Journal of Ayurveda Physicians & Surgeons (JAPS)
(Peer Review International Journal)

Review Article
Case Report

Standardization of Matsyakshi Ghrita: A Herbal Ghee Based Ayurvedic Medicinal Preparation

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Access this article on online: www.japs.co.in
Published by International-Academy of Ayurveda-Physicians (IAAP),
7HB, Gandhinagar, Jamangar-361 002, Gujarat, India
Date of submission:15-01-2018;Date of Revision:21-02-2018;Date of Acceptance:01-03-2018

Abstract:
Standardization of drug means confirmation of its identity,qualityand purity throughout all the phases of its cyclewhich includes shelf life, storage, distribution and use of various parameters from ancient time to present day the basic source of medicines are from nature[1]. The traditional medicines cater about 85% of the world population for their health needs hence it is essential to maintain safety,quality and efficacy of the plant and their products to avoid serious health problems[2].Matsyakshi Ghrita a polyherbal Ayurvedic formulation is recommended as Chakshushya[3].The present study reveals the pharmacognostical identification of ingredients of Matsyakshi Ghrita and its physicochemical analysis. Pharmacognostical study reveals the quality and genuineness of both micro and macroscopic findings of Matsyakshi Ghrita.Acid value was 1.20,Saponification value 130.625, Refractive index value 1.45733at room temperature,Iodine value 41.52,Specific gravity 0.9480 at room temperature. Unique R_f patterns were recorded.*Matsyakshi Ghrita* was authenticated according to Ayurvedic and Indian pharmacopeial standards as its analysis was important to ensure the purity and strength of drug.

Key Words: *Matsyakshi, Althernanthera sessilis,Matsyakshi Ghrita, Standardization*

Introduction:

Standardization of Ayurvedic medicine starts from the collection of drug to the extreme clinical application. Earlier, Acharya's(Ayurveda physician) have contemplatedAyurvedic formulations based various parameters including rogi and roga bala.The quality control and standardization were based on organoleptic aspects like rupa (colour), sparsha(touch), rasa(taste) and gandha(smell)along with saviryatha avadhi(shelf life) of the same. Specific parameters were adopted in preparations of certain drug dosage forms like Ghrita (ghee), thaila(oil), avaleha, asava, arishta,vati(tablets), kashaya choorna(powder)which were inspected and tested based on above said organoleptic properties[4].

The increased growth in the usage of herbal products world wide people are aware of its potency and side effects; Due to commercialization, adulteration, extension and substitution of drugs, supply of genuine raw material has become doubtful, Properties of drug may have undergone changes due to time and environmental factors. Economy of large scale industrial production, distribution to long distances, shelf life and also to gain public trust and to bring Ayurveda in to mainstream of today's health care system have necessitated development of modern and objective standards for evaluation safety, quality and efficacy of Ayurvedic formulations[5,6].

Ayurvedic medicines come under the purview of drugs and cosmetics act, 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. Pharmacopea prescribes (numerical value) like structural, analytical, physical standards for the drugs[7, 8].

Materials and Method

Standardization of Matsyakshi Ghrita was carried out to know Physico chemical properties likeColor, Refractive index, specific gravity, viscosity,Acid value, Saponification value, iodine value, peroxide value and HPTLC[8. 9]. The results were compared with standard values of Matsyakshi and Ghrita as mentioned in AFI.

Collection of drug

Althernanthera sessilis(L) was collected and Ghrita was prepared from the Ashtanga Ayurvedics(p)LTD,Thayanur Chandai Trichy garden in the month of December 2016.The collected drug was identified and Authenticated at the teaching pharmacy of Department of Dravyaguna (Ayurveda pharmacology)**S**ri Dharmasthala Manjunatheshwara College Of Ayurveda and Hospital, Hassan – 573201,Karnataka,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.Sample coded as: 17090401is being maintained for further reference.

Organoleptic characters:

Organoleptic characters like colour,odour and taste of the Ghrita were documented.

Refractive index

To know the Refractive index of drug a drop of water was placed on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centreand noted the reading. Distilled water has a refractive index of 1.33217 at 28˚C. The difference between the reading and1.33217 gives the error of the instrument. If the reading is less than 1.3320, 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

Specific gravity was assessed by following procedure, the bottle was cleaned by shaking with acetone, then with etherand the bottle was driednoted the weight. Sample solution was cooled to room temperature and filled carefullyinto the specific gravity bottle which contains test liquid, stopper was inserted and then the surplus liquid is removed from it and weighed again. Later, The procedure was repeated using distilled water in place of sample solution.

Determination of Acid value

To determine the Acid value, Weighed 2- 10g of Matsyakshi ghritha was taken in a conical flask. 50 ml of acid free alcohol-ether mixture (25 +25ml) previously neutralised with the 0.1M potassium hydroxide solution added to it and shaken well;again,1ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solutionby adding to it. Pale pink colour appeared as the end point. Experiment was repeated twice to get concordant values.

Determination of Saponification value

To determine Saponification value,Weighed 2g of the Matsyakshi ghrithawas taken into a 250 ml RB flask which is fitted with a reflux condenser 25ml of 0.5M alcoholic potash is added to it, Refluxed on a water bath for 30 minutes. Cooled and added 1 ml of Phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). The operation was repeated omitting the substance being examined (blank) (b ml). Experiment was repeated twice to get concordant values.

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.

Determination of Unsaponifiable matter

Weighed 5g of the Matsyakshi ghrithawas taken in the flask and 50ml alcoholic KOH was added into the sample. Boiled gently but steadly under reflux condenser for one hour. The condensor was washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 50ml of water was added to the separating funnel followed by an addition of 50ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 50ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25ml of aqueous alcohol and shaken vigorously. The alcohol-water layer was drawn off after each washing. The ether layer was again washed repeatedly with 25ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath.

The flask was placed in an air oven at 85°c for about 1 hour to remove the last traces of ether. A few ml of acetone was added and evaporated to dryness on a water bath. Cooled indesiccators to remove last traces of moisture and then weighed.

Peroxide value

5g of the Matsyakshi ghritha was weighed accurately and taken into a conical flask, 30 ml of mixture of 3volumes of glacial acetic acid and 2 volumes of chloroform is added to it, along with 0.5ml of potassium iodide and allowed it to stand for 1 minute, 30ml of water was titrated gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow color disappears. Titration was continued by adding 0.5ml of starch indicator until blue color disappeared.

Peroxide value= 10(a-b)/W

Where W= weight in g of the substance

Viscosity

The given sample is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the formula

$$\eta 1 = \frac{\rho 1 t 1}{\rho 2 t 2} X \eta 2$$

$\eta 1$ – Viscosity of sample

$\eta 2$ - Viscosity of water

t1 and t 2- time taken for the sample and water to pass the meniscus

$\rho 1$ and $\rho 2$ – Density of sample and water

X= Specific gravity of sample x 0.9961/specific gravity of water

Π= XxTime for samplex1.004/specific gravity of waterx70sec

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.

Sample preparation for HPTLC:

Sample obtained in the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform this was followed for all the sample of Matsyakshi ghritha and chloroform soluble portion was used for HPTLC.

HPTLC:

4, 8, 12µl of the above sample of *Matsyakshi ghritha* 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 short UV, long UV, and after derivatisation in vanillin-sulphuric acid spray reagent it was visualized under white light and scanned under UV 254nm, 366 nm and 620nm. R_f, colour of the spots and densitometric scan were recorded.

Results and Discussion:

Pharmacognostical study reveals Authentification of drug Matsyakshi and is cross verified. Standardization is the process of developing and agreeing upon technical standards of Matsyakshi Ghrita Physiochemical partameters of Matsyakshi Ghrita is detailed in table – 2.TLC photo documentation of Methanolic fraction of Matsyakshi Ghrita is shown in fig – 2.R_f values of Matsyakshi Ghrita is detailed in table – 3.Densitometric scan of Matsyakshi Ghrita is shown in fig.3 and 4. The physicochemical standards would serve as preliminarytest for the standardization of the formulation, Tests such as Refractive index,Specific gravity,Rancidity,Boiling point/Melting point,Acid value,Sponification value,Iodine value,HPTLC,results of TLC photo documentation,the unique Rgf values,densitometric scan and densitogram obtained at different wavelengths which can be used as finger print to indentify the herbal drug of Matsyakshi Ghrita, All the results show that the prepared Ghrita formulation is not rancid (after 10 months of preparation) and the quality of Ghrita is standard.

Part C: Results

TABLE 1: ORGANOLEPTIC CHARACTERS OF MATSYAKSHI GHRITA

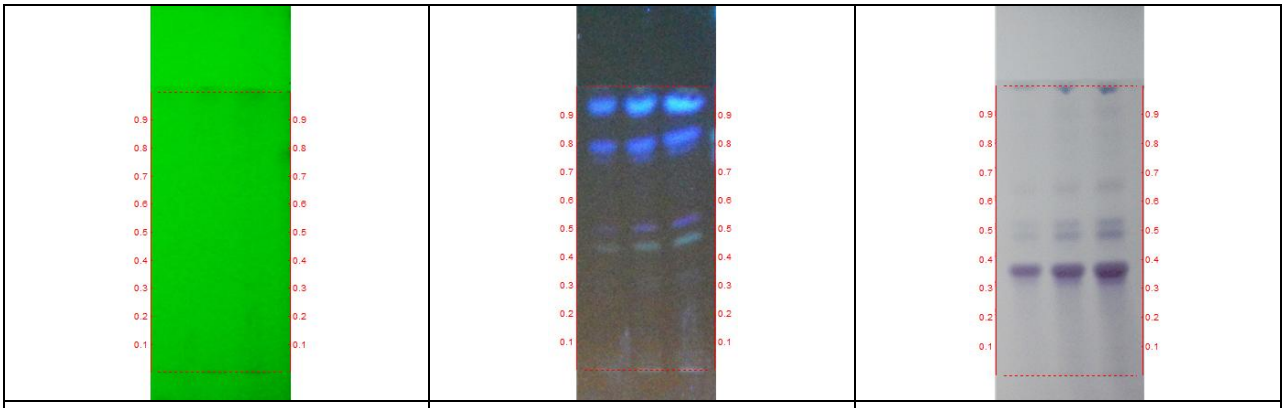
PARAMETERS	RESULT
Colour	Green
Odour	Characteristic
Taste	Acrid

TABLE 2:

PHYSICO-CHEMICAL CHARACTERS OG MATSYAKSHI GHRITA

Parameter	***Results n = 3 %w/w***
	Matsyakshi ghritha
Refractive index	1.45733
Specific gravity	0.9480
Acid value	1.20
Saponification value	130.625
Iodine value	41.52
Unsaponifiable matter (%)	0.96
Peroxide value	1.75
Rancidity	Fat is not oxidized

FIGURE –1:
TLC PHOTO DOCUMENTATION OF CHLOROFORM FRACTION OF MATSYAKSHI GHRITHA



Short UV	Long UV	At post derivatosation

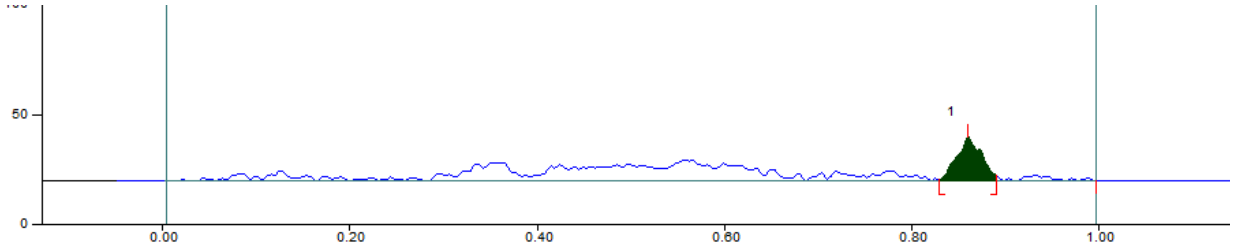
Track 1- Chloroform fraction of Matsyakshi ghritha- 4 µl
Track 2 - Chloroform fraction of Matsyakshi ghritha- 8 µl
Track 3- Chloroform fraction of Matsyakshi ghritha- 12 µl
Solvent system- Toluene: Ethyl acetate (9.0:1.0)

TABLE 3:
R_F VALUES OF THE SAMPLEOF MATSYAKSHI GHRITHA

Short UV	Long UV	Post derivatisation
-	-	0.37 (D. purple)
-	0.44 (FL. green)	-
-	-	0.49 (L. purple)
-	0.51 (FD. purple)	-
-	-	0.54 (L. purple)
-	-	0.66 (L. purple)
-	0.80 (F. blue)	-
-	-	0.84 (L. purple)
-	-	0.91 (L. purple)
-	0.94 (F. blue)	-

***D – dark; L – light; F - fluorescent**

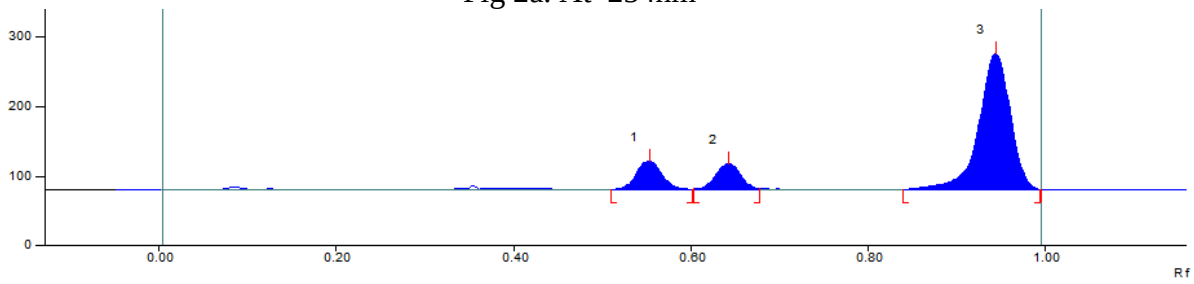
FIGURE – 2:
DENSITOMETRIC SCAN OF MATSYAKSHI GHRITHA



Track 7, ID: Matsyakshi ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Ar %
1	0.83 Rf	0.6 AU	0.86 Rf	20.1 AU	100.00 %	0.89 Rf	2.7 AU	382.8 AU	100.(

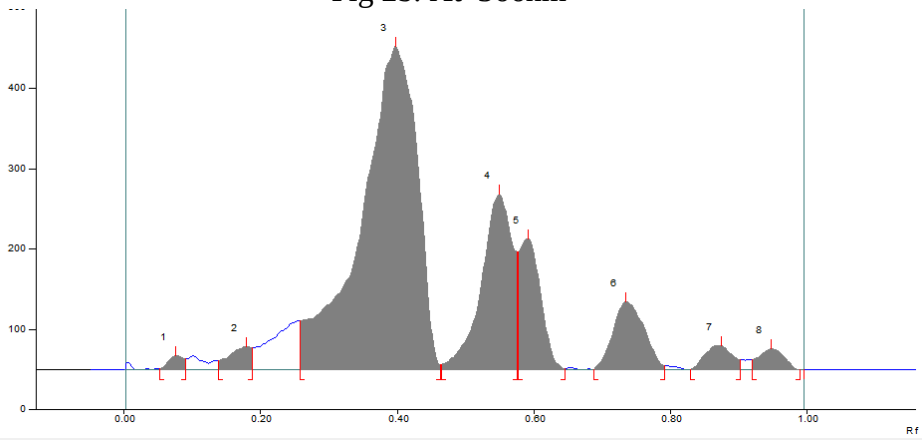
Fig 2a. At 254nm



Track 7, ID: Matsyakshi ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.51 Rf	0.6 AU	0.55 Rf	40.8 AU	14.90 %	0.60 Rf	0.9 AU	862.9 AU	12.99 %
2	0.60 Rf	1.0 AU	0.64 Rf	37.7 AU	13.79 %	0.68 Rf	1.5 AU	766.1 AU	11.54 %
3	0.84 Rf	0.6 AU	0.95 Rf	195.0 AU	71.31 %	1.00 Rf	0.2 AU	5012.2 AU	75.47 %

Fig 2b. At 366nm



Track 7, ID: Matsyakshi ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	1.5 AU	0.08 Rf	17.7 AU	1.83 %	0.09 Rf	13.6 AU	285.7 AU	0.72 %
2	0.14 Rf	11.2 AU	0.18 Rf	28.7 AU	2.96 %	0.19 Rf	27.5 AU	688.1 AU	1.73 %
3	0.26 Rf	61.0 AU	0.40 Rf	402.1 AU	41.47 %	0.46 Rf	6.0 AU	23759.0 AU	59.61 %
4	0.47 Rf	6.4 AU	0.55 Rf	218.2 AU	22.50 %	0.58 Rf	45.8 AU	7082.3 AU	17.77 %
5	0.58 Rf	145.8 AU	0.59 Rf	162.9 AU	16.80 %	0.65 Rf	0.9 AU	3711.5 AU	9.31 %
6	0.69 Rf	0.5 AU	0.74 Rf	84.5 AU	8.72 %	0.79 Rf	5.0 AU	2789.4 AU	7.00 %
7	0.83 Rf	0.2 AU	0.88 Rf	29.8 AU	3.07 %	0.90 Rf	11.8 AU	855.4 AU	2.15 %
8	0.92 Rf	12.3 AU	0.95 Rf	25.8 AU	2.66 %	0.99 Rf	0.1 AU	684.7 AU	1.72 %

Fig 2c. At 620nm

CONCLUSION:

In Ayurvedic system of medicine, standardization of Ayurvedic formulation is a big challenge even though advancement ofanalytical techniques has come up in standardization of Ayurvedic Formulations,these technique serve as a rapid and specific tool in the herbal research,allowing the manufacturers to set quality standards and confirmation of its identity,quality and puritythroughout all phases of its cycle regulatory authorities for therapeutic efficacy,safety storage, shelf life distribution and use of herbal drugs,for the quality assured Ayurvedic products,the physic-chemical standardization is most important including standardization by HPTLC for qualitative identification of active compounds in the formulation.HPTLC finger printing profile of Matsyakshi Ghrita reveals many active constituents at different R_f values. In the present investigation the set of data obtained can serve as standard for the identification of Matsyakshi Ghrita. To ensure the safety,efficacy and quality of the drug as well as in sustaining and reproducibility of drug the result will be helpful.

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Cite this article as: Shilpa Ghatge B.R(2018): Standardization of Matsyakshi Ghrita- A Herbal Ghee Based Ayurvedic Medicinal Preparation, Journal of Ayurveda Physicians and Surgeons; Volume 5(2), Page No 70.

Financial assistance: Nil; Conflict of interest: Not declared