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STANDARDIZATION OF SAPTA AVARTHITA ASHWAGANDHA GHRITA FORMULATION BY TLC METHOD

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INTRODUCTION

Ghrita is one of the Ayurvedic drugs that contain ghee as the base to dissolve or extract or hold the active therapeutic principles from the ingredients. *Ghritas* are medicated ghee preparations containing the fat-soluble components of the ingredients used in these preparations. The principle of preparation is the protracted boiling of ghee with prescribed *kashayas* (decoctions) and *kalkas* (a fine paste of the drug/drugs) to dehydration or near dehydration thereby effecting the transference of the fat-soluble principles to the *ghrita*, from the drug ingredients or *kashayas* or *swarasas* as the case may be according to the formulation.[1]

Ghrita's lipophilic property facilitates drug delivery to mitochondrium microsome and nuclear membrane of the cell and are also used as *rasayana*. *Ashwagandha* is *brumhaniya, balya*, its active component is alkaloids and withanoloids which has medicinal characters and helps in maintain general health and wellness.[2]

Various studies carried out on different formulations of *ashwagandha* such as *ashwagandha* granules, *ashwagandha arishta* and its standardization. Here in this study *Sapta avarthita Ashwagandha ghrita* was prepared and its physico-chemical study was carried out. Ashwagandha ghrita is best rasayana and useful in vata vyadhis and in sapta avrthana it gets more potencified. Ashwagandha is one of the reputed herbs in Ayurveda and has many actions on body like anti-ageing, adaptogenic, immunomodulatory, anxiety, depression, stress, cardiovascular protection, hypothyroidism to name a few.[3] It contains alkaloids and steroidals lactones, many bio-chemical heterogeneous alkaloids, including choline, tropanol, pseudotopanol, cuscokygrene, 3-tigioyloxytropana, isopelletierine and several other steroidal lactories, withanolides and several sitoindosides.[4,5]

Method of Preparation

In preparation of *Ashwagandha ghrita* and *Sapta avartita Ashwagandha ghrita*, *Ashwagandha*, *Triphala*, *Musta*, *Haridra*, *Goghrita*, *Godugdha* was used.

Ashwagandha ghrita was prepared by *snehapaka vidhi*. *Murchana* of *ghrita* was done before preparing *Ashwagandha ghrita* to remove impurities of *ghrita* and to make it fragrance and colour full.[6]

Preparation of *Murchita Ghrita*[7]

In the ratio of 1:4:16, *kalka*, *Ghrita* and *drava dravyas* are taken and subjected to *snehapaka* till the appearance of *sneha siddhi lakshanas*. *Kalka dravyas* added are *Tripahala*, *Haridra*, *Musta* and *Nimbu swarasa*. The quantity of ingredients are *Kalka*- 3.150kg in coarse form, *ghrita*- 12 kg, *Drava dravya*- water 44 lit.

These ingredients were taken into a big sufficient vessel and subjected to medium flame. Continuous stirring was done to prevent the sticking of *kalka* to the bottom of the vessel.

Preparation of *Ashwagandha ghrita*[8]

The ingredients are *Ashwagandha kalka* 2.570 kg, *Murchita ghrita* 10.300kg, *Ashwagandha Kwatha* 40lit and *ksheera* (milk) 11 lit.

The above ingredients were taken into a big vessel (15 kg) and subjected to mild flame and continuous stirring was done till getting *sneha siddhi lakshanas*. After the evaporating of water only ghee and *kalka* remained, which became like brown mud paste. This was the stage to observe intensely, after this slowly ghee separates from the *kalka* and good quality smell generates. Once the *kalka* becomes bolus form, it should be taken into the hand and rolled in between the fingers to make *varti* (roll) and subjected to fire (candle light) to assess *madhyama sneha siddhi lakshana*.

After *samyak paka*, the ghee was taken off from the fire and filtered when it is in liquid form. The final product was measured and subjected for further process.

Preparation of *Sapta avrthita Ashwagandha ghrita*
The ingredients are *Ashwagandha kalka* 1.300 gm, 6 *avarthita ashwagandha ghrita* 6.500gm, *Ashwagandha Kwatha* 24lit and milk 6.5 lit.

The *snehapaka vidhi* mentioned earlier in *Ashwagandha ghrita* was carried out during *sapta Avartana*. All the formulations prepared in KLEU's GMP certified Pharmacy, Belgaum.

Analytical Study
Authentication of all ingredients and analysis of *Sapta avarthita Asshwagandha ghrita* was done at AYUSH approved drug testing laboratory, KLEU's Shri BMK Ayurveda Mahavidyalaya, Belgaum. The following analysis were done by adopting standard protocols.[9]

Refractive Index
The refractive index is measured at 25^0C (±0.5) with reference to the wavelength of the D line of sodium (λ 589.3 nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.

The Abbe's refractometer is convenient for most measurements of refractive index. To achieve accuracy, the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 25^0C. After every step part was cleaned with Diethyl ether, which was evaporated by own. Thin layer of *Sapta avarthita Ashwagandha ghrita* sample was applied by a cotton swab.

Specific Gravity
Empty Specific Gravity bottle was weighed and the bottle filled with distilled water and again weighed, the same bottle was then filled with 1% of sample and weighed. All these 3 weights were noted at 25^0C temperature, the Specific Gravity of sample was calculated.

Determination of Saponification Value
2g of *ghrita* taken into a 250 ml RB flask fitted with a reflux condenser. Added 25 ml of 0.5 M Alcoholic potash and reflux on a water bath for 30 minutes. Cool, add 1 ml of Phenolphthalein solution and titrate immediately with 0.5 Hydrochloric acid. Repeat the operation omitting the substance being examined. The values of samples were calculated.

Determination of Acid Value
10 g of *ghrita* sample taken in a conical flask and added 50 ml of acid free alcohol ether mixture (25 +25 ml) previously neutralized with the 0.1 M potassium hydroxide solution. Shaked well. Added one ml of Phenolphthalein solution and titrate against 0.1 M Potassium hydroxide solution. End point is the appearance of pale pink colour. The values were calculated.

Determination of Moisture Content (Loss on Drying)
5.140 g of *ghrita* in a previously weighed petri dish was taken and kept in an oven at 105^0C for 5 hours. Cooled in desiccators and weighed. the percentage of loss in weight of the sample was calculated.

Determination of Iodine Value
Ghrita 2gm taken in dry 500 ml iodine flask, added 10 ml of carbon tetrachloride and dissolved. Added 20 ml of iodine monochloride solution, inserted the stopper and allowed to stand in the dark at a temperature between 15^0C and 25^0C for 30 minutes. Placed 15 ml of potassium iodide solution in the cup top, carefully removed the stopper, rinsed the stopper and the sides of the flask with 100 ml of water, shake and titrate with 0.1 M sodium thiosulphate using starch solution, added towards the end of the titration, as indicator. Noted the number of ml required. Repeated the procedure omitting the substance being examined and noted the number of ml required.

Qualitative Analysis by Thin Layer Chromatography[9]
The T.L.C is the important and simple analytical tools for the qualitative analysis of the raw materials.

Thin Layer Chromatography
T.L.C is one of the most widely used techniques for rapid identification of drugs and its formulations. It is equally applicable to the drugs as raw material state and pure state.

Chromatographic conditions
The Alcoholic extract of *Sapta avartita Ashwagandha ghrita* was subjected for thin layer chromatography as follows,

Preparation of TLC: Pre coated Silica Plate was used.

Sample Preparation: The extracts obtained after Alcoholic extraction and used for TLC. Plate/Stationary Phase-Silica gel G. Solvent front run up to 8 cm. Applicator – Capillary tube. Solvent/Mobile phase – Toluene: Ethyl acetate (7: 3) through trial-and-error method. This solvent system holds good and clear appearances of bands (spots) were seen. Detection was done by keeping plate in Iodine vapour chamber.

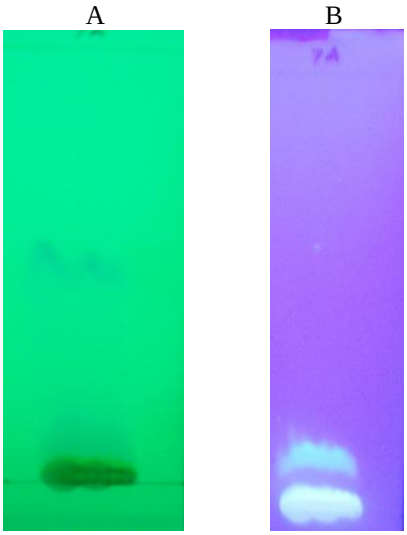
RESULTS

Table 1: Showing the results of Physico-chemical analysis of *Sapta avarthita Ashwagandha ghrita.*

Samples	Refractive Index	Specific Gravity	Saponification Value	Acid Value	LOD	Iodine Value
Sapta avarthita Ashwagandha ghrita	1.45	0.901	217.62	1.455	0.408	25.82

Table 2: Showing the Rf value of *Sapta avarthita Ashwagandha ghrita.*

Sample	Extract	Solvent system	Spots at 254nm	Spots at 366nm
Sapta avarthita Ashwagandha ghrita	Methanol Extract	Toulene: Ethyl Acetate (7:3)	0.08, 0.13, 0.52	0.05, 0.11, 0.15



A- Sapta avarthita Ashwagandha ghrita TLC at 254nm
B- Sapta avarthita Ashwagandha ghrita TLC at 366nm

Fig. No. 1: Showing the TLC of Sapta avarthita Ashwagandha ghrita.

DISCUSSION

Ghrita is one among best *Ajasrika rasayana*, it is *Ayuvardhaka balavardhaka ojavardhaka, vayasthapaka dhatuposhaka* and best among the *sneha dravyas*.[10]

Ashwagandha is an Ayurvedic herb and many studies have been done on its therapeutic potential and is very reputed drug in immunomodulation, ant-ageing and tonic for the body.

In the present study *Ashwagandha ghrita* was prepared by traditional method and was processed seven times to get *Sapta avarthita ashwagandha ghrita*. Physico-chemical analysis was performed and the values are discussed here.

Refractive index of *Sapta avarthita Ashwagandha ghrita* is 1.45 which indicates length of fatty acids. Specific gravity indicates the solute content in solvent and the value is 0.901. Saponification value 217.62. It indicates the average molicular weight/chain length of fatty acids present.[9]

The acid value of *Sapta avarthita ashwagangha ghrita* is 1.455. It is due to the drug effect which is added to the ghrita, due to the breaking in fatty acid chains acid value may decrease. Moisture content/Loss on drying 0.408. It indicates the water content.

Iodine value of *Sapta avarthita ashwagandha ghrita* is 25.82. The iodine value indicates the degree of unsaturation. The above quality parameters/ standardization parameters of *Sapta avathita ashwagandha ghrita* were shown in Table No 1. In which average values can be taken as standards.

TLC is mentioned as a primary tool for identification as part of monographs in all medicinal plants.[11] The spotted TLC was run with the solvent system Toulene (7ml) and Ethyl acetate (3ml). And it was viewed under long wave UV light at 366nm and short wave UV light at 254nm. Rf values at 254nm UV noted at 0.08, 0.13, 0.52, Rf values at 366nm UV noted at 0.05, 0.11, 0.15 (Table No.2) (Fig No.1)However, this formulation should be standardized by HPTLC, HPLC and pharmacokinetic profiling methods by using markers. These studies are suggested for future.

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