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Standardization of Bhadrikadi Ghrita: A Herbal Ghee based on Ayurvedic Medicinal Preparation

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Abstract

Bhadrikadi Ghrita also known as Marmakshata Ghrita is an Ayurvedic herbal formulation specially indicated for pain management in Marmabhighata (Injury to vital structures). Standardization is necessary to ensure the quality, efficacy and uniformity of the products to get a desired action. No research work is carried out in this direction so this article highlights the standardization of Bhadrikadi Ghrita.

Objectives: To standardize Bhadrikadi Ghrita on various parameters, in order to assure its safety and efficacy.

Materials and Methods: The Raw drugs required for the preparation of Ghrita (Herbal Ghee formulation) were procured from KLE GMP certified Ayurveda pharmacy and the Ghrita was prepared as per the Ayurveda classical methods. The Ghrita was analysed for its organoleptic, physico-chemical, Microbial counts, Phyto-constituents and chromatographic analysis to meet the required standards at Central research facility, AYUSH approved central drug testing laboratory of KLE Shri BMK Ayurveda Mahavidyalaya.

Results: The analysed ghrita shown loss on drying (1.143%), Saponification value (292.10), Iodine value (20.827 w/v), Refractive Index at 40 (1.4563), Acid Value (1.560), Specific Gravity (0.9133), pH (6.00).TLC of Ghrita with mobile phase (Toluene: Ethyl Acetate) ratio (7:3) showed refraction value of short wave at 0.17, 0.23, 0.51,0.73 and Long wave at 0.06, 0.11, 0.15, 0.22, 0.28, 0.36, 0.43, 0.47, 0.53, 0.57, 0.73, 0.79,0.85,0.90. The above parameters can be taken as standard for the analysis of the formulation.

Conclusion: The standardization of any formulation is depended on various parameters like physicochemical, Chromatographic and Microbial count standards which serve as preliminary quality index parameters. The obtained results can be kept as a reference for the assurance of quality control, which would further help in the establishment of quality standards.

Keywords: Bhadrikadi ghrita; Marmakshata Ghrita; Standardization; Marmabhighata.

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INTRODUCTION

There are various Ayurvedic herbal based dosage forms, which are mentioned in classical texts Sahsrayogam such as Kashaya (Decoction), ghrita (Ghee), Taila (Oils), churna (Powders), Gutika (Tablets), Asava-Aarishta (self generated alcohol) etcfor various ailments.[1] Ghrita (medicated ghee), is prepared by heating proper quantity of cow ghee

and decoction prepared with the crude drug paste, so that the active ingredients are assilimiated into the ghee.[2] Ghrita is lipid based polyherbal formulations and believed to possess the capacity to cross blood brain barrier and show beneficial effects on brain tissue. Numerous medicated or herbal formulations in various form shave been already in practiceand are considered as effective and safe for Marmabhi ghata or Kshata (injury) which have similar aspects as comparable to common sports injuries (CSI).[2] According to traumatic effect Ruja (pain), Shopha (Swelling) and Vaikalyata (Deformity) are the common signs of marmabhi ghata. A single herbal formulation Bhadarakadi ghrita (BG) is mentioned under Ghrita Prakarnam of Sahsrayogam specially to treat Marmaghata or kshata i.e trauma to vital points.

The therapeutic effect of any formulation mainly depends on the quality of drugs and standard preparation techniques. A standard protocol is published by the Central council of Research in Ayurveda and Siddha in order to maintain same standard parameters for the quality production of Ayurveda medicine.[3] Formulations with chemical based agents has many harmful effects and the concern in public is increasing, so in order to provide scientific grounds for traditional plant based formulation good research parameters are to be adopted. The first step for scientifically based researchis to provide the standardization of drug which is need of hour. The ghrita was prepared by purified and non-adulterated herbal drugs as per the method and the formula mentioned in Sahsrayogam.[4] With this referencing as background the present study was undertaken to ascertain the authenticity of all the ingredients of Bhadrikadi ghrita by various parameters.

MATERIALS AND METHODS

Collection of Raw Drugs

Raw Drugs were procured from *Ayush* approved GMP certified KLE Ayurveda Pharmacy, Khasbag, Belagavi at the time of preparation of the drug. Drug authentication, Physico-chemical analysis of the finished product was carried at Central research facility, *Ayush* approved drug Testing laboratory, B.M.K Ayurveda Mahavidyalaya, Shahapur, Belagavi. Go Ghrita and Go-kshira was procured from Nandini Milk Factory ISO 8086:2004 Belagavi and processed sugar from the local sources.

Method of Preparation

The required ingredients of Bhadrikadi ghrita (Table 1) were procured and ghrita was prepared according to the standard Ayurvedic procedure of 'Ghrita Paka Kalpana'.[5] The required quantity of ghee (Sneha Dravya) was taken in a large stainless steel vessel. Further addition of Kalka dravya (paste of drugs) in equal proportion, Go Kshira and Decoction of Bhadrika (Drava Dravya) along with ghee (Sneha Dravya) were combined in specified ratio of 1:16:8 respectively. The mixture was kept on mild to moderate flame until the complete evaporation of moisture and appearance of Sneha Siddhi Lakshana' (characteristic features of prepared ghee). The 'Sneha Siddhi Lakshana' was characterized by burning of paste (Varti) without crackling sounds and disappearance of froth (Phena) in ghrita. Processed sugar was added to ghrita and labelled as FG-13/19-20.

Table 1: Contents of Bhadrikadi Ghrita or Marmakshata Ghrita.

S.no	Name of the ingredients	Botanical Name	Proportion	Quantity
1.	Bhadrika	*Bergenialin gulate* wall	16 parts	10 liters
2.	Go-Kshira	Cow milk	16 parts	10 liters
3.	Murchita Ghrita	Processed Ghee	8 parts	5 liters
4.	Nirmali	Strychnos potatorum Linn.	1 part	625 grams
5.	Sugar (Processed)		1 part	625 grams

Organoleptic Study

Organoleptic Characteristics for various sensory characters like colour, taste, odour etc was carefully noted down as illustrated in Table 2. The raw drugs were separately studied by organoleptic and morphological characters like Family, Rasa (Taste), Guna (Properties), Virya (Potency) Vipaka (Post-digestion effect), Karma (Pharmalogical action).[6]

Physico-chemical Analysis: The prepared formulation was labelled as Finished good 13. (FG-13)

Table 2: Organoleptic study of Finished good.

S. no	Characters	Results
1.	Form	Ghrita
2.	Colour	Lemon Yellow
3.	Odour	Rich and Characteristic
4.	Taste	Sweet

Loss on Drying at 1100C

Place the weighed crucible with 5 gm material in plain lid position in the oven at 105°C for at least 3 hours. Place the crucible in desiccator by using tongs or gloves and allow cooling for at least 30 minutes. It was taken out and weighed again and again at regular interval of time till consistent weight as achieved. The percentage of difference was considered as loss on drying at that particular temperature. Deterioration time of the plant's material depends upon the amount of water present in that. If the water content is high, the plant can easily be deteriorated due to fungus.[7]

Saponification Value

Saponification value is amount of KOH required to completely hydrolyse (saponify) one gram of fat/oil.

2 grams of fat in a tared beaker was weighed and dissolved in about 3 ml of the fat solvent (ethanol/ ether mixture). The contents of the beaker were quantitatively transferred 3 times with further addition of 7 ml of the solvent. 25 ml of 0.5N alcoholic KOH was added and mixed well, which was attached to a reflux condenser. Another reflux condenser as the blank with all other reagents was present except the fat was set. Both the flasks were placed in a boiling water bath for 30 minutes. The flask was cooled at room temperature. Now added phenolphthalein indicator to both the flasks and titrated with 0.5N HCL. Noted down the end point of blank and test. The difference between the blank and the test reading gives the number of ML. of 0.5N KOH required to saponify 1 gm of fat.[8]

The long chain fatty acids present in fats have a low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of fat, when compared to short chain fatty acids. Medium chain triglycerides are considered as good biologically inert source of energy that is easy to digest for human body to metabolize.[9]

Iodine Value

10 ml of the fat sample was pipetted out and dissolved in chloroform to an iodised flask labelled as FG-13/19. 20 ml of the iodised monochloride reagent was added into the flask. The content in the flask were thoroughly mixed. Then the flask is allowed to stand for ½ an hour in the direct sun light protected area. Then another iodised flask by adding 10 ml chloroform was taken as FG-13/20 and 20 ml of iodine monochloride reagent and the contents were mixed in the flak mixed thoroughly and again set up in direct sun light protected area. 10 ml of potassium iodine solution was added after receiving the FG-13-B.

The stopper and the sides of the flask was rinsed using 50 ml of distilled water. The FG-13/19 against the standardized sodium Thiosulphate was titrated until a pale straw colour is observed. About 1ml starch indicator was added into the contents in the flask, and purple colour is observed. The titration is continued until the colour of the solution in the flask turns colourless. The disappearance of blue colour is recorded as the end point of the titration.[10] Heat increases the disassociation and unsaturation of the molecules of the compounds.[11]

Refractive index at 400C

Abbe's refracto meter is used to measure the refractive index of the FG-13/19. Using a monochromatic light source, the apparatus is calibrated with the water as liquid. The micro-meter screw is adjusted to focus the boundary between the bright and the dark regions, and then the refractometer scale is adjusted to the place cross wire to the telescope exactly on the boundary between the bright and dark regions. The same process was repeated after the equipment was calibrated. A drop of water placed on the prism and the drive knob was adjusted in such a way that the boundary line intersects the separatrix exactly at the centre and the reading was noted.

Distilled water has been found to have a refractive index of 1.3325 at 250C. The difference between the reading and 1.3325 gives error of the instrument. If the reading is less than 1.3325, the error is the minus (-) then the correction is plus (+). But if the reading is more than 1.3325 then the error is the plus (+) then the correction is minus (-).

Refractive index of ghrita was determined using 1 drop of the sample. All the fats are composed of various types of fatty acids and their derivatives, such as esters in the ratios. With changes of temperature or any other ingredients, the basic constituents of particular fat vary. Such variation may be due to any adulteration. Refractive index varies with temperature and wave length.[12]

Acid value

10 ml of ghrita was taken in a conical flask. To it was added 50ml of acid free alcohol ether mixture (25 ml + 25 ml) previously neutralized by the addition of 1ml of phenolphthalein solution and titrated against 0.1N potassium hydroxide solution. End point is figured out with the appearance of pale pink colour with persistence for 15 seconds.[13]

The acidity is affected with the process of

oxidation as triglycerides get converted into fatty acids and glycerol. Liberation of fatty acid is the out come of hydrolysis, thermal effects and lipolytic enzymes as lipase. So, of acid value varies linearly with rancidity.[14] In present work, acid value of ghrita was found below 2 which indicate the Ghrita of best quality.

Specific Gravity

Specific gravity bottle was cleaned and shaken with acetone and the with ether. Bottle was dried and weight was noted down. The specific gravity with the ghrita and weight were noted. The procedure was repeated using distilled water in place in place of Ghrita.

The weight of the lipid material is affected by the factors such as basic constituents, dissolved constituents used in the preparation of the formulation, or any other compound or excipient, which may be used during the process etc.[15]

pH

The value characteristic of an aqueous solution is its pH value, which represents conventionally its acidity or alkalinity. pH of the A.S is a negative logarithm for hydrogenion activity After the apparatus was cleaned and electrode was thoroughly washed. Calibration is done by standardizing the apparatus with only single sample of 10 ml ghrita was taken in glass beaker and tested to obtain a preliminary value (± 0.04) for pH after the ghrita was buffered (Fig. 1-2). Standard solutions and distilled water were kept at the temperature of measurement for more than

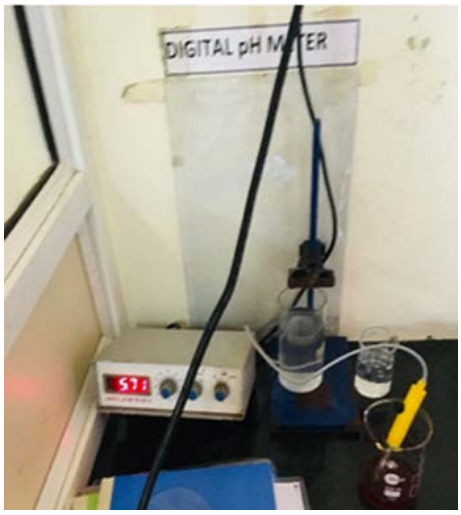

Fig. 1: pH of Bhadrikadi Ghrita

2 hours prior to making the measurement in order to reduce the negligible value effects of thermal electrodes. The pH of FG-13/19 was found to be 6 (Fig. 2) which indicates that the ghrita is under the standard parameter.[16]

Fig. 2: pH of Bhadrikadi Ghrita

Chromatographic Analysis

TLC: Thin Layer chromatography was performed for ghrita using Solvent Toluene and Ethyl acetate in 6:4 ratio.[17]

TLC profile of the unsaponifiable matter, the TLC profile of unsaponifiable matter of sample showed two prominent spots Rf 0.4 Blue. Thin layer chromatography profile of ghrita was developed by using Benzene and Ethyl acetate in 9:1 ratio as solvent system and the plate were visualized in UV chamber (Fig. 3) at long wave length (366nm). The stationary phase is applied to the plate uniformly and then allowed to dry and stabilize. However, these days ready made plates are preferred.

Fig. 3: TLC of Bhadrikadi Ghrita

With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots. Then samples solutions are applied on the spots marked on the line in equal distances. The mobile phase is poured into the TLC chamber to a levelled few centimetres above the chamber bottom. A moistened filter paper in mobile phase is placed into the inner wall of the chamber to maintain equal humidity. Now, the plate is prepared with sample spotting in TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid and the plate is then immersed, such that the sample spots are well above the level of mobile phase for the development.[17] Allow sufficient time for the development of spots. Then the plates are removed and allowed to dry. The sample spots can now be seen in a suitable UV light chamber, or any other methods as recommended for the FG-13. The Rf of the spots exhibited was measured using scale.[17]

Microbiological parameters

It includes a total viable content, total enterobacterial, total fungal and their count. Limiters are utilized as quantitative or semi-quantitative tool for ascertains and controls the amount of impurities like the reagents used during manufacturing vessels and the solvents etc (Table 4).[18]

Table 4: Microbiological parameters of Bhadrikadi Ghrita

Organisms	Limits (as per IP)	Results
E. Coli	Absent/100 ml	Absent
S. aureus	Absent/100 ml	Absent
P. aeruginosa	Absent/100 ml	Absent
S. abony	Absent/100 ml	Absent

RESULTS

Table 5: Rasapanchaka of Bhadrikadi ghrita

Ingredients	Rasa	Guna	Virya	Vipaka	Karma
Bhadrika	Tikta Kashaya	Laghu Snighdha	Sheeta	Katu	Shothahara Vranahara
Nirmali (kataka)	Kashaya	Guru	Sheeta	Madhura	Vatakaphahara Shoolaaghna Shophagna
Go-Kshira	Madhura	Snigdha	Sheeta	Madhura	Sandhaneeya, Jeevaniya Balya
Go-Ghrita	Madhura	Guru, Snigdha	Sheeta	Madhura	Vata-Pittahara Raktaprasadana Ooj Vardhan Deepana
Sita	Madhura				

DISCUSSION

Raw herbs were authenticated and analyzed before processing because the quality of finished product depends on the purity and origin of raw materials. The Lemon green color of ghrita was due to Murchita ghrita which contains Triphala along Pashanabheda decoction, saccharine etc. Ghrita was rich and characteristics in odor due to base of go ghrita and go-kshira.[19] Taste is sweet due to added Sita (Saccharine) as a Kalka Dravya. As it is semiliquid product in ghrita preparation, it is viscous and sticky. Loss on drying, Saponification, Iodine value, refractive index at 400C, Acid value, pH and specific gravity were all in normal range of limits as per API. No standards were given in API for finished products so the obtained results for the different value of Bhadarakadi ghrita were found to be with in the normal limits, which indicates the FG was of standard quality. In addition, Microbial count for E. coli, S. aureus, P. aeruginosa, S. abony were absent in per 100 ml of the given sample which means that the finished product is standard quality.[21] The refractive index was 1.4563. The specific gravity of the sample was 0.9133 which implies that prepared ghrita was not too dense in volume. The saponification value was found to be 292.102 w/v. It gives the molecular weight of an oil/fat, and the oil contained a long chain of fatty acids. Phytochemical screening (Table 3). Quantity

Table 3: Physico-chemical Standards of Bhadrikadi Ghrita

Physico-chemical Standards	
Tests	Results
Loss on drying 110	1.14%
Saponification value	292.102
Iodine value	20.827 w/v
Refractive Index at 40	1.4563
Acid Value	1.56
Specific Gravity	0.9133
pH	6

TLC of Ghrita with mobile phase (Toluene: Ethyl Acetate) ratio (7:3) showed refraction value of short wave at 0.17, 0.23, 0.51,0.73 and Long wave at 0.06, 0.11, 0.15, 0.22, 0.28, 0.36, 0.43, 0.47, 0.53, 0.57, 0.73, 0.79,0.85,0.90.

of unsaturated fatty material present in the Ghrita is estimated by Iodine value. The iodine value if it is higher in formulation it implies that it contains more unsaturated bonds in fat. Unsaturated fat supplementation has no adverse impact on the

Fig. 4: Bhadrikadi Ghrita finished product

blood lipids and also increases the total dietary energy in take to the recommended levels. It also improves the nutritional status and reduces systemic inflammation.[20] A high iodine value indicates that the fats are a rich source of polyunsaturated fatty acids that possess health benefits such as regulating blood cholesterol levels.[21] The findings of TLC, which was performed for the confirmation of the phytoconstituents presents sounds similar for standard ghrita preparation in API, which mentions the same solvent in the same ratio.[22] It shows the presence of two major components in the ghrita, which were soluble in non-polar solvent as hexane. Ghrita, is a lipid based formulation in order to separate its constituents it requires selection of solvent system which has increased non-polarity.[23] The Rf value and the colour of the bands spots were also approximately same. It can be assumed that it may be due to constituent of Bhadrika decoction which was synthesized for the formulation.

CONCLUSION

It is inferred that the formulations meet the maximum qualitative standards based on several properties and parameters. Bhadrikadi ghrita has been standardized using scientific quality parameters. The result of this may be used as reference standard in further research under takings of its kind for the benefit of the end user without any unwarranted complications. HPTLC or Gas chromatography is been recommended to explore its hidden potential. The obtained values can be adopted to lay down new pharmacopoeia standards to be followed in its preparation.

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