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Pharmaceutico-Analytical Study of *Vatavidhvamsana Rasa*

Research Article

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Abstract

Vatavidhvamsana rasa (VVR) is a well-known herbo-mineral formulation explained in classical ayurvedic text Yogaratnakara and also quoted in Ayurveda Formulary of India (AFI) and is mainly indicated for *Vatika* disorders. There is a lack of data regarding the standardization of pharmaceutical process and analytical profile of VVR. Aim: To prepare *vatavidhvamsana rasa* and analyze it using various physicochemical parameters. Materials and methods: Total three batches of *Vatavidhvamsana Rasa* was prepared as per the classical method explained in the reference to evaluate the standard procedure. During the pharmaceutical process, all ingredients were mixed thoroughly and triturated thirty times with ten *bhavana* drugs for an average of 90 hours. The pharmaceutical and analytical parameters were completed and the data was recorded. Results: Three batches of *Vatavidhvamsana Rasa* after preparation showed an average increase of 12.93% yield. Pharmacognostical evaluation showed the presence of the ingredients used. Average values of physicochemical parameters of *Vatavidhvamsana Rasa* were as follows: loss on drying 6.19, pH 7, total ash value 36.46, acid insoluble ash 7.16, water soluble extractive value 17.24, alcohol soluble extractive value 7.13. HPTLC of *Vatavidhvamsana Rasa* revealed a total of 11 and 12 bands at 254 nm and 366 nm. Conclusion: Data generated from pharmaceutical, analytical studies and HPTLC can be used to develop a preliminary standard profile for the formulation *Vatavidhvamsana Rasa.*

Key Words: *Vatavidhvamsana Rasa, HPTLC, Herbominerals, Bhasma, Standardization.*

Introduction

Vatavidhwamsana Rasa(1), a well-known herbo-mineral Ayurvedic preparation mentioned in Yogaratnakara *vatavyadhi chikitsa* chapter, which has even been quoted at Ayurvedic Formulary of India[2] and is mainly indicated for *Vatika* disorders. It contains *Shodhita Parada* (Purified Hg-Mercury),*Shodhita Gandaka* (Purified Sulphur,*Naga Bhasma* (incinerated lead), *Vanga Bhasma* (Incinerated Tin), *Tamra Bhasma* (incinerated Copper), *Loha Bhasma* (incinerated Iron), *Abhraka Bhasma* (incinerated Mica), and other herbal ingredients. The dose of *Vatavidhvamsana Rasa* as per the reference is 2 *gunja* (250 mg).

Drug standardization mainly intended to guarantee the quality, efficacy, and uniformity of the final product (3). So to maintain the quality of the final product in every batch, standardization plays a major role. Quality approved raw materials, proper in-process quality checks and finished product quality checks were important in production. Standardization starts from the raw drug collection and extends up to the manufacturing of final products. This study aims to develop pharmaceutical standardization of *Vatavidhvamsana Rasa* (VVR) by preparing three batches as per the classical reference and to establish the quality checking parameters for the formulation.

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Aims and objectives

This study aimed to develop Standard Manufacturing Procedure of *Vatavidhvamsana Rasa* and to develop analytical profile of *Vatavidhvamsana Rasa.*

Materials nad methods

Raw materials collection and authentication

All the raw drugs both herbal and minerals for the preparation of *Vatavidhvamsana rasa* (VVR) were collected from the Dept. of Pharmacy attached with the Institute for postgraduate teaching and research for Ayurveda, Gujarat Ayurveda University, Jamnagar. All the raw materials were identified and authenticated by the Pharmacognosy department of the institute authenticated as per the standards of Ayurvedic Pharmacopia of India. The ingredients used were listed below in table 1 and *bhavana* drugs used were listed in table 2. Three batches of VVR were prepared for standardization viz, VVR 1, VVR 2 and VVR 3 in the Department of Rasashashtra & Bhaishajya Kalpana, Laboratory, IPGT&RA, Jamnagar.

Table No.1- Ingredients and composition of *Vatavidhvamsana rasa*

Sr. No.	Ingredients	Latin Name	Part Used	Composition
Metals / minerals used				
1	*Shodita Parada*	Purified Mercury	----	1 Part
2	*Shodita Gandaka*	Purified Sulphur	----	1 Part
3	*Vanga Bhasma*	Incinerated Tin	----	1 Part
4	*Naga Bhasma*	Incinerated Lead	----	1 Part
5	*Tamra Bhasma*	Incinerated Copper	----	1 Part
6	*Loha Bhasma*	Incinerated Iron	----	1 Part
7	*Abhraka Bhasma*	Incinerated Mica	----	1 Part
8	*Shodita Tankana*	Purified Borax	----	1 Part
Herbal drugs used				
9	*Pippali*	*Piper longum* Linn**.**	Dried Fruit	2 Parts
10	*Shundi*	*Zingiberofficinale* Roscoe.	Dried Rhizome	2 Parts
11	*Maricha*	*Piper Nigrum* Linn.	Dried Fruit	1 Part
12	*Shodita Vatsanabha*	*Aconitum chasmantum* Staff.ex.Holmes	Root tuber	4 ½ Parts

Table No.2- *Bhavana dravyas* of *Vatavidhvamsana Rasa*

Sr. No.	Ingredients	Botanical name /Latin Name	Part Used	Form	Composition
Three bhavana in each of the following drugs					
1	*Trikatu*	*Kwatha of Trikatu*		*Kwath* (Decoction)	Q.S
	Shundi	*Zingiber officinale* Roxb.	Dried Rhizome		
	Maricha	*Piper nigrum* Linn.	Dried Fruit		
	Pippali	*Piper longum* Linn**.**	Dried Fruit		
2	*Triphala*	*Kwatha of Triphala*		*Kwath* (Decoction)	Q.S
	Amalaki	*Embilica officinalis* Gaertn.	Dried Fruit		
	Bibheetaki	*Terminalia bellerica* Roxb.	Dried Fruit		
	Hareetaki	*Terminalia chebula* Retz.	Dried Fruit		
3	*Shoditha Chitraka*	*Plumbago zeylanica* Linn.	Root	*Kwath* (Decoction)	Q.S
4	*Bhringaraja*	*Eclipta alba* Hassk.	Whole plant	*Swarasa* (Juice)	Q.S
5	*Kushta*	*Sausserea lappa* C.B.Clarke	Root	*Kwath* (Decoction)	Q.S
6	*Nirgundi*	*Vitex negundo* Linn.	Leaf	*Swarasa*(Juice)	Q.S
7	*Arka ksheera*	*Calotropis procera* (Ait.)R.Br.	Latex	*Ksheera*(latex)	Q.S
8	*Bhumi amalaki*	*Phyllanthus niruri* Linn.	Whole plant	*Swarasa*(Juice)	Q.S
9	*Chandrasura*	*Lepidium sativum* Linn.	Seed	*Kwath* (Decoction)	Q.S
10	*Nimbu Swarasa*	*Citrus limon* (Linn.) Burm.f	Fruit	*Swarasa*(Juice)	Q.S

1. Pharmaceutical study: Method of preparation of *Vatavidhvamsana rasa*

Three batches of *Vatavidhvamsana Rasa* were prepared as per the method of *kharaleeya rasayana* preparation. The amount of ingredients taken in all the three batches was listed in table-3. All herbal drugs were washed with water and dried and powdered finely. At first, *Kajjali* (a combination of mercury and sulphur) was prepared by grinding processed mercury and sulphur using mortar and pestle, till it attains a lusterless black fine powder form. Add *Naga Bhasma, Loha Bhasma, Vanga Bhasma, Tamra Bhasma, Abhraka Bhasma,* processed *Tankana* powder, *Maricha* powder, *Pippali* powder, *Shundi* powder in the prescribed quantity mentioned in table-3 was added to the *kajjali* and mixed well using a *khalvayantra*. After that 90 g of *shodita vatsanabha* powder was added and triturated well to form a uniform fine mixture. This is triturated three times each in the following ten media in sufficient quantity (total thirty triturations): 1.Decoction of *Trikatu, 2.* Decoction of *Triphala,* 3. Decoction of *Chitraka,* 4. Juice of *Bhringaraja,* 5.Latex of *Arka,* 6. Juice of *Nirgundi,* 7. Juice of *Tamalaki,* 8. Decoction of *Chandrasoora,* 9. Decoction of *Kushta* and 10. Juice of *Nimbu.* Duration of each *bhavana* was 3 hours. Then the final product was dried in shade and stored in an airtight glass container.

Table 3- Quantity of ingredients used in all the three batches of VVR

Sr. No.	Ingredients	Quantity (g) in three batches		
		VVR 1	VVR 2	VVR 3
1	*Shodita Parada*	20	20	20
2	*Shodita Gandaka*	20	20	20
3	*Vanga Bhasma*	20	20	20
4	*Naga Bhasma*	20	20	20

5	*Tamra Bhasma*	20	20	20
6	*Loha Bhasma*	20	20	20
7	*Abhraka Bhasma*	20	20	20
8	*Shodita Tankana*	20	20	20
9	*Pippali*	40	40	40
10	*Shundi*	40	40	40
11	*Maricha*	20	20	20
12	*Shodita Vatsanabha*	90	90	90

Table 4: Quantity of *bhavana* drugs used for the preparation

Sr.No.	Drugs used	Time taken for one *bhavana*	Batch 1 *bhavana* (ml)	Batch 2 *bhavana* (ml)	Batch 3 *bhavana* (ml)
			Three bhavana		
1	*Trikatu kwath*	9 hours	600	600	600
2	*Triphala kwath*	9 hours	540	540	540
3	*Chitrak Kwath*	9 hours	540	540	540
4	*Bhringaraj swarasa*	9 hours	540	540	540
5	*Kushta kwath*	9 hours	540	540	540
6	*Nirgundi swarasa*	9 hours	540	540	540
7	*Arka ksheera*	9 hours	540	540	540
8	*Bhumiamalaki swarasa*	9 hours	540	540	540
9	*Chandrasura kwath*	9 hours	540	540	540
10	*Nimbu swarasa*	9 hours	540	540	540

2. Pharmacognostic study

Pharmacognostic evaluation of the fine powder of final product was in the Pharmacognosy Lab. I.P.G.T.&R.A., Gujarat Ayurveda University., Jamnagar.

Organoleptic study**:** Powdered sample of VVR was evaluated for its organoleptic characters including taste, touch, colour and odour.

Microscopic characteristics

A small quantity of fine powder of VVR was dissolved in distilled water. Few drops from the mixture were spread over the glass slide and covered with a coverslip. Then the slide was microscopically evaluated and microphotographs were taken by using the Carl-Zeiss Trinocular microscope attached with camera (4). The microphotographs of VVR were shown in figure 1.

3. Analytical study (5)

Physico-chemical parameters like the determination of pH, loss on drying, total ash value, determination of acid-insoluble ash, determination of water-soluble extractive, determination of alcohol-soluble extractive of three batches of VVR were performed. Analytical study was carried out in the pharmaceutical chemistry laboratory of I.P.G.T. and R.A., Gujarat Ayurveda University, Jamnagar.

4. High-Performance Thin Layer Chromatography (HPTLC):

Methanolic extract of VVR was spotted on Precoated Silica Gel GF254 aluminium plate (20 cm × 10 cm with 250 m thickness) using Camag Linomate V sample applicator fitted with a 100µ L Hamilton syringe. HPTLC was done to develop a chromatographic pattern of VVR by following the standard procedure with a Camag TLC scanner III in reflectance absorbance mode at 254 nm and 366 nm equipped with Win CATS software (v 1.2.1camag) and the solvent used was Toluene: Ethyl acetate (9:1) v/v (6).

Observations and results

Pharmaceutical Study:

The yield obtained after the preparation of three batches were shown in table 5. Total 90 hours of *bhavana* was carried out and the average gain in the yield of VVR is 12.93%. Total time taken for the mixing and thirty bhavana was approximately 99 hours.

Table 5: Results of three batches of VVR

Batch code	The total quantity of ingredients taken (gm)	Final weight after 30 *bhavana* (gm)	Weight gain after *bhavana* (gm)	% gain in the final product	Duration for the whole process (Hr.)
1	350 gm	400 gm	50 gm	14%	98
2	350 gm	395 gm	45 gm	12.8%	100
3	350 gm	390 gm	42 gm	12%	99
Avg.	350 gm	395 gm	45.67 gm	12.93%	99

Pharmacognostical evaluation:

Powdered sample of final product VVR was microscopically evaluated under Carl-Zeiss Trinocular research microscope revealed the presence of starch grains of *sundi*, disturbed sclerides of *amalaki*, sclerides of *bibheetaki*, collapased stone cells of *hareetaki*, oil globules and fragment fibres of *kushta*, stone cells of *chitraka* accumulated by the mineral deposition, simple trichome of *nirgundi*, disturbed exodermal cells of *vatsanabha*, black debris of minerals or *bhasma* uniformly spread all over the finished product. The microphotographs of VVR is displayed in figure 1.

Table 6 : Organoleptic characters of *Vatavidhvamsana Rasa*

Characters	Results
Rupa (colour)	Brownish black
Rasa (taste)	Salty followed by pungent
Gandha (Odour)	Aromatic
Sparsha (Consistency)	Fine

Figure 1: Microphotographs of *Vatavidhvamsana rasa*

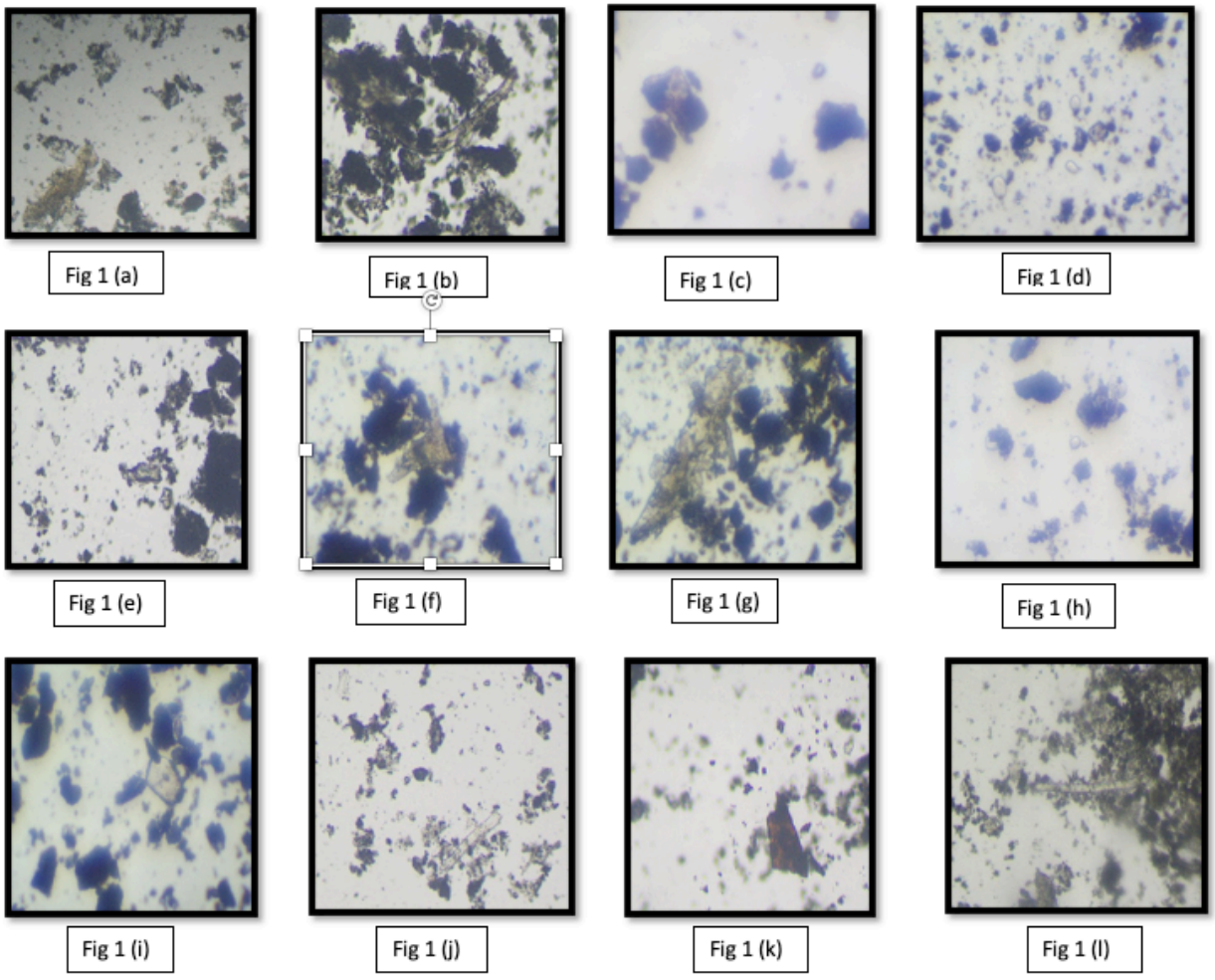

Figure 1: (a). Exodermal cells of *Vatsanabha* (b). Fibers of *Sundi* coated with minerals (c).Stone cells of *Hareetaki* (d).Starch grains of *Sundi* (e).*Silica* deposition of *amalaki* (f).Sclerides of *Bibeetaki* (g).Sclerides of *Amalaki* (h).Oil globules of *Kushta* (i).Trichomes of *Nirgundi* (j). Fibers of *Kushta* (k).Tannin content of *Chitraka* (l).Trichomes of *Bhringaraja.*

Analytical study

Physicochemical evaluation of all the three samples of VVR was carried out and the data was arranged in table 7. HPTLC evaluation revealed eight common spots in 254 and 366 nm (Table 8 and Figure 2(a),2(b),2(c)).

Table 7: Results of the physicochemical evaluation of samples of VVR

Parameters	Results			Average
	VVR 1	VVR 2	VVR 3	
Loss on drying at 110 (%w/w)	4.02	4.56	4	4.19
Total ash (%w/w)	36.13	37.25	36	36.46
Acid insoluble ash (%w/w)	7.25	7.15	7.08	7.16
pH of 5 % aqueous solution	7	7	7	7
Water soluble extractive (%w/w)	17.12	17.53	17.09	17.24
Alcohol soluble extractive (%w/w)	7.1	7.2	7.1	7.13

Table 8: Results of HPTLC of all samples of VVR

	Batch	245 nm	366 nm
Max Rf values	VVR 1	0.02,0.26,0.30,0.32,0.41,0.49, 0.54, 0.77,0.83,0.16,0.40	0.03,0.26,0.31,0.36,0.41,0.45, 0.54,0.77,0.83,0.17,0.73,0.43
	VVR 2	0.02,0.26,0.30,0.32,0.41,0.49, 0.54, 0.77,0.83,0.16,0.40	0.03,0.26,0.31,0.36,0.41, 0.45, 0.54,0.77,0.83,0.17,0.73,0.43
	VVR 3	0.02,0.26,0.30,0.32,0.41,0.49, 0.54, 0.77,0.83,0.16,0.40	0.03,0.26,0.31,0.36,0.41, 0.45, 0.54,0.77,0.83,0.17,0.73,0.43
No. of spots	VVR 1	11	12
	VVR 2	11	12
	VVR 3	11	12

Figure 2: 3 Dimensional HPTLC images of showing the Rf values at 254 nm and 366 nm Of samples VVR1, VVR2 and VVR3

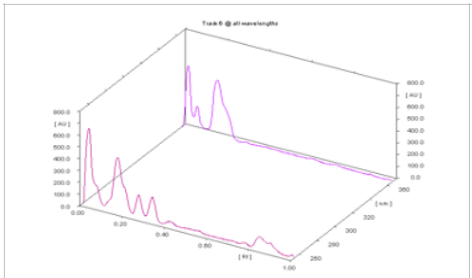

Figure 2(a)- VVR1

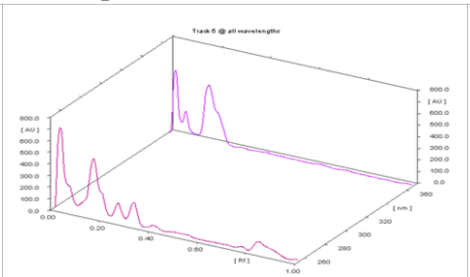

Figure 2(b) VVR 2

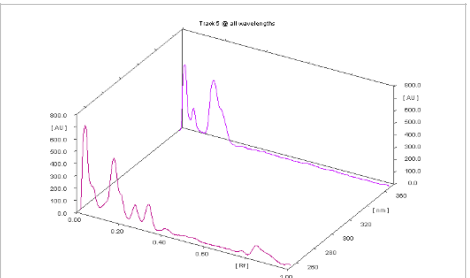

Figure 2(c) VVR 3

Discussion

VVR was prepared as per the method explained in Yogaratnakara. The formulation consisted of eight eight *bhasmas* and four herbal drugs, which are properly collected and authenticated. The drug was prepared as per the classical method of preparation of *kharaleeya rasayana* in which the drugs are properly levigated using various *bhavana dravyas*. Three batches of VVR prepared showed the average percentage gain is 12.93 % and this increase in gain is due to the thirty *bhavana* using ten herbal drugs. These *bhavana* reduces the particle size of the final product and increases the bioavailability of the formulation (7,8). The final product was analyzed for organoleptic characters, pharmacognosy and various quality checks of standardization as per Ayurveda Pharmacopeia of India. The characteristic brownish-black colour of the final product was obtained due to the repeated *bhavana* with various herbal juices and decoctions. The pharmacognosy evaluation showed the presence of distorted stone cells, sclerides, trichomes of various ingredients and *bhavana dravyas*. Thirty *bhavana* collapsed various plant structure and increases the bioavailability.

To validate the pharmaceutical process these different physicochemical parameters were used. Decreased loss on drying showed less chance of microbial contamination and the quality of the final product. Less moisture content, in turn, showed the stability of VVR. The high total ash value is due to the presence of various inorganic salts and metallic ingredients in the final product. This high value is a good indicator of the stringent pharmaceutical processes involved to justify the identity and quality of the final product. Acid insoluble ash value showed the percentage of insoluble inorganic contents of VVR in acids (9). This is also a good indicator to justify the identity of the product. The high water-soluble value

can be due to the presence of various herbal drugs in the herbomineral product indicating the solubility of formulation in water (10). It also signifies the presence of phytochemicals such as sugar and inorganic compounds. pH values are nearly neutral in all the three samples which shows the neutral nature of the final product. Neutral pH may be due to the presence of alkaline and acidic nature of various ingredients. Borax is alkaline and previous studies observed the pH of naga bhasma was almost alkaline (11). Previous studies showed the acidic nature of *kajjali* (12) and *vatsanabha* (13). Since there are no standard records for comparing the analytical values of VVR, every analytical test was repeated for three times to reduce the errors for samples VVR1, VVR 2 and VVR 3. HPTLC analysis of all the samples of VVR was carried out under 254 and 366 nm wavelengths. It showed 11 spots in 254 nm and 12 spots in 366 nm in all samples and eight Rf values are common in both wavelengths.

Conclusion

This study deals with the pharmaceutical, pharmacognostic and physicochemical evaluation of VVR. Final product sample of VVR was fine, brownish black in colour with an aromatic smell and salty taste followed by pungent. The pharmacognostic evaluation indicated the presence of various distorted ingredients of VVR due to thirty repeated *bhavana* for 90 hours. Physico-chemical analysis help to generate a preliminary standard analytical profile for VVR as there is no standard for VVR is available in the pharmacopoeia. So data generated by this study can be used as a tool for the identity and purity of the formulation *Vatavidhvamsana Rasa.*

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Conflicts of interest

There are no conflicts of interest.

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