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Standardization of *Shatpala Ghrita* w.s.r Pharamaceutico-Analytical and Antioxidant Study

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ABSTRACT:

Sneha kalpana is one of the unique and commonly prescribed *Ayurvedic* dosage form in day to day practice having increase potency, palatability, shelf life etc. Although lots of verities of *Snehas* are described in *Ayurvedic* texts, the most common amongst them are *Taila* & ***Ghrita kalpana***. It is a pharmaceutical process to prepare oleaginous medicament from the substances like ***Kalka, Sneha Dravya* and *Drava Dravya*** in specific proportion by subjecting to unique heating pattern and duration, to fulfil certain parameters according to need of therapeutics (i.e. ***Mridu, Maddhya & Khar***). *Sneha Siddha* (fat soluble) drugs have better pharmacokinetic action in comparison to other dosage forms, because the use of *Ghrita* as a base is presumably to extract or hold lipid soluble active ingredients from the herbal drugs used and these lipid soluble substances readily permeate into the bio membrane of cells due to its lipid nature. The conceptual study suggests that *Shatapala Ghrita* is used to treat *Jwara* mainly *Vata* and *Kapha Dosha* predominant. Standardization is the process of developing and agreeing upon technical standards and provides numerical value which quantifies the parameter and thus denotes the quality of formulations. *Murchana* performed on *Go-Ghrita* makes it a better medium for the solubility of the drug and imparts all the specific properties to the *Ghrita* which can be used to increase the efficacy of the drugs. Hence, it can be inferred that the medicated Ghrita should be prepared by taking the *Murchhita Ghrita* as ingredient rather without *Ghrita Murchhana.* In so many *Ghrita kalpanas*, ***Shatapala Ghrita*** is a ghee based *Ayurvedic* formulation described in *Samhitas* and various *Rasa texts* with different name and slightly changes in contents. It has broad indication such as enlarge spleen (splenomegaly), *Vishamajwara, Mandaagni, Rajyakshma* etc.

Keywords: *Sneha Kalpana, ghrita Kalpana, Sneha dravya ,Ghrita Murcchana ,dravya shatpala ghrita*

INTRODUCTION

Ayurveda, the science of life, uses natural resources to fulfil the fundamental objectives i.e. *Swasthaya Rakshanama* and *Vikara Prashamana* [1]. Ayurveda has given greatest emphasis to comprehensive knowledge and judicious use of drugs. The science of manufacturing drugs

is divided into two branches viz. *Rasa Shastra & Bhaishajya Kalpana*. *Bhaishajya Kalpana* comprises of two words- Bhaishajya and Kalpana. Bhaishajya means drug and Kalpana means preparation. *Bhaishajya* in accordance with time and requirement has flourished with different modulations. Kalpana is to carry out the preparation in proper order to enhance the therapeutic potency of the drugs. In *Bhaisajya Kalpana* there are two types of *Kalpana*- Primary *Kalpana* (*Panchavidha Kasaya Kalpana*) and Secondary Kalpana (developed from primary Kalpana). *Panchavidha Kasaya Kalpanas* are the Basic Kalpana viz. *Swarasa, Kalka, Kwatha, Hima & Phanta. Acharya Charaka* has mentioned *Panchvidha Kasaya Kalpana* for the first time2 . These are the basic pharmaceutical preparations described in Ayurvedic Pharmaceutics and used since ancient times in some or other form to treat various diseases. Thus, in this way these different dosage forms are serving human by fulfilling the aim of Ayurveda to keep human being healthy additionally, enhancing the quality of life. The drug having quality to produce Arogya is the best drug as per ancient Acharyas. Keeping this viewpoint in the mind a number of preparations known as secondary *Kalpanas* have been derived from these five basic preparations e.g. *Asavarishta, Lepa, Churna, Vati, Sneha Kalpana*, etc.

Shatapala Ghrita is a *Ghrita* based Ayurvedic formulation described in *Samhitas*[7,8] and various Rasa Shastra texts[9,10] with different name and slightly change in its contents. It has broad indication for a variety of diseases such as enlarged spleen (spleenomegaly), *Vishamajwara, Mandaagni*[11], *Rajyakshma* etc. Therefore, considering the wide array of therapeutic activity of *Shatpala Ghrita*, in the present study it has been decided to standardize and analyze the antioxidant activity.

Need Of Study

Till date no studies have been carried out on this formulation viz. *Shatapala Ghrita*. Therefore, standard parameters are not available for assurance of quality of the *Shatapala Ghrita* formulation. Hence, to establish the quality of the formulations with an eye on standardization physico-chemical evaluation as well as antioxidant activity of the prepared formulation is also necessary. Considering all these facts present study entitled "***A Study on Standardization of Shatapala Ghrita W.S.R to Pharmaceutico-Analytical and Antioxidant Activity***" has been planned.

AIMS AND OBJECTIVES

1-To compile the literary review regarding *Sneha Kalpana* wsr to *Shatapala Ghrita*.
2-To prepare *Shatapala Ghrita* as per textual guidelines.
3-To develop SOP & SMP for *Shatapala Ghrita.*
4-To analyze the prepared samples as per standard parameter laid down in API.
5-To analyze the antioxidant activity of prepared samples of *Shatapala Ghrita*.

MATERIALS & METHODS

The study was carried out in three different stages.
1-Pharmaceutical Study
2-Analytical Study
3-Antioxidant Activity

Collection Of Raw Drug

All the raw materials were procured from the pharmacy of National Institute of Ayurveda, Jaipur. The physical impurities were removed from the herbal drugs, and they were dried and made into a coarse powder to use for the pharmacognostical study. Three samples of *Shatpala Ghrita* were prepared in the Dept. of Rasa Shastra and Bhaishajya Kalpana laboratory, National Institute of Ayurveda, Jaipur. *Shatpala Ghrita* was prepared as per the classical reference of ***Yogaratnakar & Bhaishajya Ratnawali***. A physicochemical analysis and antioxidant study of the final product was conducted at Drug Testing Laboratory, Department of Rasa Shastra and Bhaishajya Kalpana, National Institute of Ayurveda, Jaipur and Ayush approved S.R. LABS Pratapnagar Jaipur.

Method Of Preparation Of *Shatpala Ghrita*

Murcchna Of Ghrita

Preparation of *Ghrita Murchhana* Reference : *Bhaishajya Ratnavali*[12]
Principle : *Sneha Paka*Equipments : Pounding instrument, Grinder, spatula, Thermometer, Gas stove, Stainless steel vessel with 40 ltr capacity(40 cm diameter and 32 cm depth) for Sneha paka, measuring cylinder, clean cotton cloth, PET jar.Table No 1. Showing Ingredients of *Ghrita Murchana*

2 – 7 drugs mentioned in Table no. 1 were cleaned properly and made into coarse powder separately and is added *Matulunga Nimbu Swarasa* (obtained by cleaned-cut pieces of *Matulunga Nimbu* by squeezing it) mixed

properly, finally small quantity of water is added till the mixture become *Kalka* form. *Goghrita* was heated over mild heat till it become moisture free at Goghrita on 140˚C temperature for 30 minutes. After that, *Goghrita* taken out from the heat and subjected to cool at temperature around 70˚C, then added to prepared *Kalka* little by little and stirred well with running hand , after that again heated with adding mentioned water quantity. The heating process was carried till *Sneha Siddhi Lakshana* appeared. Then filtered through double layered muslin cloth at that time when temperature was 80˚C. Final product i.e *Murrchitta Ghrita* (4578 gm with 8.44 % loss) was stored in a clean dry wide mouth PET jar after cooling.(Fig 1)

***Shatpala Ghrita* Preparation**

Reference : *Yogratnakar vishamjwar prakran*[13] .

Materials Table No. 2: Showing Ingredients of *Shatpala Ghrita* for each sample

Murchhita Goghrita was heated over mild heat till it become moisture free.The vessel which was containing *Goghrita* taken out from the heat and allowed to cool temperature around 70˚C, then added prepared *Kalka* little by little and stirred well, after adding the Kalka temperature was around 600C after that again heated, *Kalka Paka* was done for ½ an hour after that mentioned water quantity was added. The temperature falls to 500C when *Godugdh* was added. On heating with observing the boiling mixture for subsidence of froth (*phena-shanti*) and constantly check the *Kalka* for formation of *Varti* (*madhyama paka lakshana*). The varti and Ghrita were exposed to flame for confirming the absence of crackling sound that indicates absence of moisture.When the *Kalka* forms a *varti* and the froth subsides ,eventually heating was stopped then filtered through double layered muslin cloth.

Organoleptic Study

"Organoleptic evaluation" of a drug refers to the evaluation of a drug by Colour, Odour, Size, Shape, Taste and special features including touch, texture etc. with the help of *Gyananendriya*. The obtained results were shown in following tables.

Table No: 3: Showing results of organoleptic characters of *ShatpalaGhrita*

Physicochemical Analysis

Table no. 4: Showing the physicochemical parameters of *Shatpala Ghrita Samples.*

A. Determination Of Viscosity[14]

It is a property of liquids, that measures of its frictional resistance (Resistance to flow). A liquid may consisting of molecular layers arranged one over the other. When a shearing force is applied to a liquid, it flows. However, the forces of friction between the layers offer resistance to this flow.

All fat and oil shows decrease in viscosity with increase in temperature. The viscosity of fats and oils decreases slightly with an increase in unsaturation, therefore the results shows *Shatapala Ghrita*. Possibly having the nearby values of viscosity confirms that the base materials are having the same properties and applications in between.

B. Determination Of Specific Gravity[15]

The specific gravity of a substance (liquid) is the weight of a given volume of that substance at the status temperature as compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air. It is a quality control parameter to check the base difference of *Shatpala Ghrita* samples. Not such any big difference found in specific gravity results of samples.The result were complies with API specifications.

C. Determination Of Refractive Index[16]

The Refractive index (ṅ) of a substance is termed as the ratio of the velocity of light in vacuum or air, to that in the substance. The refractive index is related to the ease with which light passes through the fat. Temperature and degree of saturation affect the value. The data were found in limit for all samples of *Shatpala Ghrita* according to pharmacopieal standards. All values found nearly similar that denotes physically near to similar with each other and composition are very same in between.

D. Determination Of Ph[17]

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gram per litre. The same procedure is applied for 3 samples each time. The result of SG-2 is slightly more acidic in nature as compared to *SG-1,SG-3*.

E. Determination Of Rancidity[18]

Rancidity is a process which is accompanied by the formation of the unpleasant odour, taste and as a result of action of moisture, oxygen of air and enzymes. The result of sample SG-2 shows slightly rancidity of preparations.

F. Determination Of Iodine Value[19]

The number of grams of iodine absorbed by 100 gram of

the sample material when determined by using Wijs solution. The oil/fat sample taken in chloroform is treated with a known excess of iodine mono chloride solution in glacial acetic acid (Wijs Solution). The excess of iodine monocloride is treated with potassium iodide and the liberated iodine estimated by titration with sodium thiosulphate solution. The value of SG-2 was found more than other samples denotes more unsaturation in SG-2 than SG-1 & SG-3.

G. Determination Of Acid Value[20]

It is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. The result of Acid value of all the three samples of *Shatpala Ghrita* under the normal limit (not more than 3) according to Pharmacopoeial standard for Ayurvedic Formulation, CCRAS.

H. Determination Of Peroxide Value[21]

The peroxide value is the number of mill equivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance. The result of all samples of *Shatpala Ghrita* were found quite similar and under the standard limit of Pharmacopoeial standard for Ayurvedic Formulation, CCRAS.

I. Determination Of Saponification Value[22]

The number of milligrams of potassium hydroxide required to neutralize the free acids and saponify the one gram of the sample material. The result of Saponification value of all the three samples of *SG-1,SG-2 & SG-3* nearby to normal limit.

J. Determination Of Ester Value[23]

It is a measure of the saponifiable esters in the material. It is calculated as the difference between the saponification value and the acid value. Ester value denotes the presence of esters other than free fatty acids in the samples and use a quality control parameter for fat containing preparations. The result shows all the samples of *SG-1, SG-2 &SG-3* within the limit and complies with each other.

K. Determination Of Free Fatty Acid[24]

The free fatty acid content is expressed as oleic acid equivalents. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The results were found in similar range that confirms same quantity of fat substance in all the samples.

L. Determination Of Total Fatty Matter

Quantitative estimation of crude fatty substances (Triglycerides, phospholipids, wax ester, sterols) present in the Sample material. The results were found in similar range that confirms in formulation both base represent nearly same quantity of fat substance.

M. Test For Heavy Metal[25]

Heavy metals is a group of tests that measures the quantity of specific potentially toxic metals in ayurvedic/unani/siddha and food stuffs. These heavy metals are lead, cadmium, mercury and arsenic.The procedure used was wet digestion. The Metal content of the sample will be calculated according to the following equation by AAS in ppm unit. *Shatpala Ghrita* samples were free from Heavy metals and it was safe for therapeutic purpose.

N. Test For Afflatoxins[26]

Aflatoxins are closely related group of secondary metabolites shown to be mycotoxin. They are produced by fungus named Aspergillus flavus. There are four types of B1, B2, G1, G2. Aflatoxin residue are highly toxic and causes carcinogenicity. Aflatoxins easily separates and visualize by the application of thin layer chromatographic system with detection at UV 254nm and 366 nm. The result of the Afflatoxins shows that, prepared samples of *Shatpala Ghrita* were free from afflatoxins and it was safe for therapeutic purpose .

O. Microbiological Analysis[27]

i) **Total bacterial count** : Total aerobic bacterial count is the most important test to evaluate of microbial contamination in Ayurvedic formulation and raw material.

ii) **Total fungal count** : Total yeast and mould count is the most important test to evaluate of fungal contamination in herbal medicine and raw material.

The result of microbial analysis of the samples of *Shatpala Ghrita* were found quite similar and under the standard limit of Pharmacopoeial standard for Ayurvedic Formulation, CCRAS.

1. **Phytochemical Screening: Qualitative Test** HPTLC: High performance thin layer chromatography is a sophisticated and automated form of TLC technique. The method is used for separation of the components present in the mixture both qualitatively as well as quantitatively. Thin layer chromatography profile of *Ghrita* (Hexane Extract) was developed by using Benzene and Ethyl acetate in 9:1 ratio as solvent system and the plate were visualized

in UV chamber at long wavelength (366nm). The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize. These days, however, ready-made plates are preferred.(Fig 2) All three samples showing large number of spots which ultimately denotes that samples have large number of active principles. The scanning data shows different spots visualized get confirms the chemical nature and distribution pattern in specified mobile phase. Table No 5

2. Antioxidant Study

Antioxidant activity of particular drug extract can be evaluated by calculating the free radical scavenging activity of that drug using DPPH and spectrophotometer. Sample (*Shatpala Ghrita*) was subjected to solvent extraction using methanol as solvent.

I) **DPPH radical scavenging activity**: Radical scavenging activity of *Shatpala Ghrita* was measured according to the method of Blois.[28] Gallic acid was used as control.Sample-2 was observed significant IC50 Value.(Fig 3)

II) **Superoxide radical scavenging activity** : The superoxide anion scavenging activity was measured based on the described method[29] . Superoxide radicals were generated in a PMS-NADH system by oxidation of NADH and assayed through reduction of NBT. Quercetin was used as the positive control. The decrease in the extent of NBT reduction, measured by the absorbance of the reaction mixture, correlates with the superoxide radical scavenging activity of *Shatpala Ghrita*.(Fig 4)

RESULTS AND DISCUSSIONS

After *paka* the odour of samples lead to aromatic due to addition of *drava dravyas*. All samples are in semiliquid consistency before *Sneha paka* then leads to liquid consistency due to *agni sanyog* for long duration. Viscocity allows easy removal from its packaging, expansion and consistency. The viscosity of SG-1,SG-2,SG-3 were observed as 35.37cP to 37.75 cP which shows that the *ghrita* had a proper flowing consistency. The specific gravity of a sample is expected to alter due to the presence of dissolved substances in the sample. This will allow the user to determine if the test fluid will be heavier or lighter than the standard fluid. Results of physicochemical analysis of *Shatpala ghrita* are detailed in Table 3. Loss on drying, Specific gravity, and Refractive index, are in normal range. . If Saponification value is more than normal range, it indicates lower molecular saturated fatty acids. Higher the iodine value, the less stable will be *Ghrita* and the more susceptible it is to oxidation and free radical production. High iodine value *Ghritas* are prone to oxidation and polymerization and the sample becomes rancid, which decreases the shelf life and stability of the product. If acid value is more, then chances of photo-oxidation and rancidity are more. The obtained values of these tests were found within normal limits in *Shatpala Ghrita*, which indicate good quality of product. In addition, no rancidity was found in the finished product.

The refractive index was nearly to 1.485.The specific gravity of the samples was nearly to 0.9551, which was closer to plain *Ghrita*, for which it was 0.9, showing that the sample was not too dense. The acid value of samples SG-1, SG-2 & SG-3 are 0.05, 0.04, 0.04 respecively. Lesser acid value confirms the greater stability of *Taila.* The peroxide value shows that inspite of cooking with aqueous media there was no oxidative damage of *Shatapala Ghrita* and the final product was more stable. The peroxide value ranges from 3.48 to 3.99.

HPTLC provides qualitative information of the main constituents of the drug. In the present study, methanolic extract of the sample was used and the solventsystem was toluene and ethyl acetate in the ratio 9:1 respectively. For determination of TLC plate methanolic H2SO4 was used. Sample of *Shatpala Ghrita* were analyzed on different wavelengths 254 nm, 366 nm and 510 nm. *SG-2 & SG-3* shows maximum number of spots in visible light.The presence of these spots could be due to the addition of various *Kalka dravya and Dravadravyas*. But due to the absence of any standards, the specific compound could not be separately identified. The Superoxide Radical Scavenging activity was performed using Quercetin as standard, Sample *SG-2* shows antioxidant activity at lower IC 50 as compared to other samples. Super oxide radical scavenging methods, it was found that the radical scavenging activity of methanolic extract of *SG-1,SG-2,SG-3* increase in concentration in dose dependant manner and exhibited significant activity at 4 mg with inhibition concentration 43.80, 32.82, 22.95 & and absorbance value is 0.448, 0.395, 0.453. Sample showed good Super oxide radical scavenging, but SG-1 showed IC 50 significant.

CONCLUSION

1-*Shatapala Ghrita* is mentioned in *Yogratnakara Vaidhya Prabha Visham Jwara Prakarana*, *Bhaishjya Ratnavali* and other relevant Ayurvedic treatise. It is a well-established drug used to treat imbalanced state of *Doshas*

mainly *Vata and Kapha*.

2- The conceptual study suggests that *Shatapala Ghrita* is used to treat *Jwara* mainly *Vata* and *Kapha Dosha* predominant.

3-The pharmaceutical study revealed the SMP and SOP established for *Shatapala Ghrita*. It also concludes the amount of final product viz., *Shatpala Ghrita* is nearly reduced upto 11%- 15% with the temperature range from 50ºC to 90ºC.

4-Murchana performed on Go-Ghrita makes it a better medium for the solubility of the drug and imparts all the specific properties to the Ghrita which can be used to increase the efficacy of the drugs. Hence, it can be inferred that the medicated Ghrita should be prepared by taking the *Murchhita Ghrita* as ingredient rather without *Ghrita Murchhana*.

5-The values obtained for physico-chemical analysis can be used for establishing the standard parameters as standard marker. The results of this study may be used as the reference standards in further research. In the HPTLC profile, SG-2 & SG-3 shows maximum number of spots in visible light under 510 nm. The presence of these spots could be due to the addition of various *Kalka Dravya* and *Drava-Dravyas*.

6-The antioxidant study reveals that IC50 concentration of SG-2 is lower than the other samples in both DPPH & superoxide radical scavenging due to the theory of mass transfer mechanism i.e. the soluble active principles are shifted from *Kalka Dravya* to the *Drava-Dravya* depends on factors like agitation which increases concentration gradient. Though, the groundwork requisites for the standardization of *Shatapala Ghrita* are covered in the current study, additional echoing analysis and investigations are required for the identification of all the active chemical constituents of the test drug to substantiate the clinical efficacy.

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Table No 1. Showing Ingredients of *Ghrita Murchana*

S.No	**Name of drugs**	**English Name**	**Part of use**	**Quantity**	
1.	*Goghrita*	Clarified Butter	--	5 kg	1 part
2.	*Haritaki*	*Terminelia Chebula*	Dried Pericarp	52 gm	1/16 part
3.	*Bibhitaki*	*Terminelia Bellerica*	Dried Pericarp	52 gm	
4.	*Amalaki*	*Embelica Officinalis*	Dried Pericarp	52 gm	
5.	*Musta*	*Cyprus Rotandus*	Rhizome	52 gm	
6.	*Haridra*	*Curcuma Longa*	Rhizome	52 gm	
7.	*Matulanga Swarasa*	*Citrus Medica*	Fresh Fruit juice	52 gm	
8.	Water			20 litr	4 part

Materials Table No. 2: Showing Ingredients of *Shatpala Ghrita* for each sample

S.No	**Name**	**Latin Name**	**Part Use**	**Proportion**	
1.	*Pippali*	*Piper longum*	Fruit	41.66gm	¼ part kalka
2.	*Pippali Moola*	*Piper longum*	Root	41.66gm	¼ part kalka
3.	*Chavya*	*Piper retrofractum*	Root	41.66gm	¼ part kalka
4.	*Chitraka*	*Plumbago zeylancica*	Root Bark	41.66gm	¼ part kalka
5.	*Shunthi*	*Zingiber officinalis*	Rhizome	41.66gm	¼ part kalka
6.	*Saindhava*	*Rock salt*	Salt	41.66gm	¼ part kalka
7.	*Go-Ghrita*	-	Clarified Butter	1 kg	1part-Sneha
8.	*Go-Dugdh*	Cow milk	---	4 kg	4part-Dravya

Table No: 3: Showing results of organoleptic characters of *ShatpalaGhrita*

S. No.	**Organoleptic Character**	**Murchita ghrita**	**SAMPLE-1 (SG-1)**	**SAMPLE-2 (SG-2)**	**SAMPLE-3 (SG-3)**
1.	Colour	Yellow	Light yellow	Light yellow	Light yellow
2.	Odour	Characteristic	Aromatic	Aromatic	Aromatic
3.	Consistency	Semiliquid	Liquid	Liquid	Liquid
4.	Appearance	Semiliquid	Oily Viscous	Oily Liquid	Oily Liquid

Table no. 4: Showing the physicochemical parameters of *Shatpala Ghrita Samples.*

S.NO.	***Parameter***	***SAMPLE-1 (SG-1)***	***SAMPLE-2 (SG-2)***	***SAMPLE-3 (SG-3)***
1	Organoleptic characters • Colour • Odour • Consistency • Appearance	 Light yellow Aromatic Liquid Oily viscous	 Light yellow Aromatic Liquid Oily viscous	 Light yellow Aromatic Liquid Oily viscous
2	Viscosity	37.75 cP	35.37 cP	37.00 cP
3.	Specific Gravity	0.9478	0.9595	0.9550
4.	Refractive Index	1.485	1.480	1.481
5.	pH	4.75	4.10	5.65
6.	Rancidity	Absent	Present	Absent
7	Iodine Value	27.89	31.85	28.23
8	Acid value	0.05	0.04	0.04
9	Peroxide Value	3.99	3.97	3.48
10	Saponification value	297.15	274.96	273.56
11	Ester value	297.10	274.92	273.52
12	Free fatty acid	0.05	0.04	0.04
13	Total fatty matter	97.76	96.03	96.97
14	Heavy metals Lead (Pb) Cadmium (Cd) Arsenic (As) Mercury (Hg)	 BLQ (LOQ 0.1) BLQ (LOQ 0.1) BLQ (LOQ 0.1) BLQ (LOQ 0.1)	 BLQ (LOQ 0.1) BLQ (LOQ 0.1) BLQ (LOQ 0.1) BLQ (LOQ 0.1)	 BLQ (LOQ 0.1) BLQ (LOQ 0.1) BLQ (LOQ 0.1) BLQ (LOQ 0.1)
15	Total Afflatoxins Afflatoxin B1 Afflatoxin B2 Afflatoxin G1 Afflatoxin G2	 BLQ (LOQ 0.008) BLQ (LOQ 0.008) BLQ (LOQ 0.008) BLQ (LOQ 0.008)	 BLQ (LOQ 0.008) BLQ (LOQ 0.008) BLQ (LOQ 0.008) BLQ (LOQ 0.008)	 BLQ (LOQ 0.008) BLQ (LOQ 0.008) BLQ (LOQ 0.008) BLQ (LOQ 0.008)
16	Microbial Analysis Total Bacterial Count Total Funal Count	 <10 <10	 <10 <10	 <10 <10

Table No 5: Showing the results of HPTLC of *Shatapa Ghrita*.

Sample	254 nm	366 nm	510 nm
SG-1	9 spots visualized	7 spots visualized	13 spots visualized
SG-2	11 spots visualized	6 spots visualized	17 spots visualized
SG-3	11 spots visualized	4 spots visualized	13 spots visualized

Fig 1

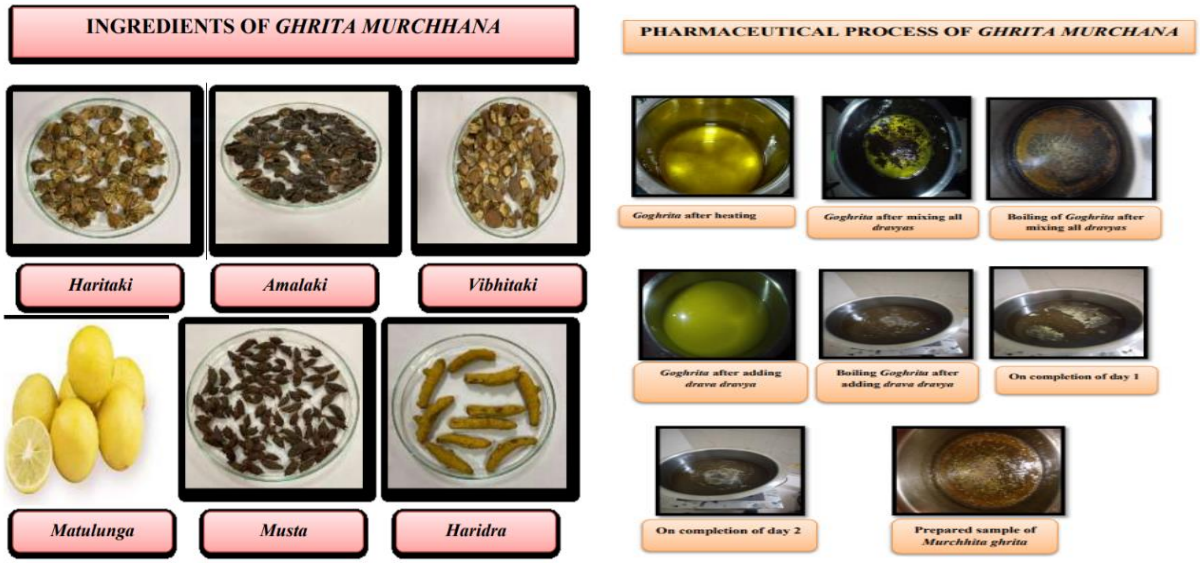


Fig 2

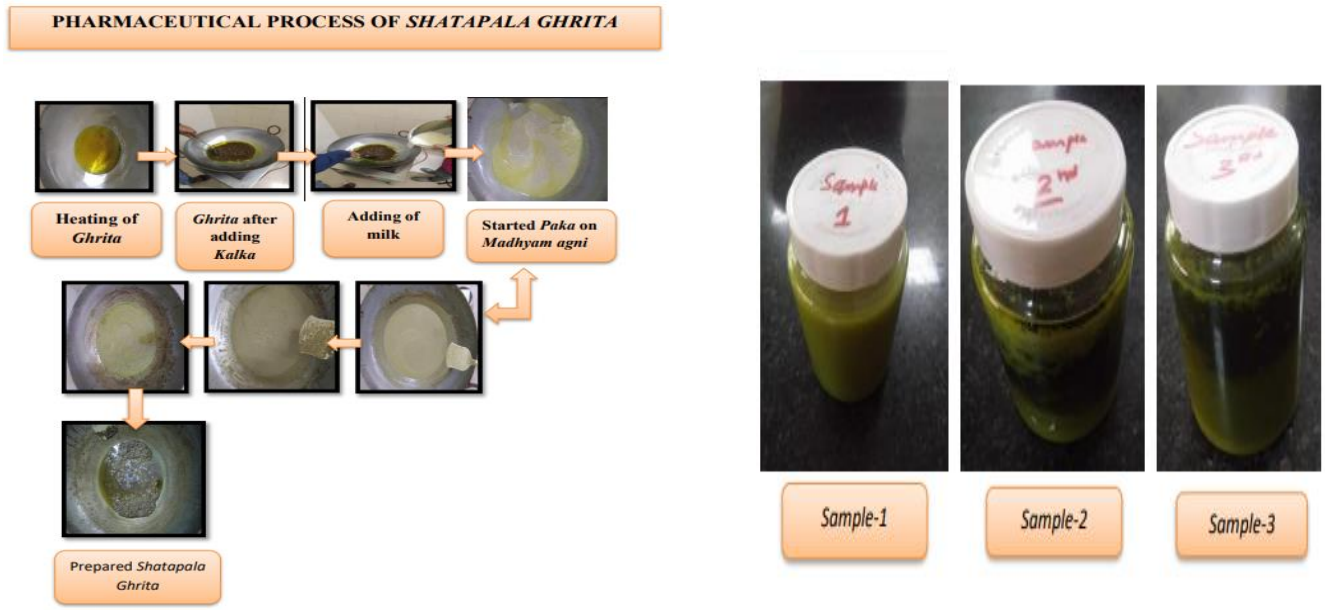


Fig 3

Showing visualization of *SG-1* at 254nm, 366nm and visible light respectively

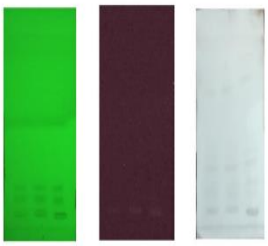


Figure 1 Figure 2 Figure 3

Showing visualization of *SG-2* at 254nm, 366nm and visible light respectively

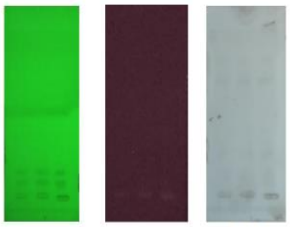


Figure 4 Figure 5 Figure 6

Showing visualization of *SG-3* at 254nm, 366nm and visible light respectively

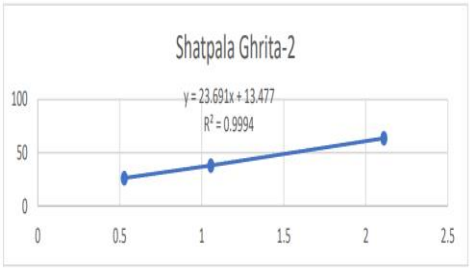
Figure 7 **Figure 8** **Figure 9**

Fig 4

Showing DPPH radical scavenging activity of *Shatpala Ghrita* (*SG-2*)

S. No	Applied Concentration (mg/ml)	Absorbance	Inhibition Concentration	IC 50 (mg/ml)
1.	0.5275	0.462	26.315789	1.5416
2.	1.055	0.389	37.958533	
3.	2.11	0.228	63.636364	

Figure 6.3:Showing DPPH radical scavenging activity *Shatpala Ghrita (SG-2).*



s

Showing superoxide radical scavenging activity of *Shatpala Ghrita (SG-2).*

S. No	Applied Concentration (mg/ml)	Absorbance	Inhibition Concentration	IC 50 (mg/ml)
1.	2.11	0.471	19.897959	6.79
2.	4.22	0.395	32.823129	
3.	8.44	0.23	60.884354	

Figure 6.7: Superoxide radical scavenging activity of *Shatpala Ghrita (SG-2).*