IJPSR (2016), Vol. 7, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 July, 2015; received in revised form, 11 September, 2015; accepted, 13 November, 2015; published 01 February, 2016

STANDARDIZATION OF ASHWAGANDHA GHRITA: A HERBAL GHEE BASED AYURVEDIC MEDICINAL PREPARATION

Deepthi Viswaroopan* Arun Raj G. R. and Shailaja U.

Department of Kaumarabhritya, SDM College of Ayurveda and Hospital, Hassan - 573201, Karnataka, India.

Keywords:

Ashwagandha, *Withania somnifera* (L.) Dunal, Ashwagandha ghrita, standardization.

Correspondence to Author:
Dr. Deepthi Viswaroopan

Research scholar,
Department of Kaumarabhritya,
SDM College of Ayurveda and Hospital, BM Road, Thanniruhalla, Hassan - 573201, Karnataka, India.

E-mail: drdeepthiviswaroopan@gmail.com

ABSTRACT: Background: The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. Since Ayurvedic medicines come under the purview of Drugs and Cosmetics Act, there is increased general awareness about the necessity for developing standards for the purpose of quality control by the manufacturers as well as by the Drug control Authorities and for quality assurance to the public. Standardization of Ayurvedic formulations is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs. **Aim:** To standardize Ashwagandha ghrita. **Materials and methods:** Physico-chemical studies like Refractive index, Specific gravity, Rancidity, Boiling point / Melting point, Acid value, Saponification value, Iodine value and HPTLC were carried out as per the WHO guidelines, Ayurvedic Pharmacopoeia and Indian Pharmacopoeia. **Conclusion:** Standardization tests done on Ashwagandha ghrita helped in authenticating and ensuring the quality of the same.

INTRODUCTION: In the present context, herbal remedies are having a vital role in health care systems.[1] The quality assessment of herbal formulations is having huge importance in order to justify their acceptability in the current understanding of the scientific faternity. [2] The issues regarding quality, effectiveness and security have raised up with the increased usage of herbal medicines. [3] According to an estimate of World Health Organization (WHO), an approximately 85-90% of the world's population consumes traditional herbal medicines. [4, 5]

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(2).819-23

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (2).819-23

The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. [6] Since Ayurvedic medicines come under the purview of Drugs and Cosmetics Act, there is increased general awareness about the necessity for developing standards for the purpose of quality control by the manufacturers as well as by the Drug control Authorities and for quality assurance to the public. [7] Standardization of Ayurvedic formulations is an important step for the establishment of a consistent biological activity, a consistent chemical profile, or simply a quality assurance program for production and manufacturing of herbal drugs.[8]

At present, the quantity of raw material is not sufficient in the market. Most of the pharmaceutical industries are using substitute drugs instead of

authentic drugs thereby compromising the quality of the drug too. So to prepare best quality drugs it is necessary to authenticate raw drugs as well as prepared formulations. Keeping the current trend in mind, Ashwagandha (*Withania somnifera* (L.) Dunal) ghrita (ghee) [9] was subjected for standardization.

MATERIALS AND METHODS:

Physico-chemical studies like Refractive index, Specific gravity, Rancidity, Boiling point / Melting point, Acid value, Saponification value, Iodine value and HPTLC were carried out as per the WHO guidelines, [10] Ayurvedic Pharmacopoeia [11] and Indian Pharmacopoeia.[12]

Plant Material:

Withania somnifera (L.) Dunal was collected from the local market of Thrissur district, Kerala state, India in the month of March 2015. The collected drug was identified and authenticated at the teaching pharmacy of Department of Dravyaguna (Ayurveda Pharmacology), SDM College of Ayurveda and Hospital, Hassan, Karnataka state, India.

Methodology:

The studies were done at SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka state, India as per standard procedure.

Organoleptic characters:

Organoleptic characters like colour, odour and taste of the ghrita were documented. The study drug, Ashwagandha ghrita is shown in **Fig.1**.

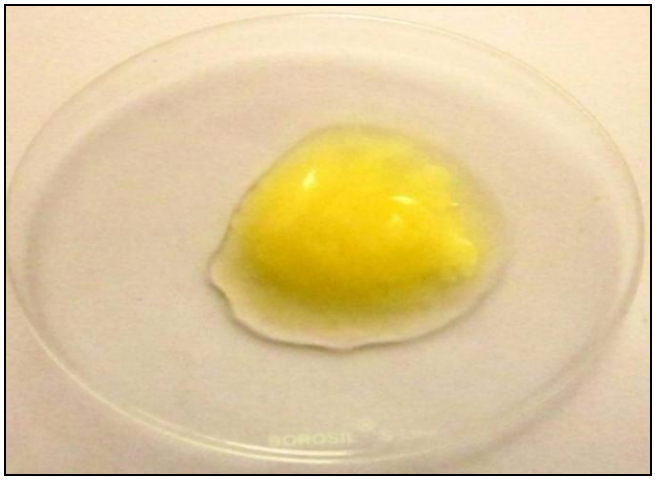


FIG. 1: ASHWAGANDHA GHRITA

Refractive index:

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre. Noted the reading. Distilled water has a refractive index of 1.3325 at 25˚C. The difference between the reading and 1.3325 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 28˚C.

Specific gravity:

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room Temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

Rancidity test:

1ml of melted fat was mixed with 1ml of conc. Hcl and 1ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

Determination of Acid value:

Weighed 10g of sample in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 + 25ml) previously neutralised by the addition of 1 ml of Phenolphthalein solution and titrated against 0.1N potassium hydroxide solution. End point was the appearance of pale pink colour which persists for 15sec. Repeated the experiment twice to get concordant values.

$$\text{Acid value} = \frac{56.1 \times \text{Titre} \times \text{Strength of Potassium hydroxide}}{\text{Weight of the Oil / Fat}}$$

Determination of Saponification value:

About 2g of the substance was weighed in tared 250 ml round bottom flask. 25ml of the alcoholic

solution of KOH was added and a reflux condenser was attached. Kept it for boiling on water bath for 1hr, the contents of the flask was rotated frequently. The flask was cooled and 1ml phenolphthalein solution was added and excess of alkali titrated with 0.5N HCl. The number of ml (a) required was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml required (b) was noted. The experiment was repeated twice to get concordant values.

$$\text{Saponification value} = \frac{56.1 \times (b-a) \times \text{Strength of Hydrochloric acid}}{\text{Weight of the sample taken}}$$

Iodine value:

The sample was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl_4, 20ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17^0 C for 30 min. 15ml of potassium iodide and 100ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

$$\text{Iodine value} = \frac{(b-a) \times 0.01269 \times 100}{w}$$

Melting point:

10gms of ghrita was taken in a 25ml beaker which was immersed in a preheated water bath the thermometer was dipped meanwhile in the beaker; the ghrita was stirred with glass rod so that uniform dissolution was obtained, the temperature at which the uniform dissolution of ghrita into a clear liquid was noted as melting point**.**

HPTLC:

1g of ghrita was partitioned with 10ml of methanol in a separating funnel; the methanol soluble portion was used further for HPTLC. 5 and 10µl of the above sample was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene – Ethyl acetate (9: 1) and the developed plates were visualized under 254 and 366 nm and after derivatisation in vanillin-sulphuric acid spray reagent and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded.

RESULTS AND DISCUSSION:

The Organoleptic characters of Ashwagandha ghrita is detailed in **Table 1**. The Physico-chemical parameters of Ashwagandha ghrita is detailed in **Table 2.** TLC photo documentation of Methanolic fraction of Ashwagandha ghrita is shown in **Fig.2**. R_f values of Ashwagandha ghrita is detailed in **Table 3**. Densitometric scan of Ashwagandha ghrita is shown in **Fig.3 and 4**. The physicochemical standards would serve as preliminary test for the standardization of the formulation. Tests such as Refractive index, Specific gravity, Rancidity, Boiling point / Melting point, Acid value, Saponification value, Iodine value, HPTLC, results of TLC photo documentation, the unique Rf values, densitometric scan and densitogram obtained at different wavelengths can be used as fingerprint to identify the herbal drug of Ashwagandha ghrita.

TABLE 1: ORGANOLEPTIC CHARACTERS OF ASHWAGANDHA GHRITHA

Parameters	Result
Color	Yellow
Odour	Characteristic
Taste	Acrid

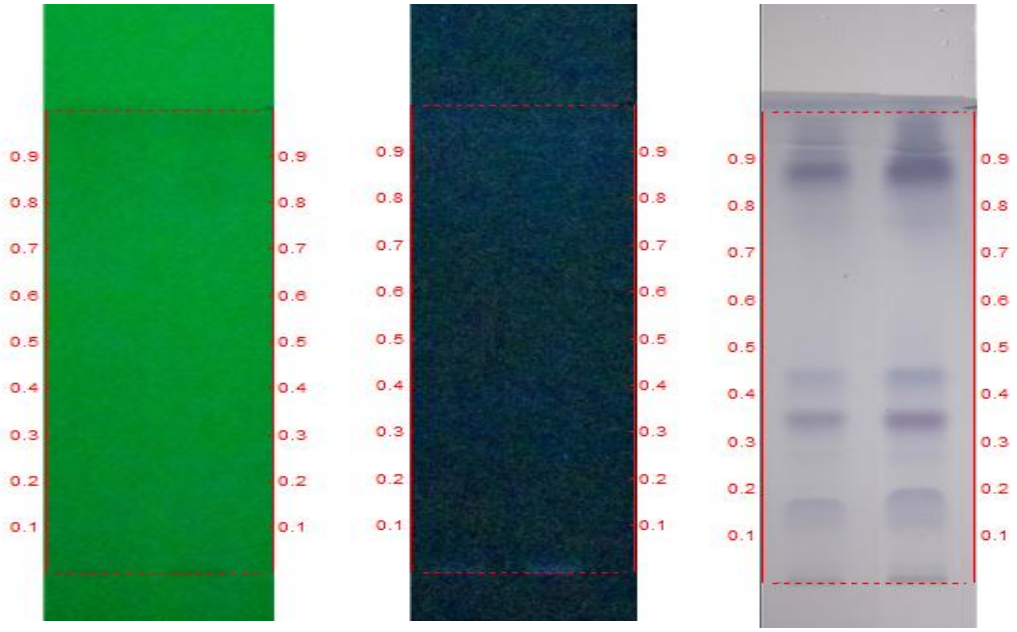
TABLE 2: PHYSICO-CHEMICAL PARAMETERS OF ASHWAGANDHA GHRITHA

S.no.	Parameters	Results n=3 %w/w
1	Refractive index	1.45682
2	Specific gravity	0.9052
3	Rancidity	Fat is not oxidised
4	Boiling point/Melting point	43ºC
5	Acid value	1.06
6	Saponification value	104.61
7	Iodine value	62.32
8	Melting point	-
9	HPTLC	-

TABLE 3: R_F VALUES OF ASHWAGANDHA GHRITHA

At 254nm	At 366nm	After derivatisation
-	-	0.13 (L. purple)
-	-	0.20 (L. purple)
-	-	0.27 (L. purple)
-	-	0.35 (D. purple)
-	-	0.44 (D. purple)
-	-	0.78 (D. purple)
-	-	0.88 (D. purple)
-	-	0.98 (D. purple)

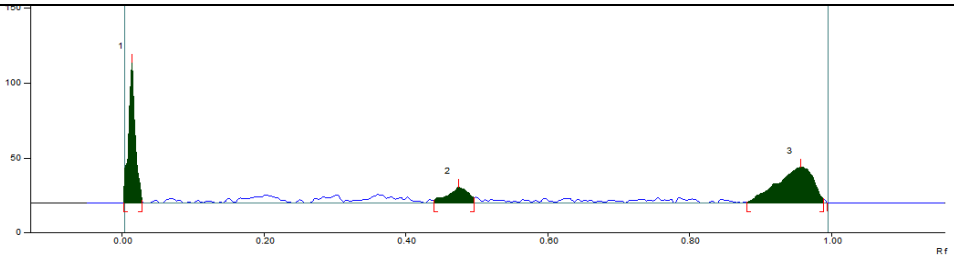
*D-Dark; L-Light



At 254nm **At 366nm** **After derivatisation**

Track 1- Methanolic fraction of Ashwagandha ghritha – 5µl
Track 2- Methanolic fraction of Ashwagandha ghritha – 10 µl
Solvent system: Toluene: Ethyl Acetate (9:1)

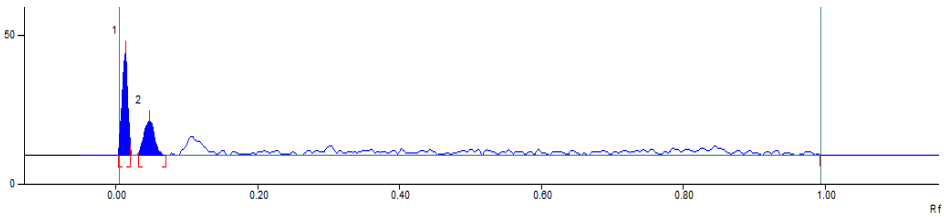
FIG.2: TLC PHOTO DOCUMENTATION OF METHANOLIC FRACTION OF ASHWAGANDHA GHRITHA



Track 4, ID: Methanol fraction of Ashwagandha ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	94.0 AU	73.10 %	0.03 Rf	2.0 AU	617.9 AU	36.01 %
2	0.44 Rf	1.6 AU	0.48 Rf	10.6 AU	8.24 %	0.50 Rf	3.7 AU	216.5 AU	12.62 %
3	0.88 Rf	0.4 AU	0.96 Rf	24.0 AU	18.66 %	0.99 Rf	3.1 AU	881.4 AU	51.37 %

At 254nm



rack 4, ID: Methanol fraction of Ashwagandha ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	34.7 AU	75.14 %	0.02 Rf	3.0 AU	193.0 AU	61.06 %
2	0.03 Rf	0.4 AU	0.05 Rf	11.5 AU	24.86 %	0.07 Rf	0.0 AU	123.1 AU	38.94 %

At 366nm

FIG. 4: DENSITOMETRIC SCAN OF ASHWAGANDHA GHRITHA

CONCLUSION: Despite the advent of modern technology in standardization of Ayurvedic formulations, only a few are standardized so far. With the current standardization procedure, we get substantial information for proper identification. The advancement of analytical techniques can serve as a specific tool in herbal drug research, thereby, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf- life of herbal drugs. The purpose of standardization of medicinal plants is obviously to ensure therapeutic efficacy. Therefore, maintaining the quality of these plant products is an essential factor. Ashwagandha (*Withania somnifera* (L.) Dunal) ghrita is an important drug with various biological properties. Hence, efforts have been made to provide scientific data on standardization of Ashwagandha (*Withania somnifera* (L.) Dunal) ghrita.

ACKNOWLEDGEMENT: Authors are highly grateful to the constant support and guidance of Dr. Prasanna N Rao, Principal, SDM College of Ayurveda, Hassan and Dr. Girish KJ, Research co-ordinator, SDM College of Ayurveda, Hassan. The Authors thank Dr. B. Ravishankar, Director, SDM Centre for Research in Ayurveda and Allied Sciences for providing the facilities and guidance.

REFERENCES:

1. Arun Raj GR, Shailaja U, Rao Prasanna N, Sharanesh T, Gokul J. Review on the contribution of Dashapushpa: a traditional medicine in the management of Cancer. Global J Res Med Plants & Indigen Med. 2013; 2(9):656-63.
2. Anand Chaudhary, Neetu Singh, Madhuri Dalvi, Asmita Wele. A progressive review of Sandhana kalpana (Biomedical fermentation): An advanced innovative dosage form of Ayurveda. Ayu. 2011 Jul-Sep; 32(3):408-417.
3. Waddar S, Rao PN, Gopi KBJ, Arun Raj GR, Waddar S. Standardization of Mulaka (*Raphenus sativus* Linn.) Kshara: a herbal alkaline preparation. J Pharmacogn Phytochem. 2014; 3(1):108-110.
4. N Kimmatkar, V Thawani, L Hingorani, R Khiyani. Phytomed. 2003; 10:3-7.
5. Arun Raj GR, Shailaja U, Prasanna N Rao, Ajayan S, Nivya P Thomas. Review on the contribution of Ura-Marunnu, A traditional baby care practice in Southern India. The Pharma Innovation. 2014: 2(11);42-70.
6. Muthamizhe Selvan K, Prakash Yoganandam G, Gopal V. Standardization of traditional medicine - Need and Urgency. Inter. J. of Phytotherapy 2013; 3(1):5-10.
7. Padmaja, Kannappan. Standardization of traditionally designed neutraceutical. International Current Pharmaceutical Journal 2012; 1(9):288-293
8. Amartya Bose, Krishanu De, Vikas Saroch. A review on latest developments in the standardization of ayurvedic drugs. IJPRBS 2012; 1(3):96-119.
9. Deepthi Viswaroopan, Arun Raj GR, Shailaja U, Dharmendra Maurya, Shradha Gawade, Shivanand P, Jithesh Raj KT. Preparation of Ashwagandha (Withania Somnifera (L.) Dunal) ghee - A practical approach inspired by traditional knowledge. The Pharma Innovation 2015; 4(4):85-89.
10. WHO. Quality Control Methods for Medicinal Plant Materials. Geneva: AITBS Publishers and Distributors, Delhi; 2002:65-67.
11. The Ayurvedic Pharmacopeia of India. Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga and Homoeopathy (AYUSH), New Delhi, Part-1; Edition 1, Vol.1-5; 2001.
12. Indian Pharmacopoeia. Ministry of Health and Family Welfare, Government of India, New Delhi, Vol. 1, 2010: 10-146.

How to cite this article:
Viswaroopan D, Arun Raj GR and Shailaja U: Standardization of Ashwagandha Ghrita: A Herbal Ghee Based Ayurvedic Medicinal Preparation. Int J Pharm Sci Res 2016; 7(2): 819-23.doi: 10.13040/IJPSR.0975-8232.7(2).819-23.