International Journal of Applied Ayurved Research ISSN: 2347- 6362

PHYSICO-CHEMICAL ANALYSIS OF *ASHWAGANDHA GHRITA*

Patil Manjiri N[1], Patil Nitin B[2]
[1]Assistant Professor, Dept of Panchakarma, Shri Acharya Deshbhushan Ayurvedic Medical College, Bedkihal, Dist:Belgaum,India.
[2]Associate Professor, Dept of Agada Tantra, Mai bhago Ayurvedic Medical College, Muktsar, India.

ABSTRACT: *Withania Somnifera*, also known as *Ashwagandha*, an important herb in *Ayurveda*, it has *rasayana* property, it promotes health and longevity by augmenting defence against disease and creating a sense of mental wellbeing. Various studies carried out on standardization of different formulations of *Ashwagandha*. Here in this study the Physico-chemical analysis of *Ashwagandha ghrita* was carried out. Pharmacognostically authenticated *Withania Somnifera* was used for the preparation of *Ashwagandha ghrita*. Fingerprints of Thin Layer Chromatography (TLC) was also carried out.

Keywords *Withania Somnifera*, *Ashwagandha ghrita*, Physico-chemical analysis, Chromatography.

INTRODUCTION: *Ghrita* formulation contains the fat-soluble components of the ingredients used in the preparations. The principle of formula is boiling of ghee with prescribed *kashayas* (decoctions) and *kalkas* (a fine paste of the drug/drugs) to the transference of fat-soluble principles to *ghrita,* from the drug ingredients 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 *Ashwagandha ghrita* was prepared and its physico-chemical study was carried out. *Ashwagandha ghrita* is best *rasayana* and useful in *vata vyadhis*. *Ashwagandha* is one among the best 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, withanolides.[4,5.]

METHOD OF PREPARATION:

In preparation of *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 liters.

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

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 packed. These formulations prepared at KLEU's GMP certified Pharmacy, Belgaum.

ANALYTICAL STUDY: Authentication all ingredients and the Physico-chemical analysis, TLC 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 was adjusted and maintained as the refractive index varies significantly with temperature.

The Abbe's refractometer is used for detecting 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 layered of *Ashwagandha ghrita* is 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.

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 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. Appearance of pale pink colour is the end point. 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 and carefully removed the stopper, the stopper and the sides of the flask were rinsed with 100 ml of water, shakes and titrated with 0.1 M sodium thiosulphate using starch solution which is 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 also applicable to the drugs as raw and pure state.

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

Preparation of TLC: Pre coated Silica Plate used.

Sample Preparation: The extracts obtained after Alcoholic extraction were 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 spots were seen. It was detected by Iodine vapour chamber.

RESULTS:

Table No 1: Showing the results of Physico-chemical analysis of *Ashwagandha ghrita*

Samples	Refractive Index	Specific Gravity	Saponification Value	Acid Value	LOD	Iodine Value
Ashwagandha ghrita	1.46	1.0904	218.02	1.600	0.460	26.80

Table No 2: Showing the Rf value of *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

DISCUSSION: In the present study *Ashwagandha ghrita* was prepared by traditional method and its Physico-chemical analysis was performed and the values are discussed here. Refractive index of *Ashwagandha ghrita* is 1.46 which indicates length of fatty acids. Specific gravity indicates the solute content in solvent and the value is 1.0904. Saponification value 218.02. It indicates the average molicular weight/chain length of fatty acids present.[9]

The acid value of *Ashwagangha ghrita* is 1.600. 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 0.460. It indicates the water content.

Iodine value of *Ashwagandha ghrita* 26.80. The iodine value indicates the degree of unsaturation. The above quality parameters/standardization parameters of *Ashwagandha ghrita* were shown in Table No 1. In which average values can be taken as standards. TLC of *Ashwagandha ghrita* was developed and Rf values at 254nm UV rays were noted at 0.07, 0.12, 0.42 and Rf values at 366nm UV rays were noted at 0.05, 0.11, 0.15(Table No. II) (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.

CONCLUSION:

Ghrita is one of best *Ajasrika rasayana*, it is *Ayuvardhaka balavardhaka ojavardhaka, vayasthapaka dhatuposhaka* and also best among the *sneha dravyas*.[10] Many ayurvedic preparations are in the form of ghee. It carries the therapeutic properties of herbs to all the body's tissues. *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. To standardize any formulation qualitative and quantitative analysis is done. The average values of Physico-chemical analysis of Ashwagandha ghrita and TLC can be considered as reference values for further studies.

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Corresponding Author: Dr. Manjiri N Patil,Assistant Professor, Dept of Panchakarma, Shri Acharya Deshbhushan Ayurvedic Medical College, Bedkihal, Dist:Belgaum,India

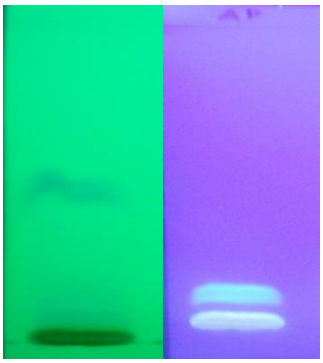
Email: vdnagasri@gmail.com,.

Source of support: Nil Conflict of interest: None Declared

Cite this Article as : [Patil Manjiri N et al : Physico-Chemical Analysis of Ashwagandha Ghrita]www.ijaar.in:IJAAR VOLUME IV ISSUE XI NOV–DEC 2020 Page No:1243-1246

Fig.No.1. Showing TLC of Ashwagandha ghrita

A B



A-*Ashwagandha ghrita* TLC at 254nm

B- *Ashwagandha ghrita* TLC at 366nm