

An Analytical Study to Standardize Baladhya Yamaka

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Abstract

Ayurveda emphasizes on 4 aspects that ensure proper management of a disease. These aspects are called chikitsa chatushpada. One among them is aushadha. Standardization of ayurvedic formulations is an important step for establishment of biological activity, consistent chemical profile or quality assurance for production. Use of sneha in different routes of management has been specifically mentioned in the treatment of vata vyadhi among which Baladhya yamaka sneha has been selected for the study pertaining to urdwajatrugata vata vikara.

Methodology : The prepared drug was analyzed using standard analytical parameters like physio-chemical parameters and HPTLC.

Result: The HPTLC showed under short UV, there were 2 bands with different intensities and under long UV, 6 bands were observed, following derivatization with VSA (Vanillin Sulphuric Acid) two bands were observed. Densitometric scan carried out for the sample of Baladhya yamaka sneha at 254nm showed up 9 peaks, At 366nm 6 peaks were evident.

Conclusion: the study drug was analysed and the values can serve as standard reference values. This study values can be used for further studies like GCMS, absorption studies.

Key words

Standardisation, analytical parameters, baladhya yamaka, urdwajatrugata vata vikara.

Introduction

Quality medicine is one among the chatushpada mentioned for the success of ayurvedic treatment.¹ On the other hand, as per current practice, Standardization of ayurvedic formulations is an important step for establishment of biological activity, consistent chemical profile or quality assurance for production². In the current scenario, the ayurvedic formulations are to be standardized to know the physical, chemical and HPTLC profile as per WHO and AYUSH testing guidelines³.

Among the different yogas, Sneha yogas are specified as highly beneficial in the management of vata vyadhi. Among the Sneha yogas, yamaka, (a mixture of any 2 snehas among four main snehas) has also been specified in vata vyadhi management.

Baladhya yamaka mentioned by Vangasena in shirorogadhikara has specific indications pertaining to urdwajatrugata vata vikaras⁴. The Baladhya Yamaka contains ingredients like jeevaniya gana drugs, bala, masha, Go-ghrita, tilataila, Go dugdha.

Method of preparation of Baladhya yamaka

For the study the yamaka was prepared in rasashala of Shri Dharmasthala Manjunatheswara college of Ayurveda, Udupi. The yamaka was used for the clinical study (nasya karma) and hence was prepared in quantity of 1.2L

Table 1. Ingredients and quantity of Baladhya Yamaka

Ghataka dravya	Quantity
Jeevaniya gana drugs	400 grams each
Bala	400 grams
Masha	100 grams
Jala	60 litres (reduced to 15 liters)
Go dugdha	10 litres
Murchitha ghritha	1.83 liters
Murchitha taila	1.83 litres

In a vessel murchitha ghritha n murchitha taila are taken and heated. To this, prepared qwatha of jeevaniya gana drugs, bala and masha is added along with go dugdha and kalka of the same drugs in 1:4:16 ratio. This is boiled for about 4 days, until sneha siddhi lakshana were seen. It is filtered and stored in air tight containers. The total quantity of taila got as end product was 2.3 litres.

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Methodology

In the present study, the research drug, Baladhya yamaka was subjected for different standard analytical procedures. The methodologies followed in the analytical study are:

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 was 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. The procedure was repeated using distilled water in place of sample solution.

Acid value

2- 10g of Baladhya yamaka sneha was weighed and taken in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25 +25ml) previously neutralised 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.

Saponification value

2g of the Baladhya yamaka sneha was weighed and taken in a 250 ml RB flask, fitted with a reflux condenser. 25ml of 0.5M alcoholic potash was added. This mixture was refluxed on a water bath for 30 minutes. Once it is cooled 1 ml of Phenolphthalein solution is added and titrated immediately with 0.5 M Hydrochloric acid (a ml). This operation was repeated omitting the substance being examined (blank) (b ml). The experiment was repeated twice to get concordant values.

Iodine value

The 0.1gm of sample was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl₄, 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 170 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 :

5g of the Baladhya yamaka sneha was weighed and poured into the flask. 50ml alcoholic KOH was added into the sample. The solution is further 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 shaken 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.

Peroxide value

5g of the Baladhya yamaka sneha was weighed accurately into a conical flask, added 30 ml of mixture of 3volumes of glacial acetic acid and 2 volumes of chloroform, added

0.5ml of potassium iodide, allowed it to stand for 1 minute, add 30ml of water titrate gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow color disappears. Add 0.5ml of starch indicator continued the titration until blue color disappears.

$$\text{Peroxide value} = 10(a-b)/W$$

Where W= weight in g of the substance

Viscosity

The given sample of oil 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

$$\eta_1 = \frac{\rho_1 t_1}{\rho_2 t_2} \times \eta_2$$

$$\rho_2 t_2$$

η_1 - Viscosity of sample

η_2 - Viscosity of water

t_1 and t_2 - time taken for the sample and water to pass the meniscus

ρ_1 and ρ_2 - Density of sample and water

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

$\Pi = X \times \text{Time for sample} \times 1.004 / \text{specific gravity of water} \times 70 \text{ sec}$

Sample preparation of 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 oil, and chloroform soluble portion was used for HPTLC.

HPTLC

4, 8, 12 μ l of the chloroform fraction of the above sample was applied on a precoated silica gel F254 on aluminium 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 and scanned under UV 254nm and 366 nm. R_f, colour of the spots and densitometric scan were recorded.

Result

Table 2. Organoleptic evaluation

Parameters	Result
Colour	Brownish
Odour	pleasant
Appearance	Oily viscous
Touch	Greasy

Table 3. Standardization parameter of Baladhya yamaka sneha

Parameter	Results n = 3 %w/w
	Baladhya yamaka sneha
Refractive index	1.4611
Specific gravity	0.9398
Acid value	7.22 $\pm$ 0.01
Saponification value	250.45 $\pm$ 0.00
Iodine value	66.46 $\pm$ 0.00
Unsaponifiable matter (%)	2.11 $\pm$ 0.01
Peroxide value	0.0 $\pm$ 0.0
Rancidity	Fat is not oxidised

Figure 1: HPTLC Photodocumentation of sample of Baladhya yamaka sneha

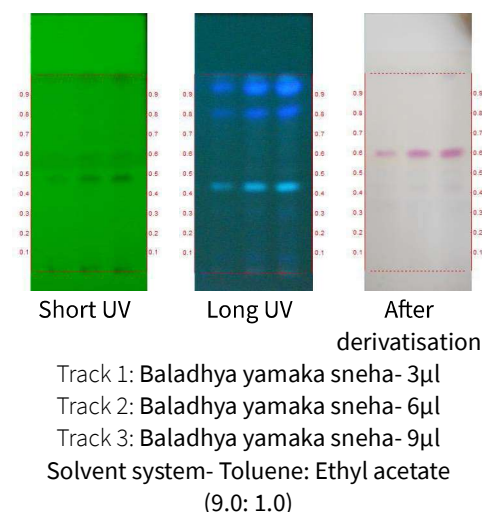
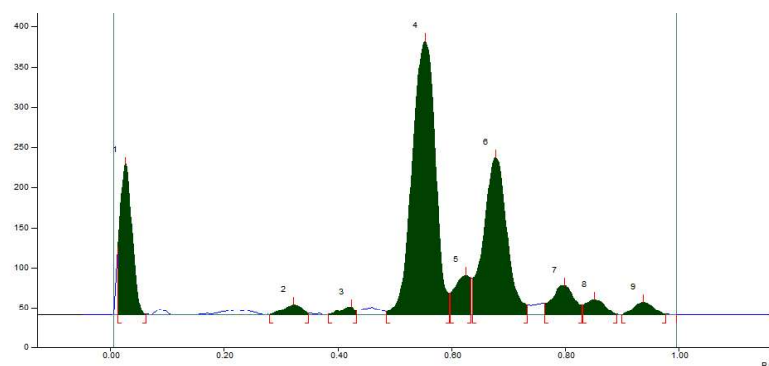


Table 4. R_f values of sample

Short UV	Long UV	After derivatisation
-	0.09 (F. blue)	-
-	0.32 (F. blue)	-
-	-	0.42 (Purple)
-	0.44 (aqua blue)	-
0.48 (D. green)	-	-
-	0.52 (F. blue)	-
0.59 (L. green)	-	-
-	0.62 (F. blue)	0.62 (Pink)
-	0.81 (F. blue)	-
-	0.93 (F. blue)	-

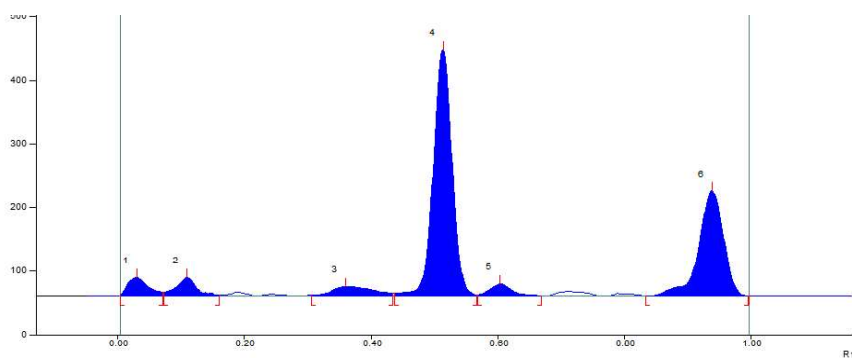
Figure 2: Densitometric scan of the sample of Baladhya yamaka sneha



Track 8, ID: Baladhya yamaka sneha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	83.4 AU	0.03 Rf	187.8 AU	21.46 %	0.06 Rf	0.7 AU	2841.1 AU	12.89 %
2	0.28 Rf	0.3 AU	0.32 Rf	13.3 AU	1.52 %	0.35 Rf	1.9 AU	321.7 AU	1.46 %
3	0.38 Rf	0.5 AU	0.42 Rf	10.6 AU	1.21 %	0.43 Rf	6.0 AU	194.5 AU	0.88 %
4	0.49 Rf	4.3 AU	0.55 Rf	341.6 AU	39.03 %	0.60 Rf	27.9 AU	9949.7 AU	45.14 %
5	0.60 Rf	28.3 AU	0.63 Rf	50.2 AU	5.73 %	0.64 Rf	46.7 AU	1015.9 AU	4.61 %
6	0.64 Rf	46.7 AU	0.68 Rf	197.6 AU	22.58 %	0.73 Rf	13.1 AU	5765.9 AU	26.16 %
7	0.76 Rf	15.1 AU	0.80 Rf	37.7 AU	4.30 %	0.83 Rf	13.3 AU	1050.4 AU	4.77 %
8	0.83 Rf	13.4 AU	0.85 Rf	20.0 AU	2.29 %	0.89 Rf	0.9 AU	484.6 AU	2.20 %
9	0.90 Rf	0.1 AU	0.94 Rf	16.4 AU	1.87 %	0.98 Rf	0.8 AU	419.3 AU	1.90 %

Fig 2a. At 254nm



Track 8, ID: Baladhya yamaka sneha

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.3 AU	0.03 Rf	29.2 AU	4.56 %	0.07 Rf	6.1 AU	670.3 AU	4.36 %
2	0.07 Rf	6.1 AU	0.11 Rf	28.7 AU	4.47 %	0.16 Rf	0.8 AU	661.3 AU	4.30 %
3	0.31 Rf	0.8 AU	0.36 Rf	14.3 AU	2.23 %	0.44 Rf	3.9 AU	683.4 AU	4.44 %
4	0.44 Rf	3.9 AU	0.51 Rf	385.9 AU	60.17 %	0.57 Rf	1.7 AU	8254.8 AU	53.64 %
5	0.57 Rf	1.7 AU	0.60 Rf	18.4 AU	2.87 %	0.67 Rf	0.2 AU	460.6 AU	2.99 %
6	0.84 Rf	0.0 AU	0.94 Rf	164.8 AU	25.70 %	1.00 Rf	0.0 AU	4660.1 AU	30.28 %

Fig 2b. At 366nm

Discussion

Refractive index of Baladhya Yamaka Sneha is indicative of its product quality and purity, where dispersed particle size is lesser than 50microns; it is one of the optical properties to be determined for liquids. The refractive index is 1.46 which is very much close to that of plastic solutions. High refractive index samples can be optically useful.

Specific gravity is another parameter which is of pharmaceutical importance to be measured keeping in mind dosage regimen to be fixed while dispensing. Specific gravity is compared with that of distilled water. It depends on base, dissolved constituents and temperature during the process. Specific gravity was found to be 0.9398 which was average of both the bases used and can be attributed to the herbal ingredients present in there.

Acid value is neutralization capacity of acid present in the formulation by the potassium hydroxide which is a base. Ghrithas and tailas are glyceryl esters of free fatty acid. Free fatty acid thus generated contributes to Quality, Stability and shelf life of formulation. Free fatty acid is formed as a result of hydrolysis, deteriorates the quality of formulation making it unstable. Since this formulation was never standardized before and it was first of its kind prepared shelf life studies have to be further conducted.

Saponification value is a measure of molecular weight and chain length of fatty acids. The herbal drugs present in the formulation are alkaloids there is chance of alkaline hydrolysis and may lead to release of short chain fatty acids. Short chain fatty acids are for the GI health. Saponification value of 250.45 is a good sign for intestinal health.

Iodine value indicates number of unsaturated fatty acids present in the formulation which reacts with iodine. PUFA have medicinal significance in medical field as they offer cardio protection by lowering LDL levels.

Peroxide value is an indicator of oxidation of the formulation which is an indicator of stability, in other words shelf life. Free radicals are hazardous to human as they can cause cell injury and cell death. High peroxide value makes formulation unfit for human consumption and use. Free radicals cause oxidative injury and are detrimental. Free radicals are formed from lipid peroxidation and hydro peroxidation. In Baladhya yamaka sneha the peroxide value was found to be nil (0) making it safe for clinical use. In order to further confirm test for rancidity was done qualitatively to make sure fat was not undergone oxidative and hydrolytic degradation.

Unsaponifiable matter amounts that part of the formulation which was not saponified by alkali but can be extracted by organic solvent.

HPTLC performed for Baladhya Yamaka Sneha under short UV there were 2 band evident (all Green) at Rf of 0.48 and 0.59. When observed under long UV total of 8 bands were seen at Rf 0.09, 0.32, 0.44, 0.52, 0.62, 0.81, 0.93. Following derivatization with VSA (Vanillin Sulphuric Acid) two bands were observed with Rf of 0.42 (Purple), 0.62 (Pink).

Densitometric scan carried out for the sample of Baladhya Yamaka. Sneha at 254nm showed up 9 peaks major ones were at

Rf 0.55 (45.14%) and 0.68 (26.16%). At 366nm 6 peaks were evident, Rf 0.51 (53.64%) and 0.94 (30.28%) were been major.

Conclusion

The baladhya yamaka was prepared as per standard operative procedures explained for yamaka preparation. Later the yamaka was subjected to different standard analysis. The values observed in the study with HPTLC fingerprints has been stored and set as monograph to identify and check the quality of baladhya yamaka. Further this can be used for comparison and for future studies as standard values.

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