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RESEARCH ARTICLE

Comparative Analytical Study of *Shatavari Ghrut* Prepared by Two Different Rules of *Snehapak*: A Quantitative Estimation of *Shatavarin* IV

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Abstract

Background: *Sneha Kalpana* is an important dosage form in *Ayurveda* known for treating various diseases. *Sneha Kalpana* involves extracting fat and transforming water-soluble active components from simple ingredients into a fatty substance known as *Sneha*. There are many rules mentioned for *Sneha* preparation, which include an essential guideline of adhering to a specific time duration for the *Snehapaka* process. This duration is not fixed and varies depending on the nature and constituents of the *Dravya* (ingredients) incorporated along with the *Sneha.* **Aim**: To prepare *Shatavari Ghrut* adhering to both the rules, develop SOP and then analyse its physicochemical properties, quantitative High-Performance Thin Layer Chromatography (HPTLC) analysis and microbial counts. **Method:** *Shatavari Ghrut* is one of the formulations which is broadly used in *Ayurveda* practice. In this formulation milk is used as liquid media and also the roots of *Shatavari* (*Asparagus racemossus* wild) are used. According to the time duration of *snehapak*, two rules are applicable here, which are, *Sneha* is prepared using milk for a duration of 2 days and *Sneha* is prepared using *Mool* (Roots) for 12 days, so accordingly *Shatavari Ghrut* 1 (SG1) is prepared in 2 days and *Shatavari Ghrut* 2 (SG2) is prepared in 12 days. **Result:** The physicochemical parameters suggest that both the SG1 (2 days) and SG2 (12 days) exhibit similar characteristics with minor variations, the HPTLC fingerprinting results for both the SG1 and SG2 samples observe that *Shatavarin* IV percentage is higher in SG2 (12 days) than SG1 (2 days). **Conclusion:** The higher concentration of *Shatavarin* IV in the SG2 (12 days) indicates that prolonged processing enhances the extraction or synthesis of this bioactive compound in *Shatavari Ghrut*. So SG2 may have better therapeutic efficacy and higher stability than SG1.

Keywords: Duration of *Snehapak,* Physicochemical Analysis, *Sneha Kalpana,* Stability

1. Introduction

Rasashastra and *Bhaishajya Kalpana*, is a distinctive branch of *Ayurveda*, specialising in herbal, mineral and herbal-mineral formulations, with *Sneha Kalpana* being a significant aspect. *Sneha* is derived from sources like plants (*Sthavara*) such as *Taila* and animals (*Jangama*) like *Vasa, Majja* and *Ghrut. Ghrut Taila, Vasa* and *Majja* are the four types of *Sneha*[1]. Among these four types of *Sneha, Ghrut* stands out due to its unique properties[2]. *Ghrut Kalpana* represents a category of formulations primarily designed to absorb both fat-soluble and water-soluble phytoconstituents from raw materials into *Ghrut*, which is recognised as an excellent base. *Ghrut Kalpana* is renowned for its diverse therapeutic effects, including properties such as *Rasayana* (rejuvenation) and *Medhya* (intellect-promoting). In the context of preparing *Ghrut Kalpana*, *Acharyas* have detailed various methods and techniques for *Sneha* preparation. Based on the detailed preparatory rules mentioned in the *Bhaishjya Ratnawali*, it is evident that the duration of the *Paka* (cooking) period varies depending on the characteristics

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and components of the *Dravya* (ingredients) combined with *Sneha* (fatty material)[3]. Additionally, there is a specific mention of the *viramkal* (rest period), which indicates that after heating the mixture for a certain time each day, it is allowed to stand overnight. It is stated that whenever *Sneha* is prepared using milk it should be prepared for 2 days and whenever *Sneha i*s prepared using *Mool* (roots) it should be prepared for 12 days, inclusive of the *viramkal*. When preparing *Shatavari Ghrut*, incorporating both *Shatavari* roots and milk as ingredients, it is possible to apply both rules. Conducting a comparative study on the efficacy of *Shatavari Ghrut* prepared using both the 2-day and 12-day *Snehapaka* rules, would be valuable in the pharmaceutical field. An analytical study for the standardisation of *Shatavari Ghrut* prepared by applying both the rules was conducted according to the organoleptic characteristics outlined in the classical texts, as well as modern analytical parameters.

2. Materials and Methods

2.1 Pharmaceutical Study

2.1.1 Ingredients

The ingredients (*Ayurvedic* name, scientific name and the morphological part) used for the preparation of SG including the quantity are shown in Table 1.

Table 1. Showing the ingredients, parts and quantity used for the preparation of SG

Sr. No.	Drug Name	Latin Name	Part Use	Proportion
1	*Shatavari*	*A. racemosus*	Root	125 g
2	*Goghrut* (Cow Ghee)	-	-	1 litre
3	*Godugdha* (Cow Milk)	-	-	10 litre
4	Water	-	-	4 litre

2.1.2 Preparation Methods

2.1.2.1 Preparation of Shatavari Ghrut (SG-1) Using 2-Days Sneha Paka[4]

Ghrut was taken in a vessel in the given proportion and heated over mild temperature. Then, the *Shatavarimool kalka* (Paste of *Shatavari* roots) was added to warm and molten *Ghrut*. The prescribed proportion of *Godugdha* (cow milk) was added to the mixture. Further water in the prescribed proportion was also added to the mixture and the mixture was heated at a mild temperature and reduced to some extent daily for 2 days. After 2 days *Sneha Siddhi lakshan* (a sign of completion) was observed. At that time the heating was stopped and then *Ghrut* was filtered through a clean cloth at its mild warm stage. Then it was stored in an airtight glass container. All 3 batches were prepared by the same method and the mean was calculated. 650ml yield was obtained and 350ml (35%) loss was observed.

2.1.2.2 Preparation of Shatavari Ghrut (SG-2) Using 12-Days Sneha Paka

Ghrut was taken in a vessel in a given proportion and heated over mild temperature. Then, *Shatavarimool kalka* (paste of *Shatavari* roots) was added to warm and molten *Ghrut*. The prescribed proportion of *Godugdha* (cow milk) was added to the mixture. Water, in the prescribed proportion, was added to the mixture and the mixture was heated to a mild temperature and reduced to some extent daily for 12 days. After the 3rd day, 1 litre of water was added to the mixture daily till the 12th day. Every day mild heat was given for 50 to 60 minutes. After 12 days *Sneha Siddhi lakshan* (a sign of completion) was observed. At that time the heating was stopped and then *Ghrut* was filtered through a clean cloth at its mild warm stage. Then it was stored in an airtight glass container. All 3 batches were prepared by the same method and the mean was calculated. 743ml yield was obtained and 256 ml (25%) loss was observed (Figure 1).

By preparing *Shatavari Ghrut* using both the rules, 2-day and 12-day *Sneha Paka* periods, the differences in the final product were observed and the impact of *Sneha Paka* duration on its properties and efficacy was assessed.

2.2 Analytical Study[4]

2.2.1 Organoleptic Characters

Organoleptic evaluation involves using the senses to assess the characteristics of *Shatavari Ghrut*. The parameters analysed typically include colour: the physical form and colour of the *ghrut,* odour: the smell, which should be pleasant and characteristic of *Shatavari,* taste: the flavour, which should be consistent with the ingredients and free from any off-tastes.

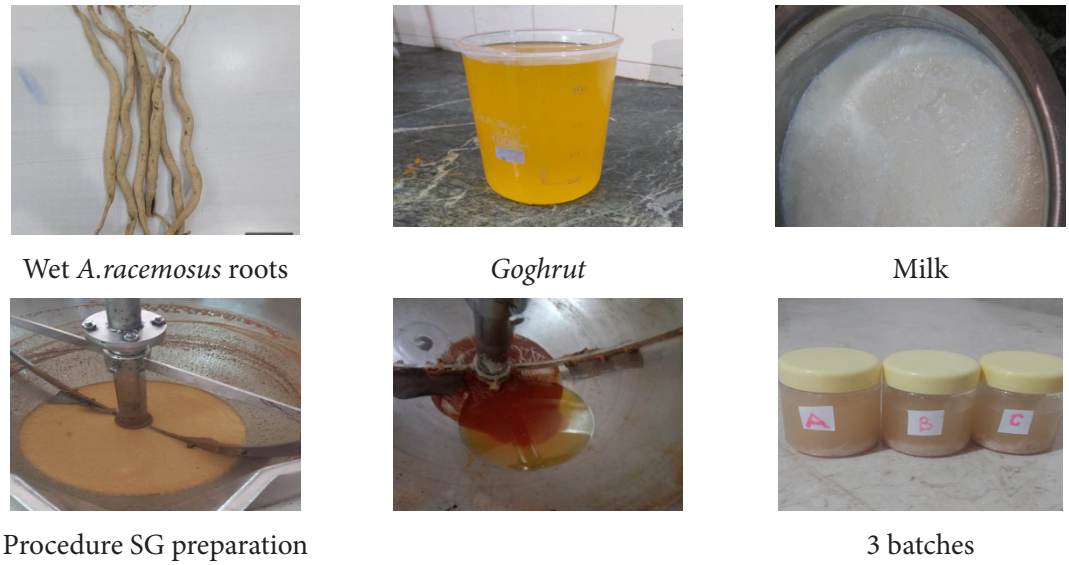


Figure 1. The ingredients and procedure of SG preparation.

2.2.2 Physico-chemical Analysis

The physico-chemical analysis involves assessing the physical and chemical properties of *Shatavari Ghrut*. Parameters analysed include pH - measurement of acidity or alkalinity. moisture - the amount of water present in the *ghrut,* acid value - indicates the free fatty acid content, which reflects the quality and freshness, saponification value - measures the average molecular weight (or chain length) of the fatty acids, iodine value - indicates the degree of unsaturation of the fats, peroxide value - reflects the extent of lipid peroxidation, which affects the stability and shelf life, refractive index - helps determine the purity and concentration.

2.2.3 Instrumental Analysis - HPTLC

HPTLC is used to identify and quantify the *Shatavain* IV % in *Shatavari Ghrut*.

2.3 Method of HPTLC

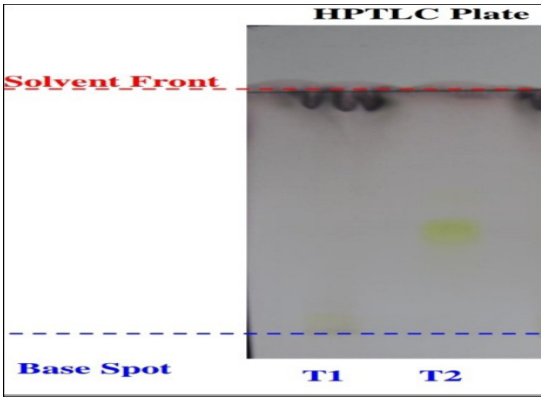
2.3.1 Preparation of Test Solution

Weigh a 10 gm sample of each *Ghrut* in an iodine flask and add 50ml methanol to each Flask. Then after reflux for 30 minutes take each *Ghrut* sample and centrifuge each sample for 10 minutes. Then take the methanol layer of each *Ghrut* sample in an evaporating porcelain dish and evaporate till the residue is obtained. Dissolve the residue of each *Ghrut* with 2ml methanol. Filter the samples with a Syringe filter (0.22 microns). Use the test solution thus obtained for HPTLC quantification. The chromatographic conditions are shown in Table 2.

Table 2. Chromatographic conditions

Application Mode	**CAMAG Linomat 5 Applicator**
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica Gel 60 F254 on Aluminum Sheets
Application (Y-Axis) Start Position 10mm	
Development End Position	80mm from plate base
Sample Application Volume	µL (*Shatavarin* IV) 10µL (each *Shatavari Ghrut*)
Distance Between Tracks	14.0mm
Development Mode	CAMAG TLC Twin Through Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Chloroform: Methanol (7:3v/v)
Visualisation	@429nm (after derivatisation)
Spray Reagent	Anisaldehyde Sulphuric Acid Reagent
Derivatisation Mode	CAMAG-Dip tank for about 1 minute
Drying Mode, Temperature and Time	TLC Plate Heater Preheated 100± 50C for 3 minutes

HPTLC Plate-SG1



HPTLC Plate –SG2

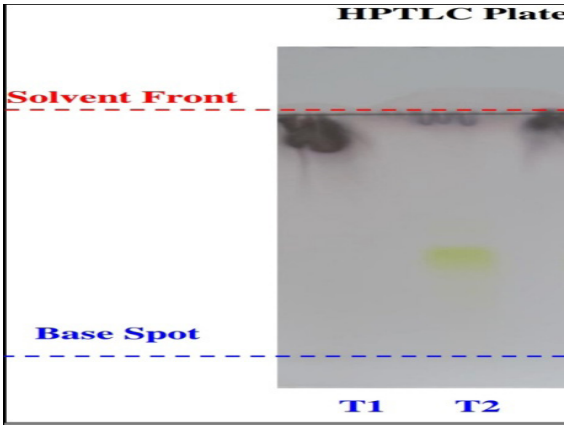
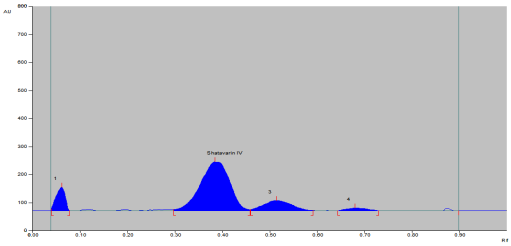
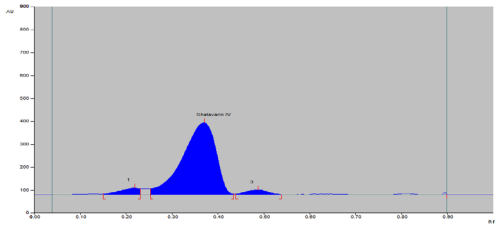


Figure 2. 3D chromatogram of SG1 and SG2.



Chromatogram of SG1



Chromatogram of SG2

Figure 3. 3D chromatogram of SG1 and SG2.

2.3.2 *Microbiological Analysis*

Microbiological analysis ensures the safety and hygiene of *Shatavari Ghrut* by checking for the presence of microorganisms. Parameters include Total Plate Count (TPC): total number of viable microorganisms, yeast and mold count: Presence of fungi, pathogen testing: specific tests for harmful bacteria like *E. coli, Salmonella, Staphylococcus aureus*, etc. Sterility testing: ensuring the product is free from microbial contamination.

2.3.3 *Phytochemical Analysis*

Phytochemical analysis identifies and quantifies the bioactive compounds in *Shatavari Ghrita*. Parameters include alkaloids: presence and concentration of alkaloids, flavonoids: important for antioxidant properties, glycosides: detection of these compounds which have therapeutic effects, saponins: measured for their role in immune modulation and other health benefits, tannins: presence of these compounds which have astringent properties, phenolic compounds: essential for their antioxidant activity.

3. Results

3.1 Organoleptic Characteristics of SG1 and SG2

The organoleptic characteristics of *Shatavari Ghrut* can be seen at both 2 days (SG1) and 12 days (SG2). The *Ghrut* remains yellow, has a characteristic odour, and maintains a pungent taste (Table 3).

Table 3. Organoleptic characteristics of SG1 and SG2

Sr. No.	Parameters	SG1 (2 days)	SG2 (12 days)
1	Colour	Yellow	Yellow
2	Odor	Characteristic	Characteristic
3	Taste	Pungent	Pungent

3.2 Physicochemical Analysis of SG1 and SG2

The physicochemical characteristics of *Shatavari Ghrut* demonstrate consistent stability over 12 days. The moisture content shows a minimal decrease from 0.62% to 0.61% and the pH slightly increases from 6.24 to 6.32, maintaining near-neutral conditions.

The specific gravity increases marginally from 0.908 to 0.916 and viscosity rises from 17.65cp to 18.58cp, indicating slight thickening. The acid value decreases slightly from 0.77 to 0.76 and the saponification value increases from 233.84 to 234.79, showing consistent fat content. The refractive index remains nearly unchanged at around 1.466-1.467, suggesting stable purity and concentration. The iodine value exhibits minimal change, remaining stable around 2.030-2.028 and the peroxide value decreases from 6 to 5, indicating reduced lipid peroxidation and good preservation. The rancidity test remains negative throughout, confirming good shelf stability and quality of the product over time (Table 4).

Table 4. Physicochemical analysis of SG1 and SG2

Sr. No.	Parameters	SG1 (2Days)	SG2 (12Days)
1	Moisture Content	0.62%	0.61%
2	pH	6.24	6.32
3	Specific Gravity	0.908	0.916
4	Viscosity by Oswald	17.65cp	18.58cp
5	Acid Value	0.77	0.76
6	Saponification Value	233.84	234.79
7	Refractive Index	1.466	1.467
8	Iodine Value	2.030	2.028
9	Peroxide Value	6	5
10	Rancidity Test	Negative	Negative

3.3 HPTLC

The HPTLC analysis of *Shatavari Ghruta* shows an increase in *Shatavarin* IV from 0.015% at SG1 (2 days) to 0.021% at SG2 (12 days) (Table 5, Figures 2 and 3).

Table 5. Instrumental analysis – HPTLC of SG1 and SG2

Parameters	Standard *Shatavarin* IV	SG1
Weight	1mg	1004.2mg
R_f Value	0.42	0.42
AUC	60436.7	8806.8
% *Shatavarin* IV	-	0.015 %
Parameters	Standard *Shatavarin* IV	SG2
Weight	1mg	1070.0mg
R_f Value	0.42	0.42
AUC	57892.9	5523.9
% *Shatavarin* IV	-	0.021 %

3.4 Microbiological Analysis

The microbiological analysis of *Shatavari Ghrut* reveals consistently low microbial counts and the absence of harmful pathogens over 12 days. Initially, at 2 days (SG1), the total microbial plate count was 11cfu/g, which decreased to less than 10cfu/g by 12 days (SG2), indicating good microbial stability and quality control measures during production. Yeast and mould were absent in both SG1 and SG2 samples, ensuring fungal safety. Additionally, tests for coliform bacteria, *Salmonella* sp., *E. coli, S. aureus* and *V. cholerae* were all negative in both samples, confirming the absence of these potentially harmful organisms. These results underscore the high standards of hygiene and processing efficacy maintained in the production of *Shatavari Ghrut*, ensuring its safety for consumption (Table 6).

Table 6. Microbiological analysis of SG1 and SG2

Sr. No.	Parameters	SG1 (2 days)	SG2 (12 days)
1	Total microbial plate count	11cfu/g	< 10cfu/g
2	Total yeast and mould count	Absent	Absent
3	Total coliform	Absent	Absent
4	*Salmonella* sp.	Absent	Absent
5	*Escherichia coli*	Absent	Absent
6	*Staphylococcus aureus*	Absent	Absent
7	*Vibro cholera*	Absent	Absent

3.5 Phytochemical Analysis

Shatavari Ghruta's phytochemical analysis shows a dynamic profile across two stages, 2 days and 12 days. Steroids, flavonoids, alkaloids, glycosides, amino acids and proteins were consistently detected in both water and alcohol extracts throughout, with some compounds showing increased presence over time. Saponins were initially present in alcohol extracts but absent later, while tannins were stable in water extracts but absent in alcohol. Sugars were absent in water at first but present in alcohol, becoming detectable in both by 12 days (Table 7).

Table 7. Phytochemical analysis of SG1 and SG2

S. No.	Parameters	Test Name	SG1 (2 days)		SG2 (12 days)	
			Water	Alcohol	Water	Alcohol
1	Steroids	Salkowski test	+	-	+	+
2	Flavonoids	Ferric Chloride reagent test	+	-	+	+
3	Alkaloids	Dragendorff test	+	-	+	+
4	Saponins	Foam test	-	+	-	+
5	Glycosides	Keller-Kilian test	+	+	+	+
6	Tannins	Ferric Chloride test	+	-	+	-
7	Amino acids	Ninhydrin test	+	-	+	+
8	Proteins	Millions test	+	-	+	+
9	Sugar	Molisch test, Barfoeds test	-	+	+	+

3.6 Statistical Observations

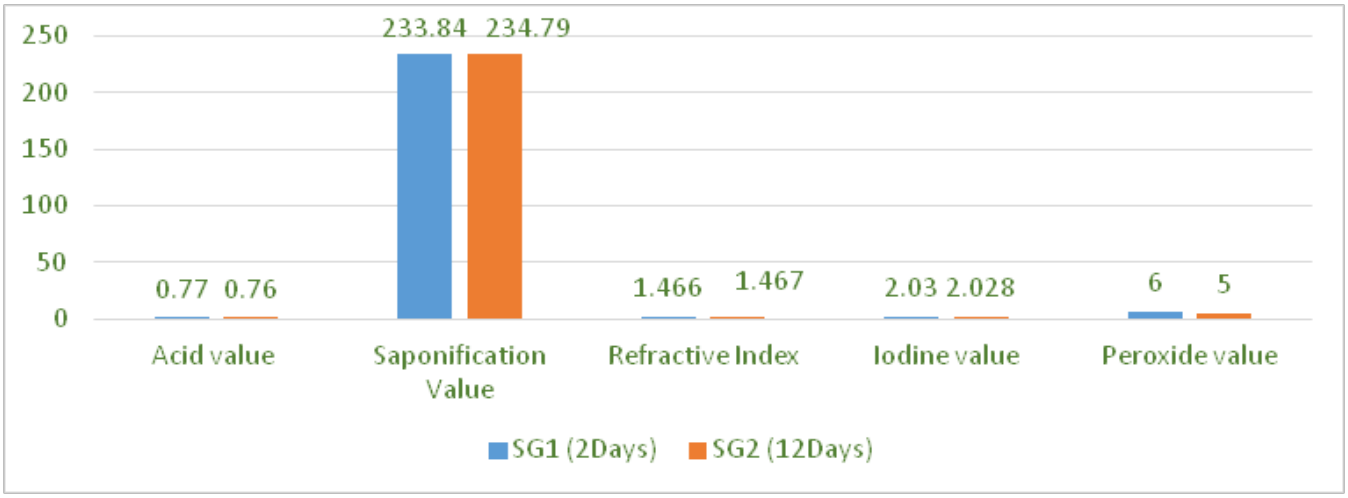


Figure 4. Comparison of physicochemical analysis of SG1 and SG2.

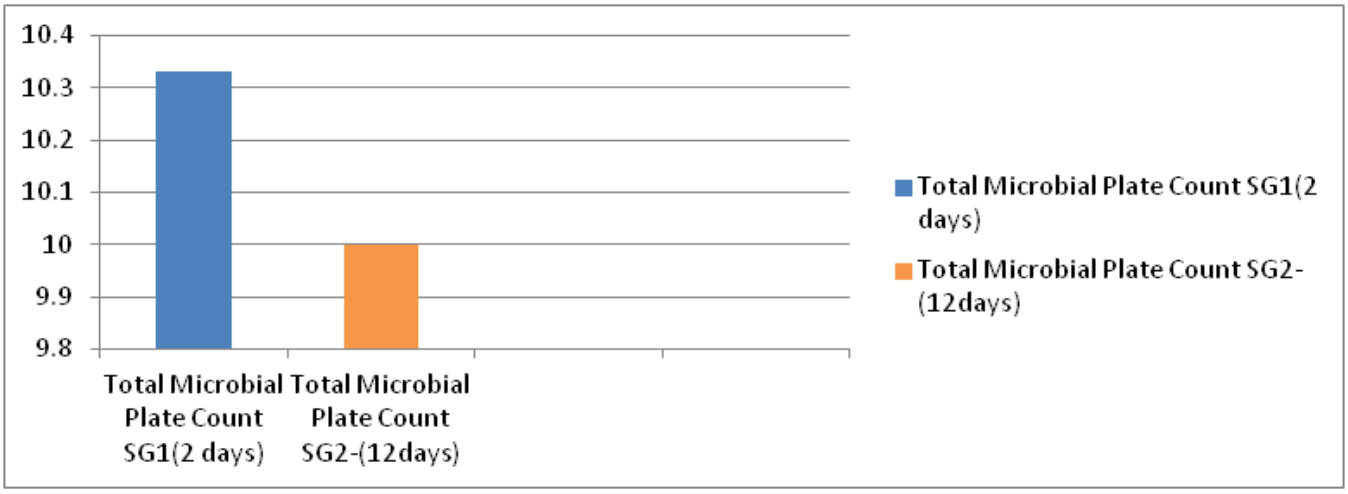


Figure 5. Comparison of microbial count comparison of SG1 and SG2.

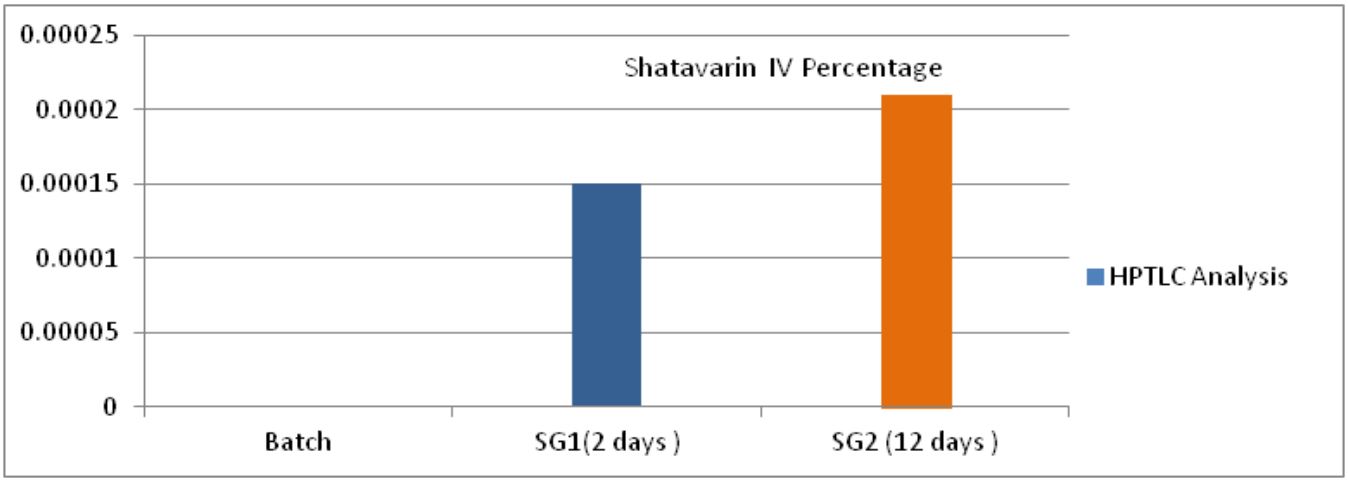


Figure 6. Comparison of HPTLC analysis (quantification of *Shatavarin* IV) of SG1 and SG2.

4. Discussion

Shatavari, an ancient medicinal plant, has been utilised for its therapeutic properties since ancient times[5]. Its medicinal usage spans various texts, including the *Sharangdhar Samhita*. Numerous formulations incorporating *Shatavari* have been documented, with *Ghrut* formulations being particularly prevalent[6].

Shatavari is employed in diverse dosage forms such as *Gutika, Churna, Ghrut* etc, in classical texts. *Ghrut* formulations, in particular, have been extensively utilised. *Shatavari Ghrut*, a formulation classified under *snehapak kalpana*, finds widespread application in managing conditions like *Vatarakta*[7], *Rasayana, Vrushya*[8] and *Yoniroga*, owing to its unique composition comprising lipid-soluble aglycone and water-soluble sugar chains within steroidal saponins.

Ayurvedic classics such as *Charaka Samhita, Sushruta Samhita* and *Ashtanga Hridaya* meticulously detail the manufacturing processes of medicated *taila* and *ghrut* (oil/ghee). However, *Sharangdhar Samhita* stands out as a comprehensive guide for pharmaceutical details of various herbal dosage forms. *Bhaishjya Ratnawali* also provides elaborate descriptions of *snehpak* rules.

One significant aspect studied is the *viramkal* (Rest Period), crucial in the preparation of medicated oils and ghee. The duration of the *Paka* (cooking time) differs depending on the characteristics and components of the ingredients combined with *Sneha.* For example, if *Sneha* is made using milk, it should be prepared for 2 days, while when prepared using roots *(Mool),* it should undergo preparation for 12 days. This implies that the *Snehapak* process should last for 12 days, with the mixture heated for a certain period each day and allowed to stand overnight.

In specific formulations like *Shatavari Ghrut*, both these rules find application since *Shatavari* roots and milk are used as ingredients. Thus, the present study aims to validate these rules in the preparation of *Shatavari Ghrut*. Both samples of *Shatavari Ghrut* are also subject to analytical profiling and quantitative estimation of *Shatavarin* IV.

On observing the result of organoleptic characteristics of both batches SG1 (2 days) and SG2 (12 days), no variations (Table 4) were found. It might be possible because the base used for both the *ghrut* preparations is the same[9]. Thus, there were no variations observed in organoleptic characteristics such as colour and taste between SG1 and SG2.

Physico-chemical parameters showed very minimum variations in both batches SG1 (2 days) and SG2 (12 days). Moisture content was 0.62% for SG1 and 0.61% for SG2, which were within normal limits. pH observed was 6.24 for SG1 and 6.32 for SG2, It indicates both samples of SG were weak acidic in nature[10]. The specific gravity of SG was 0.908 for SG1 and 916 for SG2, which indicated active constituents present in SG. The average viscosity of SG was 17.65 viscosity for SG1 and 18.58 for SG2 which denotes opposition to flow. The acid values were 0.77 for SG1 and 0.76 for SG2 which signifies the presence of free fatty acids and indicates

the rancid state. The saponification value of SG was 233.84 for SG1 and 234.79 for SG2. With this, it can be understood that SG has more stability. Medicated ghee with high saponification value has better absorption. The refractive index of SG was 1.466, for SG1 and 1.467 for SG2. It was used to determine the identity and purity of a chemical. The iodine values of SG1 were 2.030 and 2.028 for SG2 indicating less chances of rancidity and otherwise also indicating more stability of SG. The peroxide value of SG was 6 for SG1 and 5 for SG2 5, which indicates that SG2 has more stability. Rancidity determines the level of oxidation and helps to determine the shelf life of *ghrut* (Table 4 and Figure 4).

HPTLC is a major advanced technique of TLC. HPTLC was performed for normal phase separation of components of the product[11]. Quantitative HPTLC fingerprinting was done for both 2 days and 12 days of *Shatavari Ghrut*. The sample drug contains *Shatavarin* IV which is found in *Shatavari* tuber. In SG1, *Shatavarin* IV is present at 0.015%, with absorbance at 254nm, an R_f value of 0.42, and an AUC of 8806.8. In SG2 (12 days), the content of *Shatavarin* IV increases to 0.021%, with absorbance at 254nm, an R_f value of 0.42, and an AUC of 15523.9 (Table 5). It is observed that in HPTLC *Shatavarin* IV percentage is more in SG2 (12 days) than in SG1 (2 days) (Table 5 and Figure 6). HPTLC is a method employed to separate, identify and quantify components within complex mixtures. In the case of *Shatavari Ghrut* samples, HPTLC fingerprinting was performed to analyse the presence and concentration of *Shatavarin* IV, a bioactive compound found in *Shatavari* tuber. Here's a description of the HPTLC fingerprinting results for both the SG1 and SG2 samples but it is observed that the *Shatavarin* IV % is higher in SG2(12 days) than in SG1(2 days).

Microbial analysis of SG revealed a total plate count of less than 11cfu/g and 10cfu/g, respectively, with absent yeast and mould counts and total coliform pathogenic bacteria such as *Salmonella* sp, *E. coli, S. aureus* and *V. cholera* were found to be absent in both SG1 and SG2[12] (Table 6 and Figure 5).

The results from the various phytochemical analyses of SG indicated the presence of primary steroids, flavonoids, alkaloids, saponins, glycosides, tannins, amino acids, proteins and sugars. The results exhibited variations in the presence of phytochemical constituents between SG1 and SG2 and between water and alcohol solvents (Table 7). The variations could be due to differences in processing duration and solvent extraction efficiency[13].

5. Conclusion

Standard Operating Procedures (SOP) were developed for SG1 (2 days) and SG2 (12 days) by conducting physicochemical analysis. Analytical parameters were established as per Ayurvedic Pharmacopoeia of India (API).

HPTLC analysis concludes that the percentage of *Shatavarin* IV is higher in the SG2 (12 days) compared to the SG1 (2 days). The higher concentration of *Shatavarin* IV in the SG2 (12 days) indicates that prolonged processing enhances the extraction or synthesis of this bioactive compound in *Shatavari Ghrut*. So SG2 may have better therapeutic efficacy than SG1.

The physicochemical parameters suggest that both SG1 (2 days) and SG2 (12 days) exhibit similar characteristics with minor variations. However, both samples demonstrate stability and suitability for consumption, with SG2 showing slightly better stability in terms of acid value and peroxide value compared to SG1.

All the parameters discussed can indeed serve as valuable identifying tools for the quality assessment of *Shatavari Ghrut*.

6. References

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