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PHARMACEUTICO-ANALYTICAL STUDY OF ASHODHITA AND SHODHITA MADANAPHALAPIPPALI – A HERBAL DRUG USED FOR VAMANA KARMA (EMETIC THERAPY)

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ABSTRACT

Ayurveda stresses on the need of *Shodhana* (purification / processing) of crude herbal drugs. After proper *Shodhana* only, the crude herbal drugs should be administered to the patients. Purification is necessary to render chemical and physical purities to the drug. By this process, the drugs become therapeutically more effective and also less toxic. The knowledge of classical purification methods mentioned in various *Ayurvedic Samhitas* for crude herbal drugs is necessary in the present era. Keeping the above facts in mind, a comparative study was undertaken to evaluate the pharmaco-analytical changes of *Ashodhita* and *Shodhita Madanaphalapippali* by adopting standard testing protocol for AYUSH drugs such as Macroscopic, Microscopic; HPTLC, Photodocumentation, Rf Values and Densitometric Scan.

Keywords: *Ashodhita Madanaphalapippali*, *Shodhita Madanaphalapippali*, *Shodhana*

INTRODUCTION

The *Aushadha* by virtue of which, its *Prabhava* eliminates *Apakva Kapha* and *Apakva Pitta Doshas* forcefully through *Urdhva Bhaga*, is known as *Vamana Aushadha* e.g. *Madanaphala* and the process by which *Apakva Kapha* and *Pitta* are expelled out forcefully through *Urdhva-Bhaga* are known as *Vamana*[1]. The *Vamana Karma* is achieved through *Vamaka Dravyas* and among the *Vamaka Dravyas Madanaphala* is *Shreshthatama*[2].

Shodhana is a process by which unwanted impurities are separated from the substance by various pharmaceutical methods like *Mardana*, *Prakshalana*[3] etc. with specific drugs (media) there by minimization of the toxicity of substance. Mostly *Shodhana* of a drug is done for elimination of physical and chemical impurities and / or neutralization of the toxins and / or to induce and enhance therapeutic qualities and / or to make *Dravyas* suitable for administration.

In this study, *Ashodhita Madanaphalapippali* was subjected to *Shodhana*, to know and understand the pharmaco-analytical changes.

AIM: To evaluate the pharmaco-analytical changes of *Ashodhita* and *Shodhita Madanaphalapippali.*

MATERIALS AND METHODS:

Group A:

Madanaphala full dried fruits were taken and were broken to remove outer cover. *Madanaphalapippali* was taken out from dried fruits and samples of ***Ashodhita Madanaphalapippali*** in quantity of 100 gms were sent to Pharmaceutical Chemistry and Pharmacognosy Laboratory of S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka.

***Shodhana*:** *Ashodhita Madanaphala* fruits were then taken and processed for *Shodhana* by following methodology as mentioned in *Charaka Samhita Kalpa Sthana Prathama Adhyaya*[4]:

Step 1- Process of Separation of *Madanaphalapippali* from *Madanaphala* Fruits: Dry *Madanaphala* fruits in quantity of 7000gms were taken and *Madanaphalapippali* was made out by breaking the outer cover of fruits. The quantity obtained for *Madanaphalapippali* was 5000gms.

Step 2 – *Mardana* with *Go-Ghrita*: The obtained 5000gms of *Madanaphalapippali* was subjected to *Mardana* with approx. 1000ml q.s. (quantity sufficient) of *Go-Ghrita*.

Step 3 – *Mardana* with *Dadhi*: This *Ghrita Vimardita Madanaphalapippali* was then subjected to *Mardana* with *Dadhi* approx. 800ml (q.s.).

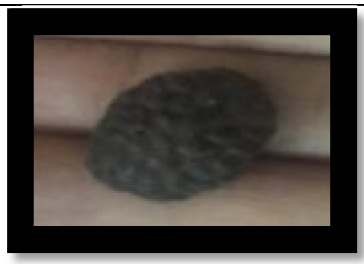
Step 4 – *Mardana* with *Madhu*: This *Dadhi Vimardita Madanaphalapippali* was then subjected to *Mardana* with *Madhu* approx. 400ml (q.s.).

Step 5 – *Mardana* with *Tila Palala*: Approx. 500gms (q.s.) of *Tila Palala* was taken and made soft by submerging in water (250ml approx). Then *Madanaphalapippali* which was subjected to *Madhu Vimardana* was subjected to *Mardana* with *Palala Kalka*.

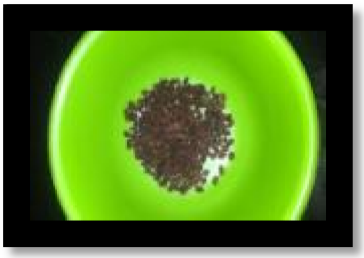
Step 6 - Drying & Sieving: After sequential *Vimardana* with *Ghee-Dadhi-Madhu-Palala*, *Madanaphalapippali* was followed by drying process. The whole *Vimardita Madanaphalapippali* was dried under sun. The full drying process took 2days. Then *Madanaphalapippali* was sieved and the *Shodhita Madanaphalapippali* seeds were separated out.

Step 7 -Packing and Preservation: After proper sieving, the *Madanaphalapippali* seeds were packed in air tight Jar.

Colour Plate 1: Showing Materials Required for *Madanaphalapippali Shodhana*

		
Ashodhita Madanaphalapippali	***Khalva Yantra***	***Go- Ghrita***
		
Dadhi	***Madhu***	***Tila Palala***

Colour Plate 2: Showing Process of *Madanaphalapippali Shodhana*

		
Mardana* with *Ghrita	***Mardana* with *Dadhi***	***Mardana* with *Madhu***
		
***Palala* Softened by Water**	***Mardana* with *Palala***	***Shodhita Madanaphalapippali***

Group B: After the process of *Shodhana*, samples of ***Shodhita Madanaphalapippali*** 100gms sample was sent to Pharmaceutical Chemistry and Pharmacognosy Laboratory of S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, to understand changes.

Analysis report for both *Ashodhita* and *Shodhita Madanaphalapippali* as per laboratory is as follows:

ANALYTICAL STUDY OF *MADANAPHALAPIPPALI*

Sample details: *Ashodhita Madanaphalapippali*, *Shodhita Madanaphalapippali*.

METHODOLOGY

Macroscopy - The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

Microscopy - Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

HPTLC - 1gm of *Ashodhitha Madanaphalapippali* and *Shodhita Madanaphalapippali* powder was extracted with 10 ml of alcohol. 4 and 8µl of the above extract was applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid

(7.0: 3.0: 0.1). The developed plates were visualized in short UV, long UV, and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. R_f, colour of the spots and densitometric scan were recorded.

RESULTS AND DISCUSSION

The results of Organoleptic Characteristics, Standardization Parameters, HPTLC, Photodocumentation, Rf values and Densiometric Scan are given in respective tables and figures.

The following changes were noticed after *Shodhana*:

Table 1: Showing changes before and after *Shodhana* of *Madanaphalapippali*

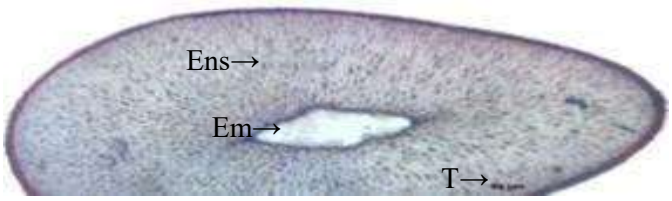
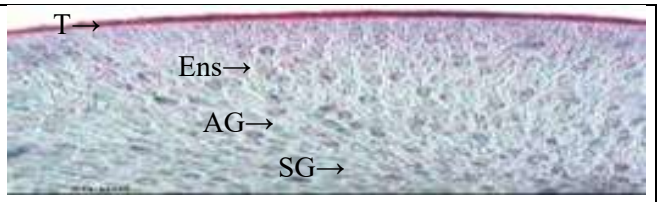
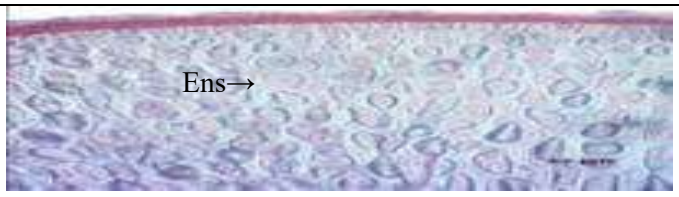
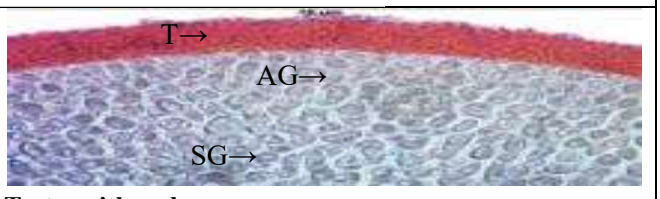
Madanaphalapippali	**Before (Unprocessed)**	**After (Processed)**
Quantity	5000gms	4600gms
Appearance	Clustered in oval shape	Dissembled small seeds
Colour	Dark brown-black	Dark brown to light brown
Odour	Light sweetish odour (*Madhu-Gandhi*)	Strong sweetish odour (*Madhu-Gandhi*)
Touch	Uneven-Silky	Smooth-Silky
Taste	*Tikta Rasa*	*Tikta Rasa*
Consistency	Hard	Soft

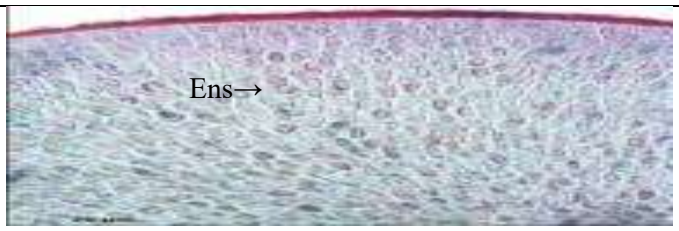
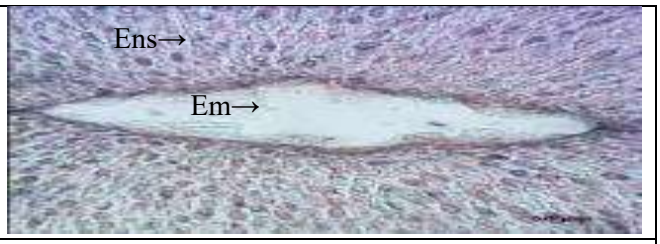
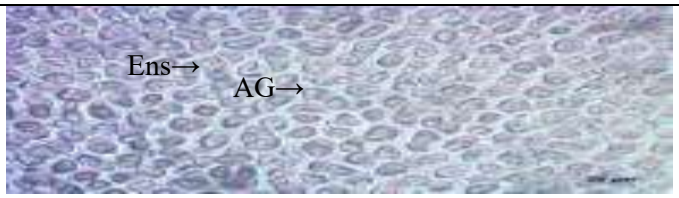
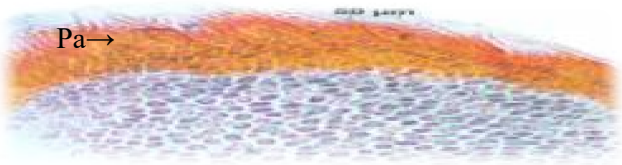
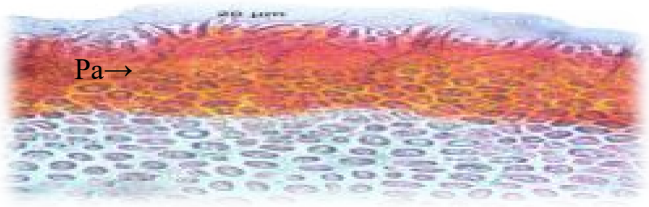
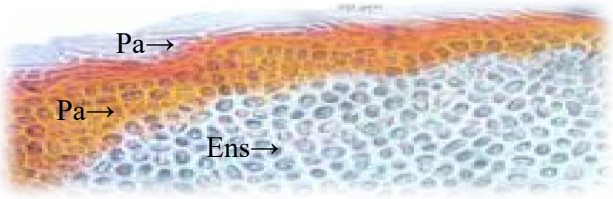
Macroscopy:

Table 2: Showing Macroscopy of *Madanaphalapipppali*

Ashodhita Madanaphalapippali	***Shodhita Madanaphalapippali***
	

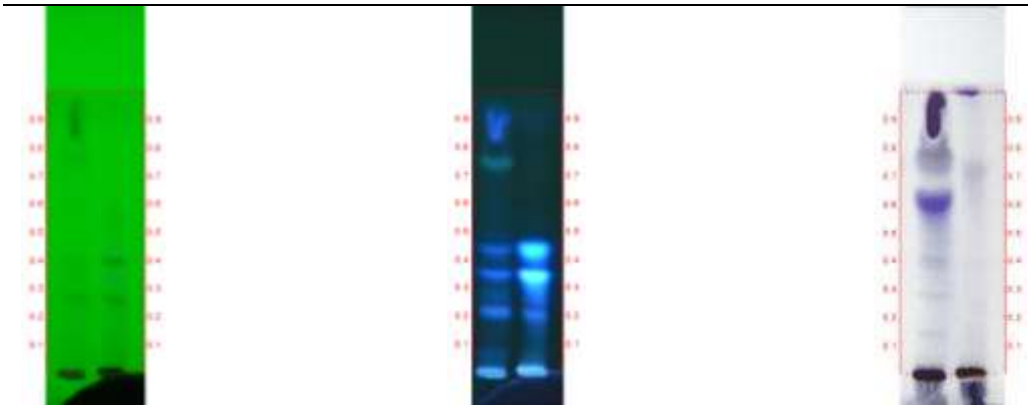
Table 3: Showing Microscopy of *Madanaphalapippali*

 **T.S of Seed**	 **Testa with Endosperm**
 **Testa and endosperm**	 **Testa with endosperm**

	
Testa with endosperm	**Narrow central embryo**
	
Endosperm containing Aleuron grains and starch, oil globules	**Outer most epidermis covering testa**
	
Tangentially elongated mucilaginous parenchyma	**Parenchyma with brown pigment**

AG – aleurone grains; Em – embryo; Ens – endosperm; SG – starch grains; T – testa.
Ens – endosperm; Pa – parenchyma.

Figure 1: TLC photo documentation of ethanol fraction of *Ashodhita Madanaphalapippali* and *Shodhita Madanaphalapippali*



Short UV Long UV Post derivatisation

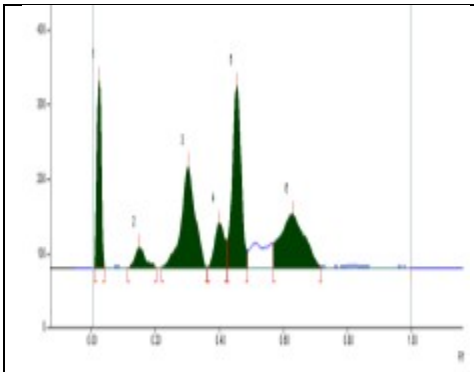
Shodhita Madanaphalapippali Sample - 4µl, *Ashodhita Madanaphalapippali* Sample- 4µl, *Shodhita Madanaphalapippali* Sample - 8µl, *Ashodhita Madanaphalapippali* Sample - 8µl Solvent system- Toluene: Ethyl acetate: Formic acid (7.0: 3.0: 0.3)

Table 4: Showing Rf values of sample of *Ashodhita Madanaphalapippali* and *Shodhita Madanaphalapippali*

Short UV		**Long UV**		**Post derivatisation**	
Ashodhita Madanaphalapippali	*Shodhita Madanaphalapippali*	*Ashodhita Madanaphalapippali*	*Shodhita Madanaphalapippali*	*Ashodhita Madanaphalapippali*	*Shodhita Madanaphalapippali*
-	-	-	-	-	0.15 (D. purple)
-	-	0.20 (F. blue)	-	-	-
-	-	-	0.22 (F. blue)	-	-
0.27 (D. green)	0.27 (L. green)	-	0.27 (F. blue)	-	-
-	-	-	0.29 (F. blue)	-	0.29 (D. purple)
-	-	-	0.32 (F. blue)	-	-
0.35 (L. green)	-	0.35 (F. blue)	0.35 (F. blue)	-	-
-	-	-	-	-	0.38 (D. purple)
0.40 (D. green)	0.40 (L. green)	-	-	-	0.40 (D. purple)
-	-	0.44 (F. blue)	0.44 (F. blue)	-	-
-	-	-	0.49 (F. blue)	-	-
0.55 (L. green)	-	-	-	-	-
-	-	-	-	-	0.61 (D. purple)
-	-	-	-	0.73 (D. purple)	-
-	-	-	0.75 (F. green)	-	0.78 (D. purple)
-	0.78 (L. green)	-	-	-	-

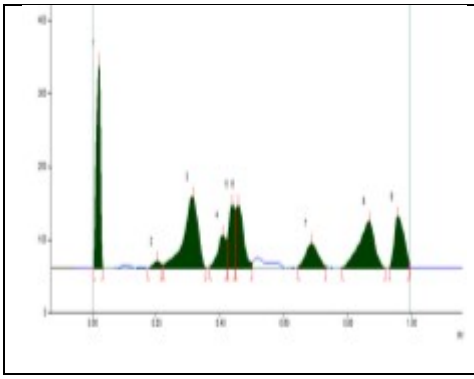
***D – dark; L – light; F – fluorescent**

Table 5: Showing the Densitometric Scan of the *Ashodhita Madanaphalapippali* Sample at 254nm



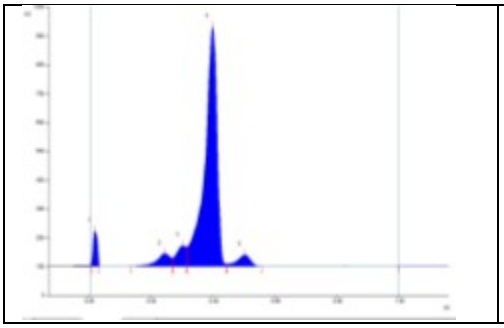
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	5.7 AU	0.02 Rf	253.0 AU	31.82 %	0.04 Rf	1.7 AU	2543.9 AU	14.41 %
2	0.11 Rf	0.2 AU	0.15 Rf	27.7 AU	3.49 %	0.20 Rf	0.0 AU	646.5 AU	3.66 %
3	0.22 Rf	1.0 AU	0.30 Rf	137.1 AU	17.25 %	0.36 Rf	0.6 AU	4319.5 AU	24.46 %
4	0.36 Rf	0.7 AU	0.40 Rf	60.2 AU	7.58 %	0.42 Rf	36.9 AU	1312.1 AU	7.43 %
5	0.42 Rf	37.1 AU	0.45 Rf	244.6 AU	30.77 %	0.49 Rf	23.6 AU	4897.4 AU	27.73 %
6	0.57 Rf	33.0 AU	0.63 Rf	72.4 AU	9.11 %	0.72 Rf	1.7 AU	3940.4 AU	22.31 %

Table 6: Showing the Densitometric Scan of the *Shodhita Madanaphalapippali* Sample at 254nm



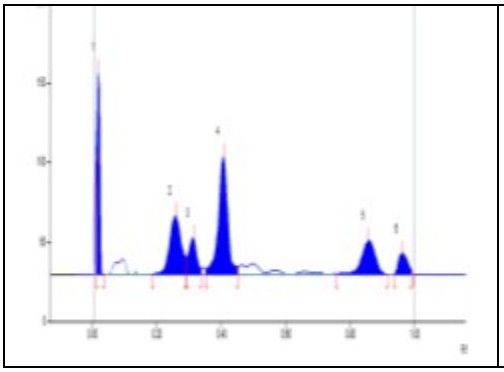
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	7.5 AU	0.02 Rf	280.1 AU	35.87 %	0.03 Rf	1.3 AU	2645.4 AU	18.15 %
2	0.17 Rf	0.1 AU	0.20 Rf	10.2 AU	1.30 %	0.22 Rf	5.3 AU	173.7 AU	1.19 %
3	0.22 Rf	5.4 AU	0.31 Rf	98.5 AU	12.61 %	0.36 Rf	0.1 AU	3220.2 AU	22.09 %
4	0.37 Rf	3.5 AU	0.41 Rf	45.7 AU	5.86 %	0.42 Rf	39.3 AU	854.8 AU	5.86 %
5	0.42 Rf	40.6 AU	0.44 Rf	88.0 AU	11.27 %	0.45 Rf	80.6 AU	1229.7 AU	8.44 %
6	0.45 Rf	81.1 AU	0.46 Rf	87.8 AU	11.24 %	0.50 Rf	9.0 AU	1544.6 AU	10.60 %
7	0.65 Rf	2.2 AU	0.69 Rf	34.3 AU	4.40 %	0.73 Rf	2.5 AU	994.7 AU	6.82 %
8	0.78 Rf	1.2 AU	0.87 Rf	64.9 AU	8.31 %	0.92 Rf	0.1 AU	2327.1 AU	15.97 %
9	0.93 Rf	0.4 AU	0.96 Rf	71.4 AU	9.14 %	0.99 Rf	11.0 AU	1585.8 AU	10.88 %

Table 7: Showing the Densitometric Scan of the *Ashodhita Madanaphalapippali* Sample at 366nm



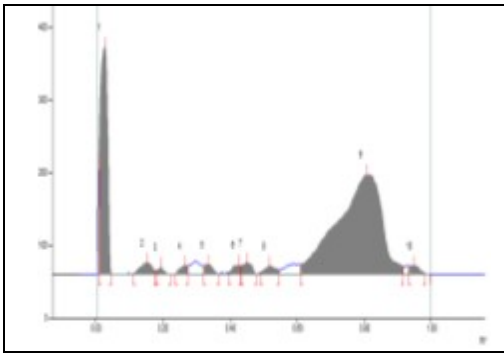
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	8.1 AU	0.02 Rf	122.6 AU	11.07 %	0.03 Rf	3.8 AU	1226.6 AU	4.23 %
2	0.13 Rf	0.0 AU	0.25 Rf	44.0 AU	3.97 %	0.27 Rf	29.4 AU	1388.3 AU	4.79 %
3	0.27 Rf	29.8 AU	0.30 Rf	71.4 AU	6.45 %	0.31 Rf	66.8 AU	1564.8 AU	5.40 %
4	0.32 Rf	66.9 AU	0.40 Rf	830.7 AU	74.99 %	0.44 Rf	7.7 AU	23574.2 AU	81.38 %
5	0.45 Rf	7.7 AU	0.50 Rf	39.0 AU	3.52 %	0.56 Rf	0.1 AU	1214.1 AU	4.19 %

Table 8: Showing the Densitometric Scan of the *Shodhita Madanaphalapippali* Sample at 366nm



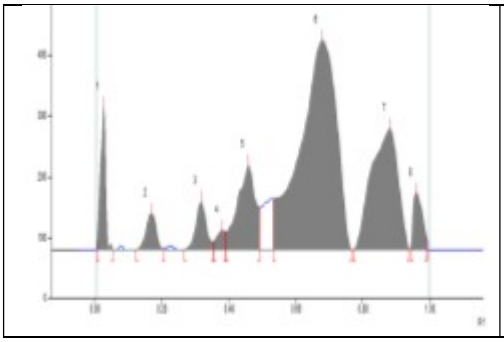
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	75.7 AU	0.02 Rf	127.0 AU	43.01 %	0.04 Rf	0.0 AU	896.4 AU	18.75 %
2	0.19 Rf	0.2 AU	0.26 Rf	37.1 AU	12.55 %	0.29 Rf	10.9 AU	965.0 AU	20.18 %
3	0.29 Rf	11.0 AU	0.31 Rf	22.8 AU	7.73 %	0.34 Rf	3.9 AU	420.5 AU	8.79 %
4	0.35 Rf	3.7 AU	0.41 Rf	74.0 AU	25.07 %	0.45 Rf	4.9 AU	1570.5 AU	32.85 %
5	0.76 Rf	0.0 AU	0.86 Rf	21.2 AU	7.18 %	0.92 Rf	0.0 AU	658.5 AU	13.77 %
6	0.94 Rf	0.1 AU	0.96 Rf	13.2 AU	4.46 %	0.99 Rf	3.0 AU	270.6 AU	5.66 %

Table 9: Showing the Densitometric Scan of the *Ashodhita Madanaphalapippali* Sample at 620nm



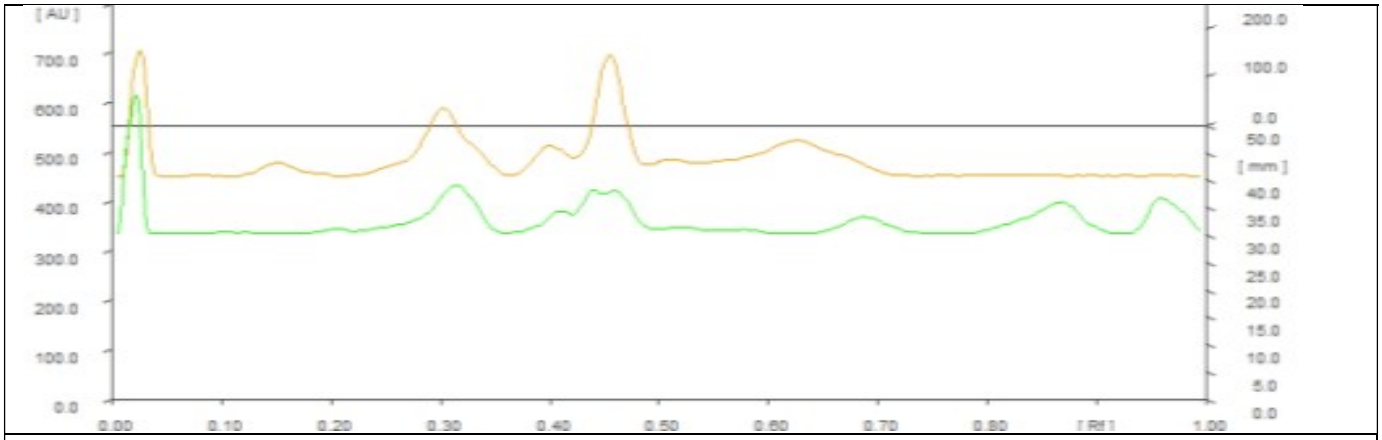
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	168.5 AU	0.03 Rf	312.6 AU	56.49 %	0.05 Rf	0.8 AU	5084.3 AU	25.29 %
2	0.11 Rf	0.8 AU	0.16 Rf	15.4 AU	2.78 %	0.18 Rf	4.6 AU	403.5 AU	2.01 %
3	0.18 Rf	4.9 AU	0.20 Rf	10.1 AU	1.83 %	0.22 Rf	0.0 AU	123.9 AU	0.62 %
4	0.24 Rf	0.3 AU	0.27 Rf	12.2 AU	2.21 %	0.28 Rf	11.2 AU	210.0 AU	1.04 %
5	0.32 Rf	10.8 AU	0.34 Rf	13.7 AU	2.47 %	0.37 Rf	0.5 AU	245.3 AU	1.22 %
6	0.40 Rf	2.8 AU	0.43 Rf	12.4 AU	2.24 %	0.43 Rf	11.0 AU	226.8 AU	1.13 %
7	0.44 Rf	11.2 AU	0.45 Rf	15.8 AU	2.85 %	0.48 Rf	0.0 AU	309.8 AU	1.54 %
8	0.49 Rf	2.4 AU	0.52 Rf	12.3 AU	2.22 %	0.55 Rf	5.8 AU	271.5 AU	1.35 %
9	0.61 Rf	13.3 AU	0.81 Rf	136.7 AU	24.71 %	0.92 Rf	10.4 AU	12966.3 AU	64.50 %
10	0.93 Rf	10.8 AU	0.95 Rf	12.2 AU	2.21 %	0.98 Rf	0.3 AU	260.7 AU	1.30 %

Table 10: Showing the Densitometric Scan of the *Shodhita Madanaphalapippali* Sample at 620nm

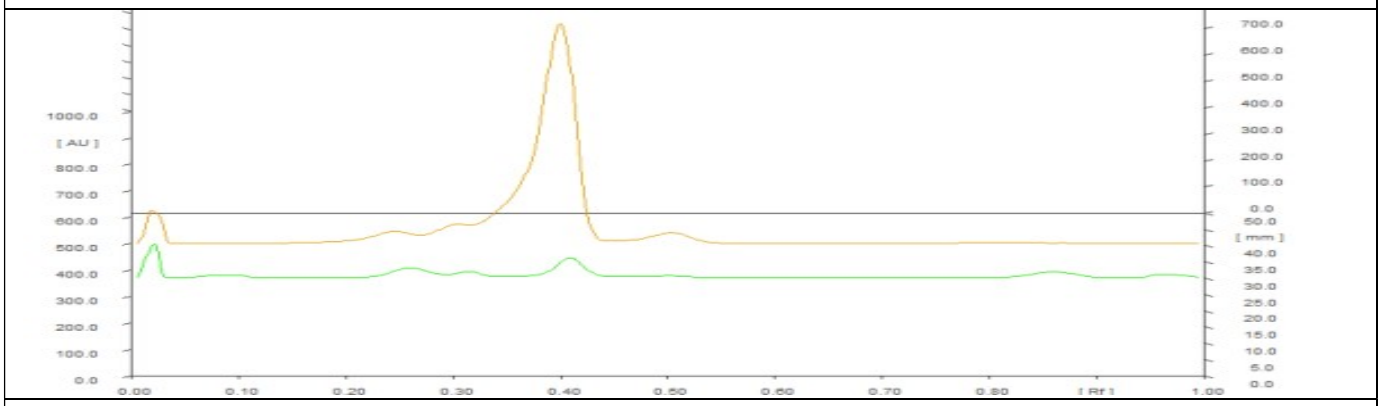


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	11.4 AU	0.03 Rf	234.5 AU	19.88 %	0.05 Rf	0.6 AU	2659.9 AU	5.12 %
2	0.12 Rf	0.0 AU	0.17 Rf	59.5 AU	5.05 %	0.21 Rf	1.0 AU	1335.8 AU	2.57 %
3	0.27 Rf	0.0 AU	0.32 Rf	79.3 AU	6.73 %	0.35 Rf	12.2 AU	1796.8 AU	3.46 %
4	0.36 Rf	12.7 AU	0.38 Rf	31.6 AU	2.68 %	0.39 Rf	29.9 AU	595.1 AU	1.15 %
5	0.39 Rf	29.8 AU	0.46 Rf	139.0 AU	11.79 %	0.49 Rf	69.5 AU	5318.8 AU	10.25 %
6	0.53 Rf	83.9 AU	0.68 Rf	343.4 AU	29.12 %	0.77 Rf	0.3 AU	26810.5 AU	51.66 %
7	0.77 Rf	0.4 AU	0.88 Rf	198.6 AU	16.84 %	0.94 Rf	0.6 AU	11491.8 AU	22.14 %
8	0.95 Rf	5.0 AU	0.96 Rf	93.2 AU	7.90 %	0.99 Rf	21.2 AU	1893.9 AU	3.65 %

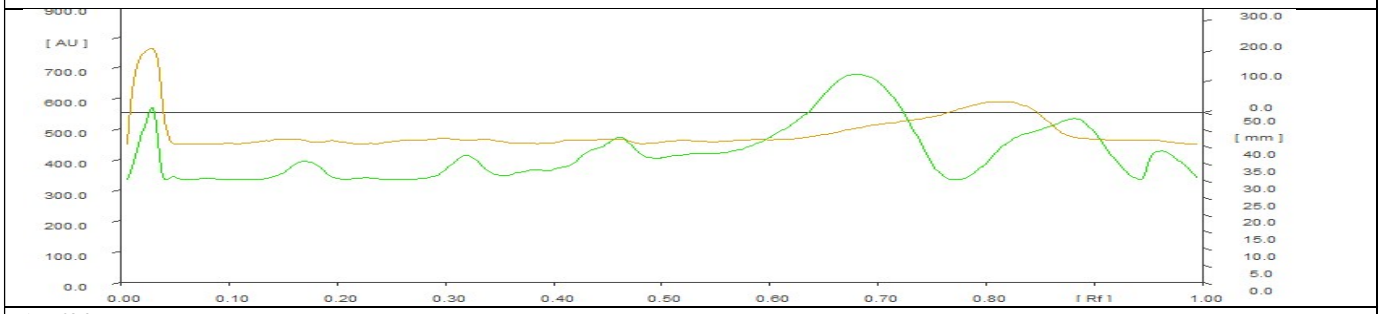
Table 11: Showing *Ashodhita* and *Shodhita Madanaphalapippali* Sample Chromatogram



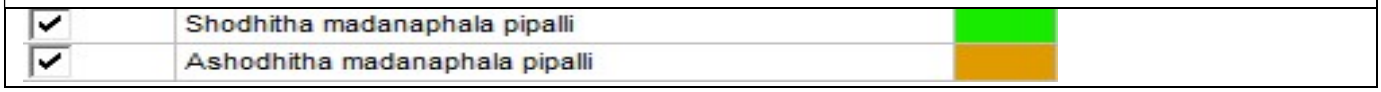
At 254nm



At 366nm



At 620nm



A. On Photodocumentation, *Ashodhita Madanaphalapippali* showed 4 major spots at Rf 0.27 (Dark green) , Rf 0.35 (Light green), 0.40 (Dark green), 0.55 (Light green) under UV 254nm; 3 major spots at Rf 0.20 (Fluorescent blue), Rf 0.35 (Fluorescent blue), Rf 0.44 (Fluorescent blue) under UV at 366nm and 1 major spots at Rf 0.73 (Dark purple) in daylight after derivatisation in Tolune: Ethyl acetate: Formic acid (7.0:3.0:0.3).

On Densitometric scan, at 254nm, 6 peaks with major peak at Rf 0.42 contributing 27.73% area; at 366nm, 5 peaks with major peak at Rf 0.32 contributing 81.38% area and at 620nm, 10 peaks

with major peak at Rf 0.61 contributing 64.50% area was noted

B. On Photodocumentation, *Shodhita Madanaphalapippali* showed 3 major spots at Rf 0.27 (Light green), Rf 0.40 (Light green), 0.78 (Light green) under UV 254nm; 8 major spots at Rf 0.22 (Fluorescent blue), Rf 0.27 (Fluorescent blue), Rf 0.29 (Fluorescent blue), Rf 0.32 (Fluorescent blue), Rf 0.35 (Fluorescent blue), Rf 0.44 (Fluorescent blue), Rf 0.49 (Fluorescent blue), Rf 0.75 (Fluorescent green) under UV at 366nm and 6 major spots at Rf 0.15 (Dark purple), Rf 0.29 (Dark purple), Rf 0.38 (Dark purple), Rf 0.40 (Dark purple), Rf 0.61 (Dark purple), Rf 0.78 (Dark purple) in daylight after derivatisation in Tolune: Ethyl acetate: Formic acid (7.0:3.0:0.3).

On Densitometric scan, at 254nm, 9 peaks with major peak at Rf 0.22 contributing 22.09% area; at 366nm, 6 peaks with major peak at Rf 0.35 contributing 32.85% area and at 620nm, 8 peaks with major peak at Rf 0.53 contributing 51.66% area was noted

CONCLUSION

Madanaphalapippali is best *Vamaka Aushadhi* for treating *Kapha Dosha Vikaras*. The methodological *Vimardana* of *Madanaphalapippali* giving due importance to *Shodhana* helps in getting desired therapeutic effects. The results of the analytical study i.e. HPTLC, Photo documentation, Rf values and Densiometric Scan with Organoleptic Characteristics, Standardization Parameters can be used as the standard quality control test to identify and check the quality as well as *Shodhana* of *Madanaphalapippali* done as per classical text which can be used for *Vamana Karma* for various indications as per the requirement.

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