrumental in determining the identity, purity, and consistency of a chemical. In the context of lipid preparations (snehas), refractive index emerges as a pivotal parameter for differentiation. SG has a refractive index of 1.466.

The iodine number serves as a gauge for the degree of unsaturation in fat, signaling the presence of unsaturated fatty acid bonds. A higher iodine number implies greater reactivity, reduced stability, and heightened susceptibility to oxidation. SG's low iodine number of 2.03 suggests minimal risk of rancidity and overall stability. Peroxide value measures the peroxides in ghee, reflecting the percentage of oxidation and indicating sample stability. A higher peroxide value indicates increased oxidation and a greater likelihood of rancidity; SG's peroxide value of 6 points to stability and the absence of oxidation. Rancidity, a key factor in oxidation, influences the shelf-life of ghee. SG, devoid of oxidation, demonstrates stability. High-performance thin-layer chromatography (HPTLC) analysis, a cost-effective and efficient tool, was utilized for quantifying Shatavarin IV in this study, revealing a concentration of 0.015%.

The outcomes of the diverse phytochemical analyses conducted on SG indicate the predominant presence of steroids (confirmed by the Salkowski test), flavonoids (evident through the ferric chloride reagent test), alkaloids (identified using the Dragendorff test), saponins (verified by the foam test), glycosides (as determined by the Keller-Kiliani Test), tannins (confirmed with the ferric chloride test), amino acids (revealed by the ninhydrin test), proteins (detected through the Millons test), and sugars (established by the Molisch test and Barfoeds test) (Gohel *et al*., 2015; Golap *et al*., 2015; Huddar, 2014; Pal *et al*., 2020; Rahman *et al*., 2021; Warrier, 2021).

5. Conclusion

The authentic raw ingredients for research formulation are widely and readily accessible. There are no preparation-related pharmacological restrictions. The analytical values obtained by this study can be considered as preparatory benchmark for SG. The various parameters of three batches like moisture content, pH, specific gravity, viscosity, acid value, saponification value, refractive index, rancidity, HPTLC and microbial analysis was done. Analytical studies including HPTLC have helped to produce preparatory standard for SG. HPTLC shows shatavarin IV present in SG which is responsible for its pharmacological action. Due to use of jacketed vessel temperature was maintained throughout the procedure, which was helpful to preserve important phytoconstituent and obtained good yield. Microbial analysis shows no microbial growth as per shelf-life of lipid formulation. The analysis of the values suggests that the formulation falls within the acceptable range of standard parameters. This indicates that the preparation was conducted in a validated manner, meeting established standards. Thus, from the present study, it was observed that the prepared SG has optimum standards. The provided data serves as a foundation for additional investigations and studies to gain deeper insights into the characteristics and properties of the formulation.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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