



Research Article

PHYSICO-CHEMICAL ANALYSIS OF PANCHAGAVYA GHRITHA PREPARED AS PER ASHTANGA HRIDAYA REFERENCE AND COMPARISON WITH MARKET SAMPLES

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ABSTRACT

Panchagavya Ghrita is a widely used Ayurvedic formulation mentioned in *Ashtanga Hridaya Utharastana Apasmara Patishedha* indicated in conditions like *Apasmara* (epilepsy), *Jwara* (pyrexia), and *Kamala* (jaundice). It contains 5 ingredients namely *Goshakrit rasa* (cow dung juice), *Amladadhi* (sour curd), *Goksheera* (cow's milk), *Gomutra* (cow's urine) and *Goghrita* (ghee). Authentic sources of cow products are not often obtained and found adulterated. Collection and processing of fresh raw materials are an important area in this formulation This study was conducted to find out the non conformances and quality issues in *Panchagavya ghrita* production in industrial level. The comparative analysis of prepared and market samples based on standard analytical parameters proposed by PLIM reveals the variation in different organoleptic characters and physico-chemical parameters. The physico-chemical parameters among prepared and different companies were statistically analysed with ANOVA test and Scheffe's pair wise comparison, showed significant difference at 0.01 levels.

INTRODUCTION

Among all of the *Snehakalapanas*, *Ghritha* is recognised as the best^[1]. *Ghritha* has the dual ability to incorporate additional pharmacological qualities while also accepting the host without losing its original properties. The medical and therapeutic benefits of *Panchgavya* are innumerable. The importance of using 'gavya' (i.e., substances derived from 'Gau' = cow) substances such as cow milk, ghee, urine, dung, and curd for the treatment of various diseases has been discussed in Ayurvedic medical system. Each ingredients have unique properties and applications in agriculture, human health, and other fields. The name "*Panchgavya*" is derived from two words: "*Panch*," which means "five," and "*Gavya*," which means "obtained from a cow." Together, these two words stand for five products made from cows. Each of the 'Gavya' exerts a different medicinal impact against various diseases.^[2]

Panchagavyaghritha is mentioned in *Astangahridaya Utharastana*.^[3] Other references of same formulation are available in *Charaka Samhitha*^[4] *Sahasrayoga*^[5], *Bhaishajya Ratnavali* and *Yogaratanakara*^[6] and, *Chakradatta* with few difference is the ratio of ingredients added in. Authentic sources of cow products are not often obtained and found adulterated. Due to their high demand and short shelf life, milk products are frequently bought in compromised, low-quality conditions. The presence of contaminants in milk products is also a major concern. Milk is mostly contaminated before to milking by the feed and fodder supplied to the cattle, by sources or materials used during milk processing, and by careless handling of the milk both before and after processing. Bovine hormones are injected into cows to increase milk output in large farms, which poses a number of health risks to people. Because of the shortage of ghee, and comparatively more demand, it is very expensive (costing 7 to 10 times more than edible vegetable oils). Therefore, ghee is prone to adulteration by the traders in the market^[7]. Admixing low-price fats or oils in ghee is done mainly for gaining more profit. Ghee is primarily adulterated by vegetable/plant oils and animal body fats^[7].

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For a medicine's therapeutic impact to be repeatable, its standard quality is crucial. The scientific validity of Ayurvedic medications has been established through analytical tests using contemporary analytical standards. To create reproducible, affordable, and effective Ayurvedic medication, the protocol criteria must be thoroughly emphasized. *Panchagavya Ghritha* being a widely prescribed and marketed formulation in India there is a need for comparing the market samples with the standard for ensuring the quality, efficacy and safety of formulation.

MATERIALS AND METHODS

Pharmaceutical Study

A total of 3 samples were prepared from the products obtained from the same cow on the same day. The reference was taken from *Astanga Hrudaya*. The various steps followed for preparation were

Collection of genuine raw drugs and its preprocessing

Fresh raw materials namely dung, urine and milk were obtained from a black coloured vechur variety of indigenous cow, that is, *Bos indicus*, aged 7 years, black in colour, having a heifer calf reared in-house *Goshala* of Illom Ayurveda hospital, Cherthala Alappuzha district, Kerala being fed a uniform diet and undergoing regular vaccination schedule. The curd and ghee were prepared by processing milk from the same source

Collection of cow urine:^[8] Fresh morning cows' urine was collected. After wiping away fecal matter from the vulva, the cow was stimulated to urinate by stroking the side of vulva for approximately 15-30 sec. 1lt of midstream urine was collected in a wide mouthed clean container previously sterilized and used immediately for the preparation.

Collection of cow dung:^[9] Fresh cow dung was collected manually by gloved hands. 1 kilogram of

Method of Preparation

PS1, PS2, PS3 were prepared in the same manner, the ingredients and their proportions are given in the table below.

Table 1: Ingredients and quantity of drugs taken

S.no	Ingredients	Quantity
1.	<i>Goshakrit rasa</i>	300ml
2.	<i>Amla dadhi</i>	300ml
3.	<i>Go Ksheera</i>	300ml
4.	<i>Go mutra</i>	300ml
5.	<i>Go Ghritha</i>	300ml

Sample PS1

The dung extract (300ml), along with 300ml each of freshly collected cow urine, were mixed with 300ml of molten cow ghee in a bronze vessel of 2lt capacity, and heated upon firewood stove on mild heat with continuous stirring, frothing was observed during the process which settled after a duration of 35minutes. At this stage curd from cow milk (300ml,

freshly collected cow dung was mixed homogeneously with 1000ml of water and mixed thoroughly using a gloved hand to form a homogenous mixture. The mixture was strained through a triple layered muslin cloth.

Preparation of Curd: 1 litre fresh milk was taken in a steel vessel. It was heated in low heat till it boils. It was let down for self-cooling to obtain lukewarm milk. Milk was made to froth by transferring it into another vessel 4-5 times. 10ml of curd starter was added to this milk and left undisturbed for 24 hours. Well-formed sour curd obtained.

Preparation of ghee: A large heavy bottomed iron vessel was taken to which 2kg of butter was added. The vessel was placed over the stove and heated on medium-low to medium heat. Melting of butter was observed. As the temperature increased, contents became thicker and bubbles were formed. Stirring was done at intervals. Once the butter melted completely, milk solids were formed on the surface of the vessel. Colour changed gradually to form a buttery, pale yellow to light golden with the butter bubbling and shimmering. After sometimes milk solids began to settle down. After complete settling of milk solids and the entire mixture became frothy. Heating was stopped and the mixture was filtered through a clean cloth. Liquid golden yellow coloured ghee weighing 920ml was obtained.

Genuinity Testing of Raw Materials

The genuinity of procured samples of milk, curd, ghee, urine and cow dung were analyzed in Drug Standardization Unit, GAVC Trivandrum and Biogenic Labs Kochi on the same day of procurement. The values are included in the following sections.

Preparation of *Panchagavyaghritha* Samples

Panchagavyaghritha was prepared as per *Astanga Hridayam Utharastana* reference.

night old) and cow milk (freshly collected in the morning), were added to vessel little by little with continuous stirring. The process was continued uninterruptedly for about 3-3.30 h, the processing was continued on mild heat. When all the *Dravadravyas* had evaporated the solids from milk, curd and dung separated as *Kalka*. At this stage, stirring was done

more often and carefully to ensure that *Kalka* does not stick to the bottom of the vessel. The *Kalka* was taken out of the ladle and tested from time to time till *Siddhi Lakshanas* (process termination signs) were obtained for *Mridu Paka*, *Kalka* was waxy and when rolled between the fingers, rolls like lac without sticking. In *Madhyama paka*, *Kalka* was harder, can be rolled into a *Varti* and when put in fire burned without any crackling noise. Heating was stopped and The *Ghritha* was filtered through a 2 layered clean muslin cloth and stored in a clean, dry, wide mouthed airtight containers.

In this manner 3 samples of *Panchagavya ghritha* were prepared with same ingredients, following the same manufacturing method and were coded as PS1, PS2, PS3. From 12 available market

samples manufacturing *Panchagavyaghritha* with same ingredients and same ratio, 5 GMP certified companies manufacturing *Panchagavyaghritha* were selected by Simple random sampling method and used for analytical study.

There were total 8 samples of *Panchagavya ghritha* for analytical study

1. *Panchagavya ghritha* PS1
2. *Panchagavya ghritha* PS2
3. *Panchagavya ghritha* PS3
4. Market sample M1
5. Market sample M2
6. Market sample M3
7. Market sample M4
8. Market sample M5

RESULTS

Pharmaceutical Study Results

Table 2: Results obtained during *Panchagavyaghritha* preparation

Observations	PS1	PS2	PS3
Initial quantity of <i>Ghritha</i>	300ml	300ml	300ml
Final quantity of <i>Panchagavya ghritha</i>	295ml	295ml	300ml

Analytical study of *Panchagavya ghritha* involved

1. Analysis of Raw drugs
2. Organoleptic characteristics of prepared and market samples of *Panchagavya Ghritha*
3. Physico- chemical analysis of prepared and market samples of *Panchagavya Ghritha*

Cow's Milk (FSSAI standards)

Description- White, easily flowing, non slimy liquid with a pleasant smell

Table 3: Analytical parameters of cow's milk^[8]

S.no	Parameters	Test values (gm/100gm)
1.	Moisture	86.1
2.	Total solids	13.9
3.	Sugar	3.90
4.	Fat	3.09
5.	Protein	4.70
6.	Solid no fat	10.8
7.	pH at 25°C	7.10
8.	Specific gravity at 25°C	1.0290
9.	Titration acidity	0.10

Cow's Ghee (API)

Description- Bright yellow coloured with well developed granules dispersed fairly, thickly and uniformly over the entire mass

Table 4: Analytical Parameters of Cow's Ghee

S.no	Parameters	Test values g/100gm
1.	Loss of drying	0.24
2.	Specific gravity at 25°C	0.9345
3.	Refractive Index at 40°C	1.4559
4.	Saponification value	226
5.	Acid value	0.89
6.	Iodine value	35.8

7.	Milk fat	99.7
8.	Unsaponification matter	0.38

Cow's Curd (FSSAI Standards)

Description: A thick, soft, white coloured solid, with a sour, astringent taste

Table 5: Analytical parameters of curd

S.No	Parameters	Test values (g/100)
1.	Milk fat	3.74
2.	SNF (Solid Not Fat)	10.9
3.	Carbohydrates	4.50
4.	Titration acidity	1.2
5.	Loss on drying	88
6.	Total ash	0.89
7.	Acid insoluble ash	0.15
8.	pH at 25°C	4.1
9.	Specific gravity at 25°C	1.0291

Table 6: Comparison between Organoleptic characters of PGG Market and Prepared samples

Sample	Colour	Odour	Taste	Texture
M1	Light yellow	Pleasant odour of ghee	Bitter	Thick, solid
M2	Brownish yellow	Unpleasant odour of urine	Bitter	Thick, semisolid
M3	Brownish yellow	Unpleasant odour	Bitter	Easy flowing, liquid
M4	Dull yellow	Unpleasant odour of cow's urine	Bitter	Thick, solid
M5	Brownish yellow	Pleasant odour of ghee	Bitter	Thick semi solid
PS1	Light green	Pleasant smell of ghee	Bitter	Thick, semisolid
PS2	Light green	Pleasant smell of ghee	Bitter	Thick, semisolid
PS3	Light green	Pleasant smell of ghee	Bitter	Thick, semisolid

Table 7: Physico-chemical analysis of prepared and Market samples of *Panchagavya ghritha*

Prepared PGG	Specific gravity	Refractive index	Acid value	Saponification value	Iodine value	Peroxide value	pH
PS1	0.9263	1.460	6.6702	222.8934	36.3437	0.60	3.27
PS2	0.9304	1.460	6.5175	213.2904	34.0583	1.58	3.24
PS3	0.9326	1.460	6.0745	229.7291	42.8472	0.60	3.18
Market PGG	Specific gravity	Refractive index	Acid value	Saponification value	Iodine value	Peroxide value	pH
M1	0.9137	1.460	7.1038	223.2001	36.0829	5.52	3.92
M2	0.9318	1.460	9.1051	197.2288	31.8889	5.64	4.14
M3	0.9228	1.465	9.2649	179.2918	52.1229	11.3	4.02
M4	0.9339	1.463	9.3674	225.9860	31.3958	3.56	3.24
M5	0.9234	1.460	7.4039	223.7931	31.585	3.64	3.39

PGG: *Panchagavyaghritha*



a)cow's milk, b)cow's urine, c)cow's ghee, d)cow dung juice, e)cow's curd

Fig 1: Ingredients of Panchagavya ghritha



a) stored butter b) ghee

Fig 2: Melting of stored butter to obtain liquid golden yellow coloured ghee



a)Molten Ghritha b)on adding Gomutra and Gomayaswarasa c)on adding Goksheera at Mridupaka d)Madhyama paka e)PGG immediately after filtration f)PGG at room temperature

Fig 3: Preparation of Panchagavya ghritha

Statistical Analysis

In order to compare the means of sample, ANOVA and for pair wise comparison Scheffe's post hoc test was employed. ANOVA showed that the Iodine value of prepared and market samples varies significantly ($F=93.014$, $P<0.01$). P has an average Iodine value of 36.17 ± 3.42 . (27.68 to 44.67). Scheffe's pairwise comparisons showed that P and M3 (50.01 per cent); P and M4 (9.17 percent) differ significantly ($P<0.01$). P and M1; P and M2; P and M5 showed approximately the same level of Iodine value. ANOVA showed that the Saponification value of prepared and market samples varies significantly ($F=30.246$, $P<0.01$). P has an average Saponification value of 222.30 ± 8.33 . (201.60 to 243.01). Scheffe's pairwise comparisons showed that P and M2 (11.20 per cent); P and M3 (18.76 percent) differ significantly ($P<0.01$). P and M1; P and M4; P and M5 showed approximately the same level of Saponification value. ANOVA showed that the peroxide value of prepared and market samples varies significantly ($F=644.054$, $P<0.01$). P has an average peroxide value of 0.93 ± 0.57 . (-0.48 to 2.33). Scheffe's pairwise comparisons showed that P and M1 (493.17 percent); P and M2 (85.93 percent); P and M3 (185.37 per cent); P1 and M4 (23.01 percent); P and M5 (132.77 percent) differ significantly ($P<0.01$).

ANOVA showed that the pH of prepared and market samples varies significantly ($F=1077.621$, $P<0.01$). P has an average pH of 3.23 ± 0.05 . (3.12 to 3.34). Scheffe's pairwise comparisons showed that P and M1 (20.95 percent); P and M2 (23.38 percent); P and M3 (19.07 per cent); P1 and M5 (4.94 percent) differ significantly ($P<0.01$). P and M4 showed approximately the same level of pH. ANOVA showed that the specific gravity of prepared and market samples do not differ significantly ($F=2.747$, $P>0.05$). P has an average specific gravity of 0.93 ± 0.00 . (0.93 to 0.93). P and M1; P and M2; P and M3; P and M4; P and M5 have approximately the same specific gravity, according to Scheffe's pairwise comparisons. ANOVA showed that the acid value of prepared and market samples varies significantly ($F=305.303$, $P<0.01$). P has an average Acid value of 6.42 ± 0.31 . (5.64 to 7.20). Scheffe's pairwise comparisons showed that P and M1 (10.66 percent); P and M3 (31.23 per cent); P and M4 (31.82 percent); P1 and M5 (10.50 percent) differ significantly ($P<0.01$). P and M2 showed approximately the same level of Acid value.

DISCUSSION

Ghee is unique among natural fats in that it contains a large proportion of fatty acids and as a consequence, many of its characteristic is quiet distinctive. Three prepared formulation, along with randomly selected market samples from 5 GMP certified pharmacies were taken for analytical study.

The parameters like specific gravity, wt/ml, refractive index, Saponification value, iodine value, peroxide value, pH and acid value.

Organoleptic features of final product were analysed and found that all the prepared samples were light green in colour. The market samples M2, M3 and M5 were Brownish yellow in colour, M1 and M4 dull yellow in colour showing wide variation from colour of prepared samples After the preparation of PGG process the yellow colour of *Ghrita* turned into light green in colour. This colour may be due to the action of lactic acid bacteria^[9] and due to drugs used in PGG like fresh cow dung which is the undigested residue of plant matter passed through the gut of cow, which is rich in minerals. On exposure to air over the colour darkens and this may be the reason for brownish yellow colour of Market sample M2, M3 and M5. The characteristic odour of *Ghrita* is converted into aromatic in case of Prepared PGG is due to the drugs in PGG like cow's milk and curd as raw milk contain 71 aromatic compounds and typical aroma of curd characterized chiefly by acetaldehyde. The specific gravities of all samples were analysed. The mean specific gravity of prepared PGG samples was 0.9302. Market sample M2 and M4 were having specific gravity more than the mean value of the prepared sample ranging from 0.9318 to 0.9339. Increase in Specific gravity is an indication of increased solid fat content in the sample Refractive index is an optical parameter which measures how much the speed of light is reduced inside a medium. Refractive indexes of all prepared samples are equal. The mean refractive indexes of prepared samples were 1.460. Market samples M3 and M5 had a higher refractive index than the mean of prepared samples. Refractive index of ghee depends on the chain length, with increase in chain length, refractive index of ghee increases.

Saponification value depends on the kind of fatty acid contained in the fat. The long chain fatty acids found in fats have a low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. When milk fat is adulterated with either animal or vegetable fat, the Saponification value changes. The saponification value for milk fat varies from 225 to 230. Adulteration of ghee with animal body fat and vegetable oil somewhat changed the saponification value. In present study mean Saponification value of PS was 222.2, market samples M3 and M2 value lesser than prepared sample. Increase in specific gravity, acid value, decrease in saponification value indicates an increase in solid fat suggestive of the process of hydrogenation of fat may have undergone.^[10]

Acid value is an important measure for the Rancidity of oils or fats. In rancidification is due to oxidation, triglycerides are converted into glycerol and free fatty acids leading to increase in Acid value. In the present study, mean value of PS was found to be 6.4207. Market samples M2, M3, M4 showed high acid value indicating more no of free fatty acids in the sample indicates rancidification of fat.

pH value indicates the degree of alkalinity or acidity of the sample. It indicates the site of absorption and action of drug. The stability and physiological suitability of a drug is also attributed by pH. pH of *Panchagavya ghritha* is usually acidic in nature owing to the presence of curd formed by fermentation process. Mean pH of prepared samples were 3.23. All the Market samples had a value greater than the mean value pH of prepared samples, indicating *Panchagavya Ghritha* is acidic in nature.

Iodine value is the method to evaluate or the unsaturation level in fatty acids. It is the number of grams of iodine consumed by 100gm of fat. A higher iodine value indicates a higher degree of unsaturation. The mean Iodine value of prepared sample was 37.7498. M1, M2, M4, M5 were having Iodine value lesser than prepared sample and M3 had a value greater than all the samples 52.1229.

The peroxide value is a parameter specifying the content of oxygen as peroxide, especially hydroperoxides in a substance. It is a measure of the oxidation present the degree of primary oxidation, and the most common cause of milk fat deterioration is rancidity which is due to oxidation, thereby affecting its flavour and quality. The acceptability of ghee largely depends on the extent to which the oxidative deterioration has occurred. It is generally considered that the first product formed by oxidation of an oil or fat is a hydroperoxide. The mean Peroxide value of the PS was noted as 0.9266. All the market samples showed higher value of Peroxide test. Market sample M3 had a high Peroxide value of 11.3 therefore it indicates the likeliness of becoming rancid.

CONCLUSION

Among the physico-chemical parameters studied, there were wide differences observed in organoleptic characters like colour, smell and consistency of prepared and market samples. Market samples differed from the prepared sample in terms of refractive index, specific gravity, acid value, iodine value, peroxide value, and pH and saponification value.

On analyzing the pH all the samples had an acidic pH. Statistical evaluation showed that the prepared and market samples had significant difference in case of Iodine value, Saponification value, peroxide value, pH, refractive index and acid value. Value of specific gravity, did not show much difference between the prepared and market samples when statistically analyzed.

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