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Research Article

THE PHARMACEUTICO- ANALYTICAL STUDY OF OLEAGINOUS FORMULATION: *ARJUNA GHRITA*

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ABSTRACT

Ayurvedic literature have a lot of unexplored or least tested medicines, *Arjuna Ghrita* is such one indicated for all types of cardiac disorder. As the incidences of cardiac disorder are increasing, need of a drug like *Arjuna ghrita* which could be used in many diseases should be formulated. Till date no work has been done at pharmaceuticoanalytical study of *Arjuna ghrita*. It is an oleaginous formulation needed to be explored in scientific light. Preparation of oleaginous dosage form is described as *Sneha kalpana* done by subjecting *Ghrita* or oil to a particular a pattern of heat treated with different *Kalka* (paste) and liquid media like *Kwath* (decoction). *Murchhana* is a pre-procedure to *Sneha kalpana* in which *Ghrita* is treated with few drugs. Three samples of *Arjuna ghrita* were prepared from three different brand cows *ghee* following the classical texts. Obtained samples were unctuous, viscous soft mass of yellow color with slightly bitter taste and characteristic bitter odour and its analytical study was done. In analytical study calculated mean value for refractive index is 1.456, specific gravity is 0.9181, iodine value is 31.026, saponification value is 131.13, peroxide value is 1.5 and acid value is 1.02. Assay of heavy metals and microbial contamination were under prescribed limit for samples of *Arjuna ghrita*. Therefore the values obtained can be considered as standard for *Arjuna ghrita*.

KEYWORDS: Analytical study, *Arjuna Ghrita*, Pharmaceutical study.

INTRODUCTION

In the gigantic *Ayurvedic* literature, many formulations are still unexplored for good pharmaceutical and clinical research. *Arjuna ghrita,* a semi solid oleaginous formulation of less ingredients for all types of cardiac disorders described by *Acharaya Chakrapani*.[1] This formulation is still untried by scientific community due to treatment of cow's ghee in it as main ingredient which was for a long considered to be unsafe for cardiac patient.

Sneha kalpana is a considerate pharmaceutical procedure in *Ayurvedic* pharmacies to obtain semi solid oleaginous dosage form used in different diseases for systemic or topical application. By subjecting *Sneha* (cow's ghee/ various oils) to a particular heat pattern with *Kalka* (paste) and *Drava* (any liquid medium, whether it could be juice, decoction, cold or hot infusion, milk etc.) in prescribed formula [2].

The rationality for *Sneha kalpana* is to extract the lipid soluble active principle of herb used in the formulation providing many nutritive and curative health benefits of *Sneha* to the patient in specific condition. The cow's ghee is believed to strengthen the *Oja*, the elixir of *Dhatus.* (body nourishing entities)

The *Arjuna ghrita*, contains *Terminalia arjuna* which is a well known cardiotonic to conventional and contemporary science while *Ghrita*, the base of the formulation is now proved to increase good cholesterol in blood stream of the patient [3]. Therefore it should be explored more at pharmaceutico-analytical, experimental and clinical level. Here's an attempt to study the pharmaceutico-analytical variables of *Arjuna ghrita* when prepared in accordance with classical Ayurvedic protocols.

Materials and Methods

Pharmaceutical study

Preparation of *Arjuna Ghrita*.

Whole of the raw material was procured from local market of Haridwar, Uttarakhand. The *Arjuna Ghrita* mentioned in *Ayurvedic* text *Chakradutta*[1] is prepared following the definition of *Sneha* pharamceutics according to *Sharangdhar Samhita*[2]. *Murchhana* was performed with *Emblica officinalis, Terminalia chebula, Terminalia bellerica, Curcuma longa* linn., *Cyprus rotandus* and *Citrus medica* as mentioned in *Bhaishajya Ratnavali*[4]. Prepare the *Kalka* (paste) of fresh bark of *Terminalia arjuna* by grinding. After that, *Swarasa* (juice) is prepared by the alternate method of juice extraction by boiling mentioned in classics measured to 8 litre. Now, *Murchhita Ghrita* was poured in a big wide mouthed stainless steel container and kept over fire for heating. *Ghrita* was heated till characteristic vapour having smoke on the heated *Ghrita* was observed. The vessel was then removed from fire and *Kalkas* and *Swaras* was added. The whole mass was again put on fire & heated on mild fire so as to evaporate the water content completely. Stir the sludge like mass continuously during whole process to avoid adhesion with

the help of ladle. This process was performed for 3 days on mild fire.

After attaining the *Sneha Siddhi Lakshan* the fire was withdrawn and the *Ghee* was filtered by help of a new previously washed and dried cloth when it is lukewarm. The same process was adopted for preparation of three sample of *Arjuna Ghrita*. The temperature ranges during complete process was fluctuating from 70-80 ^{o}C. while before adding *kalka* it was noted to be in range of 120 ^{0}C to 130 ^{0}C and after adding *kalka* it was noted between 100-110 ^{0}C.

Note: Same procedure was adopted for preparation of other two samples of *Arjuna Ghrita* and coded as S1,S2 and S3.

Observations

- During preparation, it becomes sludge like.
- After continuous stirring, *ghrita* starts to separate from *kalka*.
- Yellow coloured *Arjuna Ghrita* was obtained.
- The *Kalka* was looking smooth dense mass accumulated in form of bolus separated from layers of *Ghrita*
- When rolled between two fingers, the *Kalka* becomes wick (*varti*) like
- On sprinkling the *kalka* on fire, no sound observed
- Characteristic color, smell and taste were obtained

Analytical study of *Arjuna Ghrita*.

Analysis of organoleptic characters [5]

For Appearnce: 1gm of prepared *Arjuna Ghrita* was taken into watch glass and placed to watch through naked eye to observe the color into white light.

For Odour: 2g sample was smelled for odour.

For Taste: Pinch of subject formulation is taken and its taste was estimated on taste buds of tongue.

ForTouch: 2g sample was taken and rubbed against thumb, index finger and middle finger gently.

Analysis of Physico-chemical parametres

Specific Gravity: A specific gravity bottle of 25 mL capacity was cleaned through with freshly prepared chromic acid, distilled water, dried and weighed. It was filled up with distilled water and weighed again. The water has withdrawn from the bottle, it was dried, cooled and filled with sample of ghee and weighed. The weighing process was performed in triplicate and from these three successive readings, the specific gravity of the ghee samples was calculated by following expression. The similar procedure was adopted for analyzing the specific gravity of the samples at 40^{0}C of the stability study. [6]

$$\text{Specific gravity of ghee} = \frac{\text{Wt. of Ghee (in gms)}}{\text{Wt. of equal Vol. of distilled water (in gms)}}$$

Refractive Index: It was determined by Abbe refractometer (portable RA-130). For this, the sample of ghee was dropped over the prism after complete cleaning of the prism. The prism was filled with the sample liquid up to the line on sample stage. The measurement was made at that position on which the crossed horizontal line dissected the two half contrasts of a circle and aligned with the scale on refractometer. The refractive index of the stability samples was performed at frequent time intervals as per stability guidelines in which the measurement was performed at 40^{0}C. For maintaining the thermal conditions, the pre-warmed water at 40^{0}C was circulated through tubing of the refractometer all-around the sample to be studied. [6]

Iodine Value: About 10 mL of the fatty sample was dissolved in chloroform, to an iodination flask and labeled as test. To this ample, a 20 mL of iodine monochloride reagent was added to this flask and mixed thoroughly. Afterwards, the flask was maintained in dark condition for half an hour for incubation. The blank was also prepared by applying the similar method using 10 mL of chloroform. To the blank, 20 mL of iodine monochloride reagent was added and the contents of the flask were mixed homogenously. Afterwards, the blank was also incubated for 30 min. After incubation, 10 mL of potasium iodide was added to the flasks containing test and blank. The stopper and the walls of the flasks were rinsed by adding 50 ml of the distilled water. The test solution was titrated against sodium thiosulphate until a pale straw colour was observed. About one ml of starch solution was added to the falsk and a purple colour was developed. The titration was continued until the purple colour of the flask was turned into colourless and it indicated the endpoint of the titration. Similarly, the end point for the blank was also determined. [7] The actual volume of sodium thiosulphate consumed by the sample was calculated: volume of sodium thiosulphate consumed by blank (ml)- thiosulphate consumed by test (ml). The iodine value of the sample was calculated by applying the following expression-

$$\text{Iodine No. of fat} = \frac{\text{Equivalent Wt. of Iodine} \times \text{Volume of } Na_2S_2O_3 \text{ used} \times \text{Normality of } Na_2S_2O_3 \times 100 \times 10^{-3}}{\text{Weight of fat sample used for analysis}(g)}$$

Saponfication Value: A 1 g of each of the sample was taken in beaker and dissolved in 3 mL of ethanol. Quantitatively the contents of the beaker were transferred by washing successively three times with 7 mL of solvent. A 25 mL of 0.5N alcoholic KOH was also added, mixed well and attached to a reflux condenser. Other reflux condenser set was also used for the blank prepared as above in which all the reagents were added except the fatty material. These flasks were placed in a boiling water bath for 30 min. afterwards; these were cooled down at room temperature and phenolphthalein indicator was added. The contents of the flasks were titrated with 0.5 N HCl. The endpoint of sample and the blank were noted down and the difference between the blank and test readings provided the number of milliliters of 0.5N KOH required to saponify the fatty material. [8] The weight of potassium hydroxide (mg) consumed by 1g of fatty sample indicated the saponifiaction value of the sample.

Peroxide Value: A 2g of the sample was taken into a 100 ml glass stoppered Erlenmeyer flask and to it 12 mL of the acetic acid- chloroform solution was added. The contents of the flask were agitated vigorously until the sample was dissolve completely. To the flask, about 0.2 mL of saturated potassium iodide solution was added. The contents of flask

were swirled for one minute. Afterwards, 12 mL of the distilled water was added and mixed homogenously to liberate the iodine from chloroform layer. The solution of the flask was titrated with 0.1 N sodium thiosulphate solutions taken in a burette. The titrant was added slowly to the flak until the colour of the titre and was turned into light colour[9]. With help of the dispenser, 1 mL of starch indicator was added. The titration was continued until the deep grey colour was disappeared from the upper aqueous layer. The peroxide value of the sample was determined by following expression-

Peroxide value =
((S − B)×normality of sodium thiosulphate)/
(weight of the sample)×1000

Where, S is the volume of thiosulphate consumed in titration of sample and B the volume of sodium thiosulphate consumed in titration of blank.

Acid Value- About 5 g of each sample was weighed accurately and transferred into a 250 mL conical flask. To this, a 50 mL of neutralized alcohol solution was added. This mixture was heated for 10 min by heating mantle. Afterwards, the solution was taken out after 10 min and 1 or 2 drops of phenolphthalein indicator was added. This solution was titrated against KOH solution from the burette. The appearance of pink color indicated the end point. The volume of consumed KOH solution was determined and the titration of each sample was carried out in triplicate and the mean of the successive readings was used to calculate the acid-value of the respective sample by following expression. Previously, KOH aqueous solution used in this study was standardized for estimation its actual strength. Briefly, a 20 mL of 0.1 N aqueous oxalic acid was taken in a 250 mL conical flask in which 1 or 2 drops of phenolphthalein indicator was added. It was titrated against KOH taken in a burette. The appearance of pink color indicated the end point. From the volume of KOH solution consumed taken in burette, the normality of KOH was calculated [6].

Acid value = (Volume of KOH X Normality of KOH X Eq. wt X 1000) / Weight of *Arjuna Ghrita* sample (g)

Assay for Heavy Metals [10] - this was done following the Ayurvedic pharmacopeia of India.

Microbial Contamination [10]**-**this was done following the Ayurvedic pharmacopeia of India

Result and Discussion

During the preparation of *Arjuna Ghrita* the juice used is obtained by boiling method as because of involvement of heat, more of therapeutically active ingredient must be extracted and less bark is required. Temperature is one of the most important factor in the procedure so it is noted at different stages as shown in Table 1. The final yield obtained after attaining the definitive signs of prepared *Sneha*, the final yield of three samples is shown in table 2. The organoleptic characterstics observed for three samples of *Arjuna ghrita* are shown in Table 3. The results for tests of refractive index, specific gravity, iodine value, peroxide value, saponification and acid values are shown in Table 4. Refractive index and specific gravity are distinctive parameters of oleaginous substances. The degree of unsaturation of an oil, fat or wax is measured by Iodine value. Peroxide value is a deteriorative change depends on level of unsaturation, packaging material and storage condition. It increases on storing ghee at room temperature as well as on increasing temperature. Other than the formation of off-flavors and odors, another reason to avoid hydrolytic rancidity is that the reactions of hydrolysis supply free oleic, linoleic, and linolenic acids that could then undergo further oxidative rancidity. Depending on the fatty acid, saponification value is expressed by potassium hydroxide in mg required to saponify one gram of fat. This parameter is indicator of free acidic groups available in the fatty matter. Acid value is a measure of the content of free fatty acids in the vegetable oil and describes the quantity of caustic potash solution which is necessary for the neutralization of the free fatty acids. Assay of heavy metals inferred all within prescribed limits shown in Table 5. The microbial study is shown in Table 6 showing complete absence of microbial contamination.

Table 1: **Temperature Variations at different stages during preparation of three samples of *Arjuna Ghrita***

Various Stages During Preparation	Temp. of S1	Temp. of S2	Temp. of S3
Before adding *Kalka Dravyas*	120^0 C	130^0 C	120^0 C
After adding *Kalka Dravyas*	100^0 C	110^0 C	110^0 C
During process of *Ghrita* formation	70^0 C	80^0 C	80^0 C

Table 2: Yield of three samples of *Arjuna Ghrita*

Sample	*MurchhitaGhrita (l)*	Amount of *ArjunaKalka*	*ArjunaSwaras(l)*	Obtained *Ghrita (l)*	% loss
S1	1.820	500g	8	1.740	4.4
S2	1.830	500g	8	1.753	4.2
S3	1.850	500g	8	1.776	4.0

Table 3: Organoleptic characters observed for three samples of *Arjuna Ghrita*

Organoleptic Character	S1	S2	S3
Appearance	Viscous soft mass	Viscous soft mass	Viscous soft mass
Colour	Yellow	Yellow	Yellow
Odour	Characteristic bitter	Characteristic bitter	Characteristic bitter
Touch	Unctuous	Unctuous	Unctuous
Taste	Slightly bitter	Slightly bitter	Slightly bitter

Table 4: Physicochemical Parameters observed for three samples of *Arjuna Ghrita*

Physicochemical Parameters	S1	S2	S3
Specific gravity	.8945	.9447	.9153
Refractive index	1.4576	1.4587	1.4543
Iodine Value	30.4561	31.3443	31.2808
Saponification Value	129.03	133.95	130.43
Peroxide Value	1	1.5	2
Acid Value	0.561	1.122	1.4025

Table 5: Heavy Metal Assay of three samples of *Arjuna Ghrita*

Heavy Metal	S1	S2	S3
Lead	0.0976ppm	0.0989pm	0.103ppm
Arsenic	0.023ppm	0.21ppm	0.26ppm
Cadmium	0.0056ppm	0.0546ppm	0.0575ppm
Mercury	0.095ppm	0.012ppm	0.010ppm

Table 6: Microbial study of three samples of *Arjuna Ghrita*

Name of Test	S1	S2	S3
Total Microbial Count	20 Cfu/gm	25 Cfu/gm	25 Cfu/gm
Total yeast & Mould count	Less than 10Cfu/gm	Less than 10Cfu/gm	Less than 10Cfu/gm
E. Coli	Absent/gm	Absent/gm	Absent/gm
Salmonella	Absent/gm	Absent/gm	Absent/gm
Pseudomonas aeruginosa	Absent/gm	Absent/gm	Absent/gm
Staphylococcus aureus	Absent/gm	Absent/gm	Absent/gm
AflatoxinB1	Absent	Absent	Absent
AflatoxinG1	Absent	Absent	Absent
AflatoxinB2	Absent	Absent	Absent
AflatoxinG2	Absent	Absent	Absent

CONCLUSION

The *Arjuna ghrita* is first mentioned in Ayurveda with least ingredients to manage all kinds of cardiac disorders. When classically prepared, it gives a good yield with average loss of 4.2 percent. *Arjuna Ghrita* is unctuous, viscous soft mass of yellow color with slightly bitter taste and characteristic bitter odour. The calculated mean for different tests values are 0.9181 for specific gravity, 1.456 for refractive index, 31.026 for Iodine value, 131.13 for saponification value, 1.5 for peroxide value and 1.02 for acid value. Heavy metal assay and microbial study comes under prescribed limit. As the formulation is still in texts only it needs pre-clinical and clinical studies to implement it as a potent cardiotonic and magnificent remedy for management of broad spectrum of cardiovascular diseases.

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