

The Physico-chemical analysis of Ashwagandha ghrita and analysis of every Avarthana upto Saptaavarthita Ashwagandha ghrita

*Manjiri N Patil, **Nitin B Patil, ***Anil

Abstract

Ashwagandha is the herb used for rejuvenation of whole body and shows immunomodulatory, adaptogenic and several other activities. 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.

Here in this study Ashwagandha ghrita was prepared and processed seven times to get Saptaavarthita Ashwagandha ghrita. The physico-chemical analysis of ashwagandha ghrita and each avarthitaghrita sample was done. By repeated processing of the same ghrita the final product gets potentified and useful for therapeutic purposes. To standardize the preparations TLC of Ashwagandha ghrita and Saptaavarthita Ashwagandha ghritawas carried out and details of its preparation and its physico chemical analysis has been discussed in this study.

Key words

Immunomodulatory, Avarthana, Physico-chemical analysis, TLC

Introduction

Ghrita is one of the Ayurvedic formulations 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 mitochondrion, microsome and nuclear membrane of the cell and are also used as rasayana. Ashwagandha is brumhaniya, balya, its active component are alkaloids and withanolides 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 Saptaavarthita Ashwagandha ghrita was prepared and its physico chemical study was carried out. Ashwagandha ghrita is best rasayana and useful in vatavyadhis and in saptaavarthana it gets more potentified. 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 steroidal lactones, many bio-chemical heterogeneous alkaloids, including choline, tropanol, pseudotropanol, cuscorygrene, 3 - tigloyoxytropina, isopelletierine and several other steroidal lactones, withanolides and several sitoindosides.^{4,5}

Method of Preparation

In preparation of Ashwagandha ghrita and Saptaavarthita Ashwagandha ghrita, Ashwagandha, Triphala, Musta, Haridra, Goghrita, Godugdha were used. The Ashwagandha ghrita, Saptaavarthita Ashwagandha ghrita prepared in KLEU's GMP certified Pharmacy, Belgaum.

Ashwagandha ghrita was prepared by snehapakavidhi. Murchana of ghrita was done

*Assistant Professor, Dept of Panchakarma, Shri Acharya Deshbhushan Ayurvedic Medical College, Bedkihal, Dist:Belgaum., **Physician, Punarnava Ayurved Panchakarma Clinic, Ichalkaranji., ***Professor, Shri J.G.Co-operative Society's Ayurvedic Medical College, Ghatprabha, Karnataka

before preparing Ashwagandha ghrita to remove impurities of ghrita and to make it fragrance and colorful.⁶

Preparation of Murchita Ghrita⁷:

In the ratio of 1:4:16, kalka, Ghrita and dravadravyas are taken and subjected to snehapaka till the appearance of sneha siddhi lakshanas. Kalka dravyas added are Tripahala, Haridra, Musta and Nimbushwarasa. 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 ghrita⁸:

The ingredients are Ashwagandha kalka 2.570 kg, Murchitaghrita 10.300kg, Ashwagandha Kwatha 40lit and ksheera(milk) 11 lit. Fresh godugdha(cow milk) was procured from local market and analysis was done for cereal starch, sugar and urea at AYUSH approved drug testing laboratory, KLEU's Shri BMK Ayurveda Mahavidyalaya, Belgaum.

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 madhyamasneha siddhi lakshana.

After samyakpaka, 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.

All the formulations were prepared in KLEU's GMP certified Pharmacy, Belgaum.

Preparation of SaptaAvartita Ashwagandha ghrita:

Table No1: Showing ingredients of Avarthita Ashwagandha ghrita during each Avarthana

Name of the Preparation	Quantity of Kalka in grams	Quantity of Ghrita in Kg	Quantity of Dravadravya in Liters		Quantity of Final Product in Kg
			Kwatha	Milk	
1Avartana	1.870	7.5	30	7.5	6.960
2Avartana	1.750	7	28	7	6.920
3Avartana	1.710	6.8	28	7	6.617
4Avartana	1.680	6.5	26	6.5	6.750
5Avartana	1.650	6.500	26	6.5	6.910
6 Avartana	1.380	6.500	24	6.5	6.740
7 Avartana	1.300	6.500	24	6.5	6.861

The snehapakavidhi mentioned earlier in Ashwagandha ghrita was carried out during every Avartana. From one to 3rdAvartana the final quantity of final product was reducing but from 4th to 7thAvartana process increase in the quantity of final product was observed. From 4thAvartana process ghee was becoming Yellowish to light yellow and became viscous. The loss of ghee due to kalka was replaced with the milk.

Analytical Study:

Authentication of all ingredients and analysis of both the formulations were 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°C (±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 was used for determining the refractive index. To achieve accuracy, the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 25°C. After every step part was cleaned with Diethyl ether, which was evaporated by own. Thin layered of each 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 at and weighed. All these 3 weights were noted at 25°C temperature, the Specific Gravity of sample was calculated.⁹

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°C 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 150C and 250C 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 Chromatography⁹:

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 Ashwagandha ghrita and Saptaavarthita 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.

Analytical study results:

Table No 2: Showing the results of Physico-chemical analysis of Ashwagandha ghrita and each avarthana till Saptaavarthita Ashwagandha ghrita

Samples	Refractive Index	Sp. Gr	Saponification Value	Acid Value	LOD	Iodine Value
Ashwagandha ghrita	1.46	0.904	218.02	1.600	0.460	26.80
1Avertana	1.450	0.589	214.41	1.232	0.628	26.340
2 Avertana	1.451	0.8986	217	1.120	0.650	25.810
3 Avertana	1.459	0.899	218.51	1.234	0.486	25.466
4 Avertana	1.45	0.9012	217.62	1.458	0.462	25.82
5 Avertana	1.45	0.9032	218.24	1.246	0.421	26.185
6 Avertana	1.45	0.899	218.51	1.237	0.419	25.46
7 Avertana	1.45	0.901	217.62	1.455	0.408	25.82

Table No 3: Showing the Rf value of Ashwagandha ghrita and SaptaAvarthita Ashwagandha ghrita

Sample	Extract	Solvent system	Spots at 254nm	Spots at 366nm
Ashwagandha ghrita	Methanol Extract	Toulene : Ethyl Acetate (7:3)	0.07, 0.12, 0.42	0.05, 0.11, 0.15
Saptaavarthita Ashwagandha ghrita			0.08, 0.13, 0.52	0.05, 0.11, 0.15

Discussion:

- Refractive Index : The refractive index increases with increasing chain length and increasing unsaturation, whereas the density decreases with increasing chain length and decreasing unsaturation. When compared to plain ashwagandha ghrita to saptaavartita there were no significant changes found in this parameter (Table No.2). This indicates there is no change in length of fatty acids.
- Acid Value : Acid value of 4th and 7thAvartanaprocess was almost similar (Table No.2). When compared to Ashwagandha ghrita the acid value was increased in 7 avartitaghrita, due to the breaking in fatty acid chains, acid value may increase. Minimal increase in Acid value may be because of drug and indicates the increase of free fatty acids in the formulation.
- Iodine value : It indicates the degree of unsaturation, which is reduced in Saptaavartitaghrita. Decrease in iodine value to a minimal level indicates that conversion of unsaturated fatty acids to saturated fatty acids has occurred¹⁰. It was not significantly changed from single processed to saptaavarthitaghrita (Table No.2).
- Specific gravity indicates the solute content in solvent, Solute refers to the active principle from the drug. Saptaavarthitaghrita specific gravity was reduced compare to plain ashwagandha ghrita. It was decreasing from 2nd to 7 avartana process (Table No.2), may be due to the fortification and due to continuous boiling the guru guna might become laghu in this parameter.
- Saponification value was not changed significantly from one to seven avartana process (Table No.2). It indicates the average molecular weight/chain length of fatty acids present; it is reduced in saptaavarthitaashwagandha ghrita.
- Moisture content/Loss on drying signifies the moisture content of the substance⁹; it was more than 0.5% in 1st and 2nd Avarthita ashwagandha ghrita and was gradually reduced in further Avartana process (Table No.2). It indicates that the minimal water content in 1st and 2nd Avartitaghrita, when it is processed further water content was evaporated completely.

- The above quality parameters/standardization parameters of Avarthita Ashwagandha ghrita were shown minimal changes, in which average values can be taken as standards. The difference could be because of difference of fatty acids composition, density of separated fats and temperature used in ghrita preparation.

TLC analysis:

TLC of Ashwagandha ghrita and Saptaavartita Ashwagandha ghrita was developed by using Toulene: Ethyl Acetate as solvent system with 7:3 ratios. Rf values at 254nm UV rays were noted at 0.07, 0.12, 0.42 in Ashwagandha ghrita, 0.08, 0.13, 0.52 in Saptaavartita Ashwagandha ghrita, and Rf values at 366nm UV rays were noted at 0.05, 0.11, 0.15 in Ashwagandha ghrita, 0.05, 0.11, 0.15 in Saptaavartita Ashwagandha ghrita (Table No.3).

Acknowledgements:

The author would like to thank Dr. B.S. Prasad sir, Principal, BMK Ayurved College of Belgaum, for providing the facilities for Analysis of the ghrita. The author would like to acknowledge helpful discussions on the manuscript with Dr.Srinath Vaidya, Dr.Ajit Lingayat, Dr.R.V.Sanapeti, Belgaum for helping to complete this study.

References:

1. Dholakia M V, Vachharajani Y R, Shah D C, Bahadur M V and Subrata De J R A S 1992 13(3-4) 173
2. Rasayanasarvaswam-Dr.Santosh Belawadi, 1st edition, Nov 2011.
3. Unknown., Withaniasomnifera Monograph, Alternative Medicine Review., 2004,9,210- 213
4. Farzana Akhtar, Isolation And Structural Studies On The Chemical Constituents Of Withaniasomnifera, Fumaria Flabellata And X-Ray Diffraction Studies, Pakistan research repository., 1997, 275.
5. Mohammad H.M, Elisabeth M., Steroidal Lactones from Withaniasomnifera, an Ancient Plant for Novel Medicine, Molecules., 2009, 14, 2373-2393.
6. Kinnari Dhruve and Anand K.Chaudhary, Snehalakshana-A probable Pharmaceutical Explanation, Aryavaidyan, Vol. XX., No.3, Feb-Apr.2007.p.181-189.
7. Govindadas Sen, Bhaishya Ratnavali, Edited by prof.Siddhi Nandan Mishra, Jwara chikitsa 1266, Varanasi: Chaukhamba Surabharati Prakashan, Reprint.p.206.
8. Chakrapanidatta, Chakradatta, Edited by Indradev Tripathi, Ramanath Dwivedy, Vata vyadhichikitsa 90, Varanasi: Chaukhamba Sanskrit Bhawan, 2011.p.141.
9. Quality control manual for ASU drugs, Govt. of India, Dept of Ayush, Ministry of family welfare, Pharmacopeia laboratory, for Indian Medicine, GhaZiabad, 2008.p.40.
10. Dhurvey Y.R., Kawatikwar P.S, Sarkar P.M, Evaluation of Physico chemical properties of cow ghee before and after Hydrogenation, JCRGG, Jan-Mar 2012.