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Research Article

VALIDATION OF WET AND DRY DRUG COLLECTION PRINCIPLE IN THE PREPARATION OF VASA GHRITA THROUGH QUANTITATIVE ESTIMATION OF VASICINE

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ABSTRACT

Principle of wet and dry drug collection is applied for collection of drugs while preparing any formulations i.e. wet state of drug is taken in double to its dry drug quantity but in case of exception, drug should be taken in wet state and also not taking in double to its prescribed quantity. Here, *Vasa* is mentioned as an exceptional drug for wet and dry drug collection principle. Hence, *Vasa Ghrita* were selected as a dosage form to validate this principle. Aim of present study was to validate the wet and dry drug collection principle in the preparation of *Vasa Ghrita* through quantitative estimation of Vasicine. Three samples of *Vasa Ghrita* were prepared as per the reference of Charaka Samhita from wet and dry state with equal quantity of *Vasa* and wet state and double quantity of *Vasa*. On comparing the data of three samples highest yield (94.16%) was found in *Vasa Ghrita* prepared from wet and equal quantity. The percentage of Vasicine was more in dry *Vasa Panchanga* (0.1914%) than wet state (0.0999%) in HPTLC quantification. Equal quantity and wet state of *Vasa Ghrita* showed more percentage of Vasicine (0.0032%) than dry state and equal quantity (0.0028%) while the percentage of Vasicine in wet state double quantity was in nearer range (0.0035%) to wet state and equal quantity of *Vasa Ghrita*. So, it can be concluded that *Vasa* should be used in wet state without taking in double quantity in *Vasa Ghrita*.

KEYWORDS: Wet and dry drug collection principle, *Vasa, Vasa Ghrita,* Vasicine.

INTRODUCTION

includes complete Bhaishaiya Kalpana knowledge of drugs including the basic principles of drug collection and pharmaceutics. Ayurvedic literature have mentioned the principle regarding the collection of drugs before preparing formulations i.e. wet state of drug should be taken in double quantity to the prescribed Mana (measurement). In Rai Nighantu^[1], as indicated by Aacharya Narhari Pandit that new and wet state of drugs are Suveerya (best therapeutic potency), dry drug have medium potency and Jeerna (old) are Nishphala (least in potency). Drugs like Aardraka, Pippali, Maricha, Kustumbari have different properties in wet and dry state as the therapeutic potency of herbal drugs additionally varies as per their state.

Charaka Samhita^[2], Sushruta Samhita^[3], Ashtanga Hridaya^[4] and Vangasena Samhita^[5] stated exceptional *Mana* (measurement) excluding exceptional drugs for wet and dry drug principle in which *Shushka* (dry state of drug) and *Aardra* (wet state of drug) should be taken in equal quantity. Later on, the texts such as Sharangdhara Samhita^[6], Bhavapraksha Nighnatu^[7], Raja Nighantu^[8], Kaideva Nighantu^[9] and Bhaishajya Ratnavali^[10] are stated

some exceptional drugs for this principle in which drugs should be used in wet state but not taken in double quantity to the prescribed quantity of dry drugs. Under the heading of exceptional drugs list, *Guduchi, Vasa* and *Kutaja,* are commonly described.

Vasa is abundantly used drug in Ayurved with having property of Kaphapittanashana^[11] and also included under the exceptional drug of principle for wet and dry drug collection. So, Vasa (Justicia adhatoda L.) as an exceptional drug for this principle and Vasa Ghrita^[12] as a dosage form were selected. The rationale for selection of this dosage form was, looking to the therapeutic importance of Vasa, it is recommended as Agreya Dravya for Raktapitta^[13] disease as well Vasa Ghrita is also used in Raktapitta Chikitsa. Vasa mainly contain alkaloid Vasicine, which is mainly shows broncho-dilatory activity^[14], antitussive activity^[15] etc.

The present study is aimed to evaluate the principle of wet and dry drug collection whether any significant difference happens if the state and quantity of drug change in preparation of *Vasa Ghrita* in context to Vasicine.

MATERIALS AND METHOD

Procurement and Authentication of Raw Drug

Wet state of drug: Total 7 kg *Vasa Panchanga* and 400 g *Vasa Pushpa* were collected from Government Ayurved Pharmacy, Rajapipla in December 2020 by adopting Good Collection Practices guidelines [16]. The physical impurities such as dust, stone and infected parts were removed by cleaning and manually sorting. Then *Yavakuta* was made in M.S. chopper at

Government Ayurved Pharmacy, Rajapipla. The raw material was identified and authenticated in the Pharmacognosy department, Food and Drug Laboratory (FDL), Vadodara.

Dry Drug: Among the three samples of *Vasa Ghrita*, one sample of *Ghrita* was prepared from dry state of *Vasa Panchanga*. Henceforth *Vasa Panchanga* and *Pushpa* were kept in separate S.S. tray on a thin layer cloth subjected to shed dry.

Table 1: Result of Drying Process of Vasa

	Resu	ılts
Parameters	Vasa Panchanga Yavakuta	Vasa Pushpa
Initially quantity of wet state of <i>Vasa Panchanga Yavakuta</i> and <i>Vasa Pushpa</i> (g)	3800	250
Total time taken for drying	10 days	8 days
Final quantity of dried Vasa Panchanga Yavakuta and Vasa Pushpa (g)	1838	55
Final yield in (%)	48.3684	22
Total loss (g)	1962	195
Total loss (%)	51.64	78
Reason of loss	Due to drying	Due to drying

Cow Ghee: Cow Ghee was procured from Khadigram Udhyoga, Bhutadijapa, Vadodara with *fssai* standard.

Preparation of Samples

Vasa Ghrita was prepared as per the reference mentioned in Charaka Samhita^[17]. In this reference, ratio is not mentioned. Hence, Anukta ratio was adopted as 1:6:24 to Kalka; Ghrita: Kwatha^[18]. Kalka^[19] and Kwatha^[20] were prepared as per mentioned in Sharangdhara Samhita. All the samples of Vasa Ghrita were prepared in pharmaceutical laboratory of PG Department of Rasashashtra evam Bhaishajya Kalpana, Government Ayurved College, Vadodara.

Samples are Labeled as

VGWE- Vasa Ghrita prepared with equal quantity of wet state of Vasa Panchanga

VGWD- Vasa Ghrita prepared with double quantity of wet state of Vasa Panchanga

VGDE- Vasa Ghrita prepared with equal quantity of dry state of Vasa Panchanga

Table 2: Proportion and Quantity For All Three Batches of VGWE, VGWD And VGDE

	Name of drug	Latin Name	Condition	Ratio	Quantity
VGWE	Vasa Pushpa Kalka	Justicia adahtoda L.	Wet	1	35 ml
	Go Ghrita	-	-	6	200 ml
	Vasa Panchanga Kwatha	Justicia adahtoda L.	Wet	24	800 ml
	Vasa Pushpa Kalka	Justicia adahtoda L.	Wet	2	70 ml
VGED	Go Ghrita	-	-	6	200 ml
	Vasa Panchanga Kwatha	Justicia adahtoda L.	Wet	48	1600 ml
	Vasa Pushpa Kalka	Justicia adahtoda L.	Dry	1	35 ml
VGDE	Go Ghrita	-	-	6	200 ml
	Vasa Panchanga Kwatha	Justicia adahtoda L.	Dry	24	800 ml

Preparation of Kalka

Kalka was prepared from *Vasa Pushpa*. The wet state of *Vasa Pushpa* was cleaned with water, ground in a mixer and made a paste. The dry state of

Vasa Pushpa was also converted into coarse powder form by grinding in a mixer. After that paste was prepared from the powder by adding sufficient quantity of water.

Preparation of Kwatha

Kwatha was prepared from Vasa Panchanga Yavakuta. As Vasa Panchanga Yavakuta was light in weight, the drug absorbed all the water and the Kwatha could not be prepared well. So, volumetric measurement was selected for the drug preparation. The drug was measured in volume and took the 4 times water quantity to the volume of drug. The mixture was heated until 1/4th quantity of water remained. It was then filtered through a cotton cloth. For uniformity in the measurement, other ingredients i.e. Kalka and Ghrita were also taken volumetrically.

Preparation of Ghrita

Go-Ghrita was taken in a S.S.vessel and heated over Mrudu Agni till complete evaporation of moisture content. Prepared Kalka was added to Go-Ghrita followed by addition of Vasa Panchanga Kwatha. Mild heat was applied with intermediate stirring. Temperature of Ghrita were in range of 90 - 99 °C. Heating duration is adjusted so as to complete the Sneha Paka and process was carried out till Sneha Siddhi Lakshana appeared. The prepared Ghrita was filtered through cotton cloth in its warm stage and stored in a container after self-cooling. (Figure 1)

Analytical Study

Standard raw material leads optimum quality of product. Cogitating this, analysis of raw material was done foremost. To ensure reliability of finished product, analytical parameters were applied three batches of each sample which were prepared with same quantity, ratio, equipment specification and process.

For Raw Drug: Organoleptic parameters, physic-chemical parameter such as loss on drying^[21], total ash^[22], acid insoluble ash^[23], water and alcohol soluble extractives^[24-25], test for heavy metals^[26], quantification of Vasicine by HPTLC.

For Finished Product: Organoleptic parameters, physicochemical parameter such as acid value^[27], iodine value^[28], saponification value^[29], pH determination^[30], specific gravity^[31] and refractive index^[32], test for heavy metals^[33], quantification of Vasicine by HPTLC.

High Performance Thin Layer Chromatography (HPTLC)

Quantification of Vasicine through HPTLC in wet and dry state of *Vasa Panchanga*, all three sample of *Vasa Ghrita* i.e. VGWE, VGDE, VGWD were done.

High Performance Thin Layer Chromatography (HPTLC) method has been carried out for determining its major bioactive marker Vasicine in CAMAG Linomat 5– Applicator with MERCK- TLC/HPTLC Silica gel 60 F_{254} on Aluminum sheets as Stationery Phase. The mobile phase consisted of Ethyl acetate: Methanol: Ammonia (8: 2: 0.2 v/v). the plates were developed to a distance of 16 mm in twin trough chamber, previously saturated for 30 minutes. Under these conditions, the retention factor (R_f) of Vasicine was 0.23 and it was quantified at 254 nm. (Figure 2, 3)

OBSERVATION AND RESULT

Kalka and Kwatha were added at ranging between 75-80°C and 90-95°C temperature of Ghrita respectively. The crackling sound was heard after adding of Kalka. Phenodgama (foams) occurs at early stage of Sneha Paka suggestive of the presence of water/moisture. Shabdahino-agninikshipta (not produces 'Chat', 'Chat' like sound while putting on fire) suggests complete loss of moisture which is observed in later stage of Snehapaka. At the end it is to be tested in Kalka^[34]. At the time of filtration, the temperature of Ghrita was 90°C. It was pale yellow in color, bitter in taste and had Characteristic smell.

Table 3: Comparative Observational Average Data of Three Sample of Vasa Ghrita

Sr.No.	Parameter	Average parameters for 3 batches		
		VGWE	VGWD	VGDE
1.	Initial Temp of <i>Ghrita</i> (°C)	40	39	41
2.	Kalka added at (Min)	05	05	05
3.	Temp.(°C) at Addition of <i>Kalka</i>	71.33	74.33	75
4.	Kwatha added at (min)	10	10	10
5.	Temp. (°C) at Addition of <i>Kwatha</i>	88.33	88.43	87
6.	Filtration (min)	129	161	125
7.	Temp. (°C) at filtration	90	92	94
8.	Maximum temp of Ghrita(°C)	99	99	99
9.	Total days of Ghrita Paka	1	1	1
10.	Total duration (min)	130	163.33	126.6

Table 4: End Point Parameters

S.No.	Parameters		Average pa	rameters for 3	3 batches
			VGWE	VGWD	VGDE
1.	Vartivata Sneha Kalka	(min)	122	153	120
		Temp. (°C)	97	93	95
2.	Phenashanti	(min)	126	157	122
		Temp. (°C)	96	94	94
3.	Shabdhahina Agni	(min)	129	162	125
	Nikshipta	Temp. (°C)	98	93	95

Table 5: Details Average Data of Pharmaceutical Preparation of VGWE, VGWD and VGDE

Sr.no.	Parameter	Average parameters for 3 batches		
		VGWE	VGWD	VGDE
1.	Kalka (ml)	35	70	35
2.	Initial <i>Ghrita</i> (ml)	200	200	200
3.	Kwatha (ml)	800	1600	800
4.	Total yield (ml)	188.33	185.66	187.33
5.	Total yield (%)	94.16	92.83	93.665
6.	Total loss (ml)	11.66 a	14.34	12.66
7.	Total loss(%)	5.8	7.17	6.33
8.	Total duration (min)	130	163.33	126.6

Analytical Study

Table 6: Organoleptic Characters of Wet And Dry State of Vasa Panchanga Yavakuta

Sr.no.	Characteristics	Wet state of Vasa Panchanga Yavakuta	Dry state of Vasa Panchanga Yavakuta
1.	Color	Dark greenish brown	Light greenish Brown
2.	Appearance	Corse powder	Corse Powder
3.	Texture	Corse	Corse
4.	Taste	Bitter and Astringent	Bitter and Astringent
5.	Odor	Characteristic	Characteristic

Table 7: Physico-Chemical Analysis of Wet And Dry State of Vasa Panchanga Yavakuta

		Results	
S.no.	Parameters	Wet state of Vasa Panchanga	Dry state of Vasa Panchanga
1.	LOD (%w/w)	53. 41	12. 6785
2.	Ash value (%w/w)	2.9320	3. 2276
3.	Acid insoluble ash (%w/w)	0.825	0.990
4.	Alcohol soluble extractive (%w/w)	0.67	2.1946
5.	Water soluble extractive (%w/w)	0.97	13.51

Table 8: Heavy Metal Analysis of Wet And Dry State of Vasa Panchanga

Sr. No	Heavy Metal Content	Wet state of Vasa Panchanga	Dry state of Vasa Panchanga	Permissible Limits as per API
1	Lead	0.5656 ppm	1.0932 ppm	10 ppm
2	Cadmium	Not Detected	Not Detected	0.3 ppm
3	Arsenic	Not Detected	Not Detected	3 ppm
4	Mercury	Not Detected	Not Detected	1ppm

Table 9: Organoleptic Characters of VGWE, VGDE and VGWD

Sr.No.	Characteristics	VGWE	VGWD	VGDE
1.	Color	Pale yellow	Dark yellow	Pale yellow
2.	Touch	Cold, unctuousness	Cold, unctuousness	Cold, unctuousness
3.	Taste	Bitter	Bitter	Bitter
4.	Odor	Characteristic to <i>Vasa</i> and <i>Ghrita</i>	Characteristic to <i>Vasa</i> and <i>Ghrita</i>	Characteristic to <i>Vasa</i> and <i>Ghrita</i>

Table 10: Average Value of Physico Chemical Parameters of Three Batches of VGWE, VGWD and VGDE

Sr. No.	Parameters	Average value of three batches			
		VGWE	VGWD	VGDE	
1	Refractive index at 40°C	1.4543	1.4541	1.4540	
2	Iodine value	35.5367	35.5713	35.5543	
3	Saponification value	208.3	209.66	210.33	
4	Acid value	1.0394	1.067	1.0691	
5	Specific gravity	0.7511	0.7402	0.7604	
6	рН	5.22	5.47	5.14	

Table 11: Heavy Metal Analysis of VGVE, VGWD AND VGDE

Sr. No	Heavy Metal Content	VGWE	VGWD	VGDE	Permissible Limits as per API
1	Lead	1.1252 ppm	1.0552 ppm	0.9028 ppm	10 ppm
2	Cadmium	Not Detected	Not Detected	Not Detected	0.3 ppm
3	Arsenic	Not Detected	Not Detected	Not Detected	3 ppm
4	Mercury	Not Detected	Not Detected	Not Detected	1ppm

Table 12: Shows R_f Value And No. of Spot Found In *Vasa Panchanga* (Wet And Dry) and 3 Samples of *Vasa Ghrita* Visualized Under 245 Nm

Solvent system (v/v)	Standard and Sample	Visualization	R _f value	No. of spots
	Vasicine		0.23	2
Ethyl acetate:	Wet state of Vasa Panchanga		0.19, 0.23	2
Methanol: Ammonia (8: 2: 02 v/v)	Dry state of Vasa Pnchanga	245 nm	0.19, 0.23, 0.72, 0.75	4
	VGWE		0.19, 0.23, 0.36, 0.75	4
	VGWD		0.19, 0.23, 0.32, 0.75, 0.86	5
	VGDE		0.19, 0.23, 0.27, 0.36, 0.75	5

Table 13: Showing HPTLC Quantification of Vasicine

Sample	Vasicine standard	Wet state of Vasa Panchanga	Dry state of Vasa Panchanga	VGWE	VGWD	VGDE
Weight (mg)	10.4	2077	2018	2384	2402	2146
Area	6367.6	1963.5	3657.2	1219.1	1328.3	967.5
% Vasicine	-	0.0999%	0.1914%	0.0032%	0.0035%	0.0028%

Table 14: Observation and Interpretation of Quantified Vasicine in Raw Material and Finished Product

Initial material and % V	Finished product and % Vasicine		Extraction of Vasicine in		
Raw material	% Vasicine	Vasa Ghrita	% Vasicine	final product (e =d/b%)	
a	b	С	d	e	
Wet state of Vasa	0.0999%	VGWE	0.0032%	3.2%	
Panchanga		VGWD	0.0035%	3.5%	
Dry state of Vasa Panchanga	0.1914%	VGDE	0.0028%	1.46%	

DISCUSSION

Ancient literature has mentioned the principle of wet and dry drug collection in context of weight measurement for the preparation of formulation i.e., wet state of drug should be taken in double quantity to the prescribed quantity. The persuaded reason behind this is mentioned in Bhaishajya Ratnavali that dry state of drug is having Guru (heavy) and Tikshna (strong) properties. So, dry drug is used half to wet state of drug. The exceptional drugs for this principle are not mentioned in former manuscripts but some formulations are available in which state of drug is specifically mentioned. Acharya Sharangadhara is the first author who has listed the exceptional drugs for this principle. Vasa is quoted as an exceptional drug for this principle in five classical texts.

Among all the dosage form of *Vasa* mentioned in various classics, maximum number of formulations is found of *Kwatha* (290) than *Ghrita Kalpana* (112). Total 13 references are found in *Samhita* and *Samgraha Grantha* regarding *Vasa Ghrita* and first ever reference found in Charaka Samhita. Another total 99 *Ghrita* are found in which *Vasa* used as an ingredient.

Standardization is need for scenario of Globalization of Ayurved. Here, to develop SMP, three batches of each sample of *Vasa Ghrita* labeled as VGWE, VGWD and VGDE were prepared as per the reference of Charaka Samhita. Method of preparation and ingredients were revealed in concerned reference that *Vasa Pushpa* and *Panchanga* were used as *Kalka* and *Kwatha Dravya* respectively but the ratio of ingredients is not defined. So, *Anukta* ratio for preparation of *Ghrita* was followed as 1:6:24 of *Kalka: Ghrita: Kwatha.* For preparation of VGWD, the ratio of *Kalka* and *Kwatha Dravya* were taken double than prescribed quantity. While method of preparation was same in all three sample of *Vasa Ghrita.* Quantity and state of drug does not affect the

final yield of all sample of *Vasa Ghrita* but VGWD consumed more time for preparation than VGDE and VGWE.

Analytical Study

Analytical study was conducted to distinguish any physical and chemical alterations happened to raw material to finished product.

Raw Material

Wet and dry state of *Vasa Panchanga* showed major difference in LOD, WSE and ASE due to wet state of *Vasa Panchanga* consists more water content than dry state while Total Ash and Acid Insoluble Ash didn't show any substantial difference between them.

Dry state of *Vasa Panchanga* contained more Vasicine (0.1914%) than wet state (0.0999%). This result may be due to the dry drug is more concentrated than wet form. This result supports that after shed drying of *Vasa Panchanga*, alkaloids of it i.e. Vasicine does not deteriorate.

Finished Product

Organoleptic parameters were similar in VGWE, VGWD and VGDE except its color i.e. VGWD was dark yellow while VGWE and VGWD were pale yellow in color.

Physico-chemical parameters like Refractive index, Saponification value, Iodine value, pH and Specific gravity of VGWE, VGWD and VGDE were about to nearer. It concludes that quantity and state of drug doesn't alter physical and chemical attributes.

Among all heavy metal, lead was detected in raw material as well as all the samples of *Vasa Ghrita* within permissible limit. As raw material contains lead, finished product also bears lead within permissible limits. The growing condition of plant, chemical treatments, type of plant species, processing steps, and storage condition are important factors affecting the levels of different metals in herbal preparations.

Quantitative HPTLC showed that the percentage of Vasicine was 0.0032% and 0.0035% in VGWE and VGWD respectively. Extraction of Vasicine from wet state of *Vasa Panchanga* to VGWE and VGWD were 3.2%, 3.5% correspondingly which was almost nearer to each other. Using double quantity of wet state of drug (VGWD) to prescribe quantity does not increase the percentage of Vasicine in finished product.

For VGDE, the percentage of Vasicine was 0.0028% in VGDE. So, based upon this quantitative method of HPTLC, it can be said that the extraction of Vasicine from dry state of *Vasa Panchanga* to VGDE was 1.46%, almost half in compare to VGWE. This was happened even though raw material of VGDE i.e. dry state of *Vasa Panchanga* had more percentage of Vasicine than raw material of VGWE i.e. wet state of *Vasa Panchanga*.

CONCLUSION

Vasa Ghrita prepared from wet state and equal quantity showed highest yield 94.16%. Organoleptic and physico-chemical attributes of all three samples of VGWE, VGDE and VGWD doesn't change except its color. Physicochemical parameters and the percentage of Vasicine content in wet (0.0999%) and dry (0.1914%) state of Vasa Panchanga showed remarkable difference which concludes that bio-active constituents are higher in dry state of Vasa Panchanga. Based on the result obtained from HPTLC method, Vasa Ghrita prepared from wet state equal and double quantity [VGWE (0.0032%), VGWD (0.0035%)] bears the almost similar Vasicine content while Vasa Ghrita prepared from dry state and equal quantity [VGDE (0.0028%)] bears the least Vasicine content than Vasa Ghrita prepared from wet state and equal quantity [VGWE (0.0032%)]. Henceforth, it can be concluded that Vasa listed as exceptional drug for principle of wet and dry drug collection should be taken in wet state without taking double quantity to its prescribed quantity.

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Preparation of Vasa Ghrita Heating of Go-Ghrita Heating it on Mandagni Addition of Kwatha Addition of Kwatha

Figure 1: Preparation of Vasa Ghrita

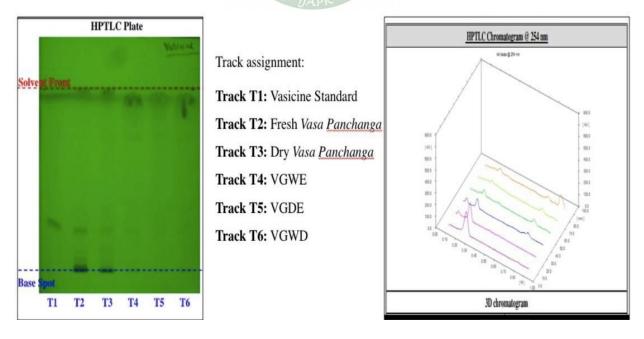


Figure 2: HPTLC Fingerprinting and 3 D Chromatogram

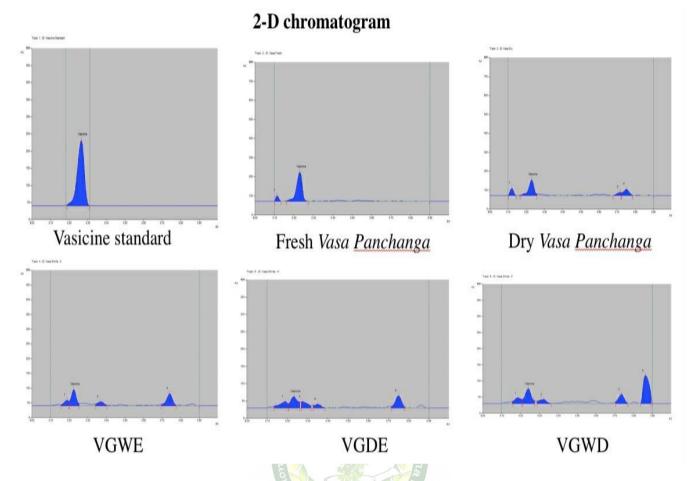


Figure 3: 2D Chromatograms