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ANALYTICAL STUDY OF *SHADBINDU GRITHA* –A POLYHERBAL PREPARATION

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ABSTRACT

The purpose of drug standardization is to find the quality, efficacy and identity of products. *Shadbindu ghrita* is a polyherbal Ayurveda formulation used in rhinitis. The drug contains *Bhringaraja* (*Eclipta alba*), *Lavanga* (*Syzigium aromaticum*), *Yashtimadhu* (*Glyzirrhiza glabra*), *Kushta* (*Saussurea lappa*), *Shunti* (*Gingiber officinalis*) and *ghrita* (clarified butter). The drug was prepared in Sri Dharmasthala Manjunatheshwara Ayurveda Pharmacy, Udupi as per the standard protocol. In the present study the prepared drug was subjected to various analytical methods of standardization to establish its quality and purity. **Aim and Objective:** To study the organo leptic and physico chemical characters of *Shad Bindu ghrita*. **Materials and Methods:** Organo leptic characters, refractive index, saponification and acid value, rancidity, HPTLC etc were done as per the standard testing protocol. Results: the results obtained from the analytical study was within standard limits.

KEYWORDS: Standardization, Shadbindu ghrita, Rhinitis.

INRODUCTION

Formulations in the form of ghee are widely used to treat various diseases in clinical practice. *Shadbindu ghrita*[1], a polyherbal Ayurveda formulation in the form of ghee is used through intranasal route to treat rhinitis. The purpose of drug standardization is to establish the

quality, efficacy and identity of the product. *Shad Bindu ghrita* contains *Bhringaraja* (*Eclipta alba*), *Lavanga* (*Syzigium aromaticum*), *Yashtimadhu* (*Glyzirrhiza glabra*), *Kushta* (*Saussurea lappa*), *Shunti* (*Gingiber officinalis*) and clarified butter.[2] The drugs are washed thoroughly and wiped with a clean dry cloth. The wiped drug is taken in equal quantity and ground well into a soft paste. This was followed by addition of clarified butter and water. The mixture was placed over mild fire and stirred. Then the ghee is strained and stored in airtight container.

MATERIALS AND METHODS

The formulation was evaluated for organoleptic characters such as colour, appearance and odour by means of examination using sensory organs. The physicochemical characters such as refractive index, saponification value, acid value, unsaponifiable matter, rancidity test and iodine value that are generally applied for clarified cow's butter were assessed.[3] It was subjected to HPTLC an analytical method of standardization.

Physico chemical analysis

1. Refractive index[4]

Placed a drop of water on the prism and adjusted the drive knob of Abbe's refractometer 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 ghee 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 was measured at 28˚C.

2. Saponification value[5]

Weighed 2g of the *Shadbindu ghrita* into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash. 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). Repeated the operation omitting the substance being examined (blank) (b ml). Repeated the experiment twice to get concordant values.

3. Acid value[6]

Weighed 2- 10g of *Shadbindu ghrita* in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 +25ml) previously neutralized with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink colour. Repeated the experiment twice to get concordant values.

4. Determination of Unsaponifiable matter[7]

Weighed 5g of *Shadbindu ghrita* into the flask. Added 50ml alcoholic KOH into the sample. Boiled gently but steadily under reflux condenser for one hour. The condenser 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 shaked vigorously. And drawing off the alcohol-water layer 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. Placed the flask 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 in a desiccator to remove last traces of moisture and then weighed.

5. Rancidity test[8]

1ml of melted *Shadbindu ghrita* 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.

6. Iodine value[9]

Shadbindu ghrita 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.

7. Sample preparation for HPTLC of *Shadbindu ghrita*[10]

Sample obtained in the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform.

HPTLC

3, 6 and 9µl of the above sample were applied on a precoated silica gel F_{254} on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: ethyl acetate (8:1) and the developed plates were visualized under short UV, long UV and after derivatisation in vanillin-sulphuric acid spray reagent and scanned under UV 254nm, 366nm and 620nm (following derivatisation). R_f, colour of the spots and densitometric scan were recorded.

RESULTS

The results of organoleptic characters, standardization parameters, HPTLC photo documentation, Rf values and Densitometric scan are given in the following tables and figures.

Table 1: Results of organoleptic characters.

	Parameters	Shadbindu ghrita
1.	Colour	Greenish yellow
2	Appearance	Granular semisolid
3.	Odour	Aromatic

Table no 2: Results of Physicochemical analysis.

Results n=3 %w/w		
	Parameters	**Shadbindu ghrita**
1	Refractive index	1.45956
2	Saponification value	179.50
3	Acid value	1.92
4	Unsaponifiable matter	0.55
5	Iodine value	30.08

HPTLC

The Rf value of sample in short wave, long wave and after derivatisation are shown in table no 3

Table no 3: Rf values of all the samples

The Rf values of the samples in short wave, long wave and derivatisation has been noted in table no.3

Table no 3

At 366 nm	After Derivatisation	366nm following derivatisation
0.24 (FL. blue)	-	-
-	0.32 (D. purple)	0.32 (L. purple)
0.46 (F. green)	-	-
-	0.48 (D. purple)	0.48 (L. purple)
-	0.60 (L. purple)	-
-	0.66 (L. purple)	-
-	0.71 (L. purple)	-
0.76 (F. blue)	-	-
-	0.78 (L. purple)	0.78 (L. purple)
-	0.87 (L. purple)	0.87 (L. purple)
0.93 (F. blue)	-	-

DISCUSSION

Saponification value

It is a measure of molecular weight and chain length of fatty acid. Short chain fatty acids are good for internal consumption. The saponification value number of *Shad Bindu ghrita* is 179.50 indicates that it is good for permeability through the nasal mucosa and subsequently the absorption will be better.

Acid value

Acid value is the neutralization capacity of acid that is present in the *Shad bindu ghrita* formulation by alkali (Potassium hydroxide)

As this ghee preparation is glyceryl ester of free fatty acid then the free fatty acid that is generated contributes to quality and shelf life of formulation. The free fatty acid is formed as a result of hydrolysis and it deteriorates the quality of the formulation making it unfit for further use. This was the first attempt to standardize one such formulation to be used in clinical practice.

Unsaponifiable matter

It is that fraction of the formulation which is not saponified by alkali but can be extracted by organic solvent.

Iodine value

Iodine value indicates the number of unsaturated fatty acids present in *Shad Bindu ghrita* which reacts with iodine. Polyunsaturated fatty acids have medicinal significance in clinical field. Higher the iodine value greater the degree of unsaturation. In *shad bindu ghrita* iodine value is high which indicates the better absorption of drug. It has protective body mechanism.

HPTLC

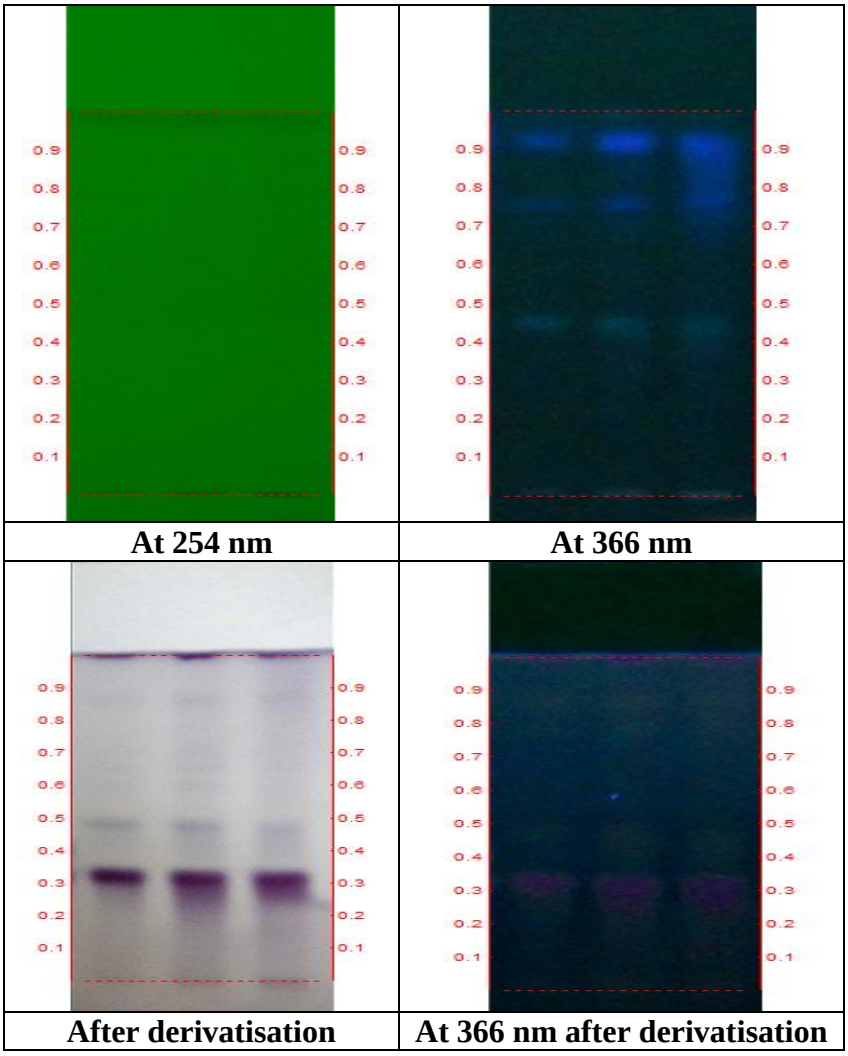
Under short UV there were no spots observed in all the 3 concentrations applied. Under long UV there were 4 bands evident namely at Rf 0.24, 0.76, 0.93 (all fluorescent blue) and 0.46 (fluorescent green). After derivatization of the plate with VSA spraying reagent observed in white light there were 4 spots identified namely at Rf 0.32, 0.48, 0.87 (all purple) in (fig 1).

Densitometric scan at 254nm

Densitometric scan of the plate at 254nm showed the presence of 5 peaks at Rf 0.06, 0.32, 0.53, 0.82, 0.90 with maximum absorption at 0.53 (47.06%) was the major peak (fig 2).

Densitometric scan at 366nm

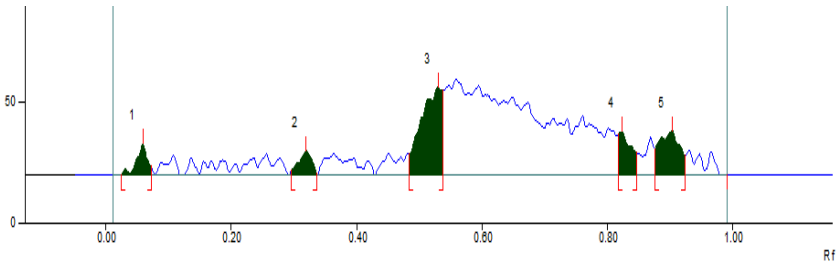
Densitometric scan at 366nm carried showed 3 peaks at Rf 0.37, 0.54, and 0.80 with maximum percentage area of 40.20 at Rf 0.80 (fig 3). After derivatisaion when the plate was scanned at 620nm total of 11 peaks were observed among which Rf of 0.35 (26.65% area) was the major peak. Rest of the peaks were identified at Rf 0.06, 0.09, 0.17, 0.22, 0.51, 0.57, 0.67, 0.81, 0.90, 0.92 respectively (fig 4).



Track 1- *Shadbindu ghritha*– 3µl; Track 2–6µl; Track 3– 9µl

Solvent system: Toluene: Ethyl acetate (8:1)

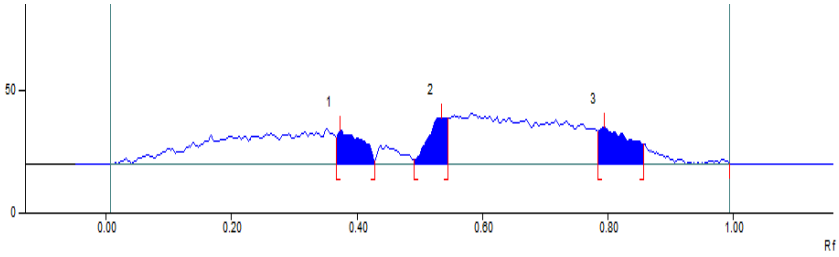
Fig 1: HPTLC photo documentation of chloroform extract of *Shadbindu ghritha.*



Track 9, ID: Shatabindu ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.0 AU	0.06 Rf	12.7 AU	13.39 %	0.07 Rf	3.6 AU	154.2 AU	8.27 %
2	0.30 Rf	1.7 AU	0.32 Rf	10.2 AU	10.69 %	0.34 Rf	0.7 AU	147.9 AU	7.93 %
3	0.48 Rf	8.7 AU	0.53 Rf	36.0 AU	37.97 %	0.54 Rf	34.7 AU	877.3 AU	47.06 %
4	0.82 Rf	17.0 AU	0.82 Rf	17.9 AU	18.87 %	0.85 Rf	9.1 AU	256.3 AU	13.75 %
5	0.88 Rf	10.8 AU	0.90 Rf	18.1 AU	19.07 %	0.93 Rf	8.0 AU	428.5 AU	22.99 %

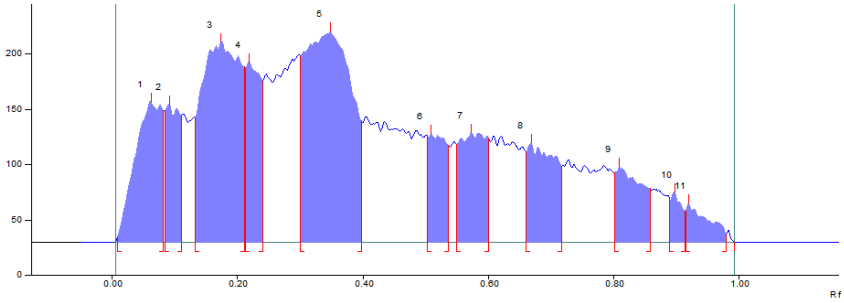
Fig 2: Densitometric scan of the sample at 254nm.



rack 9, ID: Shatabindu ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.37 Rf	10.8 AU	0.37 Rf	13.7 AU	28.89 %	0.43 Rf	1.4 AU	381.9 AU	29.02 %
2	0.49 Rf	1.6 AU	0.54 Rf	18.7 AU	39.55 %	0.55 Rf	18.6 AU	405.3 AU	30.79 %
3	0.79 Rf	13.2 AU	0.80 Rf	14.9 AU	31.56 %	0.86 Rf	7.8 AU	529.1 AU	40.20 %

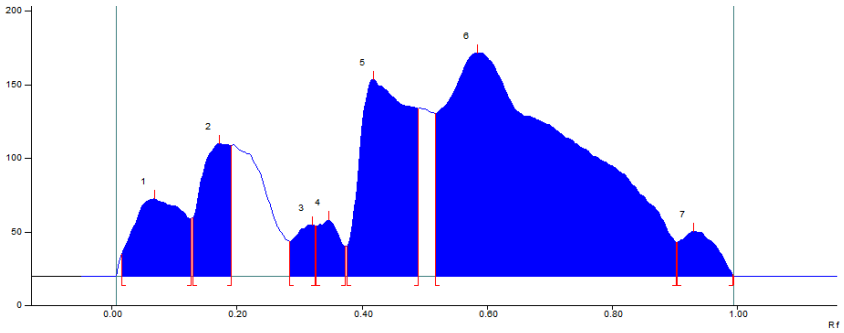
Fig 3: Densitometric scan of the sample at 366nm.



rack 9, ID: Shatabindu ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	5.3 AU	0.06 Rf	127.2 AU	10.41 %	0.08 Rf	18.9 AU	3961.2 AU	10.17 %
2	0.08 Rf	119.3 AU	0.09 Rf	125.1 AU	10.23 %	0.11 Rf	14.4 AU	2145.8 AU	5.51 %
3	0.13 Rf	112.4 AU	0.17 Rf	180.9 AU	14.80 %	0.21 Rf	58.4 AU	8071.4 AU	20.72 %
4	0.21 Rf	157.3 AU	0.22 Rf	162.7 AU	13.31 %	0.24 Rf	45.4 AU	2792.2 AU	7.17 %
5	0.30 Rf	168.9 AU	0.35 Rf	190.5 AU	15.58 %	0.40 Rf	09.6 AU	10381.3 AU	26.65 %
6	0.50 Rf	97.0 AU	0.51 Rf	98.0 AU	8.02 %	0.54 Rf	87.8 AU	2067.1 AU	5.31 %
7	0.55 Rf	88.3 AU	0.57 Rf	99.6 AU	8.14 %	0.60 Rf	94.4 AU	3114.0 AU	7.99 %
8	0.66 Rf	81.8 AU	0.67 Rf	89.7 AU	7.34 %	0.72 Rf	68.8 AU	2865.0 AU	7.35 %
9	0.80 Rf	62.5 AU	0.81 Rf	68.4 AU	5.60 %	0.86 Rf	48.4 AU	2067.5 AU	5.31 %
10	0.89 Rf	39.8 AU	0.90 Rf	45.2 AU	3.69 %	0.91 Rf	27.7 AU	592.8 AU	1.52 %
11	0.92 Rf	28.3 AU	0.92 Rf	35.3 AU	2.88 %	0.98 Rf	6.8 AU	895.1 AU	2.30 %

Fig 4: Densitometric scan of the sample at 620nm following derivatisation.



Track 9, ID: Shatabindu ghritha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	15.2 AU	0.07 Rf	52.5 AU	9.88 %	0.13 Rf	39.0 AU	2989.6 AU	7.54 %
2	0.13 Rf	39.5 AU	0.17 Rf	90.0 AU	16.94 %	0.19 Rf	88.7 AU	2973.8 AU	7.50 %
3	0.28 Rf	23.6 AU	0.32 Rf	34.8 AU	6.55 %	0.33 Rf	34.2 AU	825.5 AU	2.08 %
4	0.33 Rf	34.0 AU	0.35 Rf	38.2 AU	7.20 %	0.37 Rf	20.5 AU	958.4 AU	2.42 %
5	0.38 Rf	20.6 AU	0.42 Rf	133.6 AU	25.16 %	0.49 Rf	13.7 AU	7610.9 AU	19.19 %
6	0.52 Rf	110.5 AU	0.59 Rf	151.4 AU	28.51 %	0.90 Rf	22.7 AU	23078.0 AU	58.18 %
7	0.90 Rf	22.9 AU	0.93 Rf	30.6 AU	5.76 %	0.99 Rf	1.0 AU	1227.2 AU	3.09 %

Fig 5: Densitometric scan of the sample at 366 nm following derivatisation.

CONCLUSION

The organoleptic characters and parameters of physico chemical analysis of *shadbindu ghrita* was within the normal reference range. Under Densitometric scan at 254nm 5 peaks and at 366nm 3 peaks were found. After derivatisation at 620nm 11 peaks were observed. Hence it is inferred that the *shadbindu ghrita* meets a maximum qualitative standard.

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