



Research Article

STANDARDIZATION AND ANALYTICAL PROFILING OF *SHIRASHOOLADI VAJRA RASA*: A COMPARATIVE STUDY WITH MARKETED SAMPLES

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ABSTRACT

*Shirashooladi vajra rasa* is a potent mercurial preparation widely used in the treatment of *Shirashoola* (headache). It consists of processed mercury and sulphur, incinerated copper and iron along with other herbal ingredients like *Triphala*, *Yastimadhu*, *Pippali*, *Shunthi*, *Vidanga*, *Dashamula* and *Guggulu*. This formulation is enriched with *Vatashamaka* (mitigates *Vata dosha*) herbs like *Dashamula*, *Guggulu* etc. **Aim and objective:** This study aims at developing standard manufacturing procedure (SMP) and analytical parameters of *Shirashooladi vajra rasa* along with analysis of its available marketed samples. **Methodology:** The formulation was prepared in three batches to standardize its pharmaceutical processing. Also, six other available different samples of the *Shirashooladi vajra rasa* were collected from market and all the seven samples were assessed for quality control parameters to compare their quality. **Observations & Result:** All the quality control parameters conducted for inhouse and marketed samples were not similar, some variations were found. **Conclusion:** The average yield obtained from three batches is 99.1%. Analytically, slight variation was observed among all the samples.

INTRODUCTION

*Shirashooladivajra rasa* is a poly herbo-metallic, *Kharaliya* preparation described in the management of headache (*Shirashoola*). [1] Ayurvedic Seers describes *Shirashoola* both as a symptom in numerous diseases as well as an independent disease. Due to vitiation of *Vata dosha* in head region, pain is manifested. In Ayurveda, *Shirashooladivajra* rasa is mentioned for the management of the disease. As the name explains, it pacifies headache (*Shirashoola*) like the God *Indra's* thunderbolt kill demons. Many polyherbal formulations are widely used in clinical practice but most of them are anguish from lack of data specifically pharmaceutical and analytical data. Standardizing the formulations is one important step among the basic quality control parameters. Since, the term 'standardization' is used to indicate all the measures that are taken during the manufacturing

process and denotes the reproducible quality production. While standardizing the polyherbal or herbo-mineral preparations, numerous factors are responsible for affecting the reproducibility and to avoid these difficulties, proper attention should be given right from the identification, collection, manufacturing till its proper packaging. Analytical study is one step ahead in terms of determining the quality check. Hence, the present work aims at the pharmaceutical standardization and establishing analytical profile of the formulation prepared inhouse. Also, six different available marketed samples of *Shirashooladivajra* rasa were analyzed for the quality check and the comparative quality inference was drawn of all the samples.

MATERIAL AND METHOD

Preparation of *Shirashooladivajra Rasa*

Method adopted for formulating *Shirashooladivajra rasa* is taken from '*Bhaishajya Ratnavali*' text. Three batches of *Shirashooladivajra* rasa were prepared for the standardization in Department of Rasa Shastra and Bhaishajya Kalpana, All India Institute of Ayurveda, New Delhi.

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### Step 1: Collection and Authentication of Raw materials

All the raw herbal materials were procured from the local market of Delhi, were identified and authenticated by Dept of *Rasa Shastra*, AIIA, New Delhi. *Tamara bhasma* and *Lauha Bhasma* both were prepared classically in the laboratory of Dept of RSBK, AIIA. The ingredients of this formulation are listed in Table 1.

### Step 2: Preliminary processing

#### Preparation of *Kajjali*

*Shudha Parada* (48gm) and *Shudha Gandhaka* (48gm) were taken in equal amount in a clean mortar and pestle and triturated well till desired characteristics of *Kajjali* were obtained.

#### *Kwatha* preparation

Coarsely powdered (*Yavkuta*) *Dashamula* were taken in amount equal to quantity of all the ingredients in the formulation (648gm). To this, water was added eight times to that of the total quantity (5184ml) and was subjected to medium flame (100°C) till 1/8<sup>th</sup> liquid was left. The *Kwatha* so obtained was filtered and kept.

### Step 3: Primary preparation

*Kajjali* was taken in a clean mortar and pestle (*Khalva Yantra*), to this *Tamra Bhasma* (48gm) and *Lauha Bhasma* (48gm) were added and triturated to form a homogeneous mixture. Afterwards, fine powders of *Haritaki* (32gm), *Bhibhitaki* (32gm), *Amalaki* (32gm), *Yashtimadhu* (12gm), *Pippali* (12gm), *Shunthi* (12gm), *Gokshura* (12gm), *Vidanga* (12gm) and *Bilva* (12gm) were added to it sequentially and triturated well.

#### Addition of *Guggulu*

*Shodhita Guggulu* (192gm) was taken and melted in *Dashmoola kwatha*. After proper melting, it was poured into mixture of *Kajjali* and other herbs in quantity till it gives mud like appearance (*Panka vat*). It was then subjected to levigation till desired characteristics were obtained. Only one *Bhavna* of *Dashmoola kwatha* is indicated.

### Step 4: *Vati* Preparation

The homogeneous mass was taken and pills (*Vati*) of about 575-625 mg were rolled manually as per given in reference. Ghee was used while rolling *Vati* to avoid sticking of mixture on the hands. All the *Vati* were then dried in an oven at 40°C. After complete drying, pills were stored in airtight containers. The average weight obtained after drying was 600mg.

Two more similar batches with raw drugs in same quantities and under same conditions were prepared for standardization of this formulation. Details of the same are described in Table 2.

### Analytical Study

All the three batches were subjected to analytical parameters. Along with in-house sample of *Vati*, six other marketed samples (MS1, MS2, MS3, MS4, MS5 & MS6) of *Shirashooladivajra rasa* were analysed for quality assurance. All the samples were tested on the following parameters- Organoleptic characteristics (colour, smell, taste and appearance) described in Table 3, pH,<sup>[2]</sup> hardness,<sup>[3]</sup> friability,<sup>[4]</sup> weight variation,<sup>[5]</sup> disintegration time,<sup>[6]</sup> loss on drying,<sup>[7]</sup> water soluble extractive,<sup>[8]</sup> alcohol soluble extractive,<sup>[9]</sup> ash value,<sup>[10]</sup> acid insoluble ash<sup>[11]</sup> are mentioned in Table 4 and sophisticated analysis like HPTLC <sup>[12]</sup> and FTIR are described in picture 1 & 2.

### High performance thin layer chromatography (HPTLC)

#### Preparation of extract

1mg coarse powder of all samples were mixed with 10 ml ethanol and kept overnight. Next day, the samples were sonicated for 20 mins followed by filtration with Whatman Filter paper 1. The clear liquid so obtained is used to apply over HPTLC plate.

#### Preparation of mobile phase

Toluene: Acetone (9:1) were measured accurately, mixed well and transferred in a 20× 10 cm twin trough chamber. Chamber was then tilted to 45° for equal distribution of mobile phase. A filter paper was placed over the front side of the chamber and covered with the lid. The chamber was now left undisturbed for 20 mins for the saturation of mobile phase in the chamber.

#### Procedure

The procedure was performed by using CAMAG linomat HPTLC system attached with automatic TLC sample applicator, TLC scanner with WinCats software.

The pre-coated HPTLC plate silica gel 60 F254 of 20cm × 10cm was cut and used for analysis. 5µl of sample was applied by using TLC sample applicator. Band size of all the sample was 8mm. After proper application of samples, the plate was dried over CAMAG heater at 40°C. After drying the plate, it is kept in the pre saturated chamber along the rear side till it reaches the mark i.e., 7cm. Then, the plate was removed from chamber and dried again. The visualization of band was done by using TLC visualizer at 254nm and 366nm following by TLC scanner on two wavelengths i.e., 254nm by using D2 and W lamp and another at 366nm by using Hg lamp. Different obtained  $R_f$  were then noted down.

### OBSERVATION AND RESULT

While processing the formulation, it has been observed that after addition of *Lauha bhasma* and *Tamra bhasma* to *Kajjali*, the black colour of *Kajjali* turns to brownish colour. For melting the *Guggulu*, it was mixed in double amount of *Dashmoola kwatha* i.e.,

192 gm of *guggulu* in 384 ml of *dashmoola kwatha*. The time required for complete melting of *guggulu* is about 10 min. After addition of melted *guggulu* in the mixture of Kajjali and herbal powders, the mixture becomes sticky in nature. Time required for different processes while pill (*Vati*) formation is mentioned in Table 4.

The average yield obtained from three batches was 99.1%. All the three in-house preparations were in pill (*Vati*) form. Among the procured marketed samples, MS1, MS2, MS3, MS4 and MS5 are in tablet form, MS6 is available in pill (*Vati*) form. The pH value of all the pills varies between 3.79 to 4.72, signifying the acidic nature. Hardness of pills was observed  $8 \pm 1.5$ . Variation among weight was observed among all samples. Average weight of inhouse sample was 600 mg, prepared according to classical reference. (20% average loss) MS6 *Vati* has average weight of about 350mg. The pills of the three batches of inhouse sample along with MS1, MS2, MS4 and MS6 has passed the weight variation as per API standards whereas MS3 and MS5 didn't match the standard criteria. The friability of samples was within normal limit except MS1 (0.5-1%). Average Loss on drying of all the samples were within normal limits ( $0.85 \pm 0.31\%$ ). Only three marketed samples i.e., MS4, MS5 and MS6 have slightly higher values. The average values of water-soluble extractive of inhouse batches are similar to that of MS2 while for the rest of samples, it varies. ( $6.57 \pm 1.06$ ) In alcohol soluble extractive, average values of inhouse batches are similar to that of MS3 and varies in all other samples. Ash value and acid insoluble ash of all the samples were found to be beyond the normal limit i.e.,  $14.76 \pm 0.67$  and  $4.58 \pm 0.44$  respectively. (Table 4)

HPTLC result shows that most of the samples gives  $R_f$  values at 0.02 (blue band), 0.10 (blue and purple band), 0.14 (green band), 0.259 (light purple band), 0.38 (light blue band), 0.45 (light blue band), 0.81 (light green band), 0.92 (light purple band), 1.05 (green band). (Pic 1)

FTIR data exhibits presence of various functional groups. Alkenes, esters, alcohols, carboxylic acid groups were present in all. Maximum functional groups were found to be in MS5, whereas minimum in inhouse S2. Among all, three inhouse samples and 4<sup>th</sup> marketed sample were found to be similar and rest didn't (Pic 2).

## DISCUSSION

*Shirashooladivajra rasa* is a well-known Ayurvedic formulation, firstly described in *Rasendra Sara Sangaraha* (13<sup>th</sup> century).<sup>[13]</sup> *Bhaishajya Ratnavali*, a text of 18<sup>th</sup> century, has also mentioned the same formulation by the name of *Shirovajra rasa*. In recent texts like *Ayurveda Sara Sangraha* this formulation is mentioned by the name of *Shirashooladivajra rasa*.<sup>[14]</sup> The reference of *Bhaishajya*

*Ratnavali* is also quoted in the same text with slight variations in ingredients. Instead of *Pippali* and *Shunthi*, *Kutha* is taken as an ingredient, whereas method of preparation is same.

Many pharmaceutical companies give reference of *Bhaishajya Ratnavali* for the preparation of this herbo-metallic formulation but none strictly follows the classical reference. All of them follows the reference of *Ayurveda Sara Sangraha*. MS1, MS2, MS3, MS4 and MS5 uses *Kutha* along with *Pippali* and *Shunthi* but no such reference is available in literature. MS6 was prepared by using the reference of *Rasatantra Sara Sangaraha* which contains *Nishotha* or *Trivrutta* instead of *Tamra bhasma*.

Average yield of three batches was around 99.1%. About 0.9% loss in the final product could be due to procedural loss. Also, remaining 0.9% could be the procedural loss. pH of all the samples is acidic which may be due to the acidic nature of all the ingredients used in it. Due to different size of MS1 i.e., 125mg, hardness of the tablet is reduced whereas all the other samples have size varying between 300-350mg, thus having similar hardness. MS4 which is in tablet form, have size varying between 160-180mg, also shows varying hardness, which is possibly due to the presence of binding agents in larger proportions. The variation in the DT (Disintegration time) could be due to the reason that pills of all the three inhouse samples were hand rolled, which resembles with MS6, which is also available in pill form while the other marketed samples having DT varying from these could be due to the reason that they are compressed mechanically. LOD values are nearly similar of all the samples. In HPTLC, the  $r_f$  0.02 gives maximum area under curve which denotes the concentration of *Dashamoola* and is present in all the samples. Variation in FTIR data signifies that the three inhouse samples along with MS1 matches with each other while all other samples do not match and shows certain variations.

The probable mode of action of *Shirashooladivajra rasa* is based on its *Yogavahitva* property due to presence of ingredients like mercury (*Parad*), *Lauha bhasma* and *Tamra Bhasma*.<sup>[15]</sup> The average particle size of *Louha* and *Tamra bhasma* is found to be in micro to nano range in many published papers,<sup>[16]</sup> thus helping in the target specific action. Many research works have been conducted which shows that mercury can pass blood brain barrier (BBB) which helps other herbal drug in their actions.<sup>[17]</sup> *Shirashooladivajra rasa* mostly contains ingredients which are having *Vatashamaka* properties and hence pacifies *Vata dosha*, which is a major cause of headache. Also, ingredients like *Triphala* are described as a rejuvenator (*Rasayana*) by Acharya Charaka.<sup>[18]</sup> *Vidanga*, another ingredient, is a potent

anti-helmintic (*Krumighna*) which can be helpful in *Krumij shiroroga*, another type of *Shirashoola* mentioned by Ayurveda scholars.<sup>[19]</sup>

### CONCLUSION

*Shirashooladivajra rasa* is widely used in treatment of *Shirashoola*. Three batches of the *Shirashooladi vajra rasa* were prepared and the data

so obtained can be further used as preliminary standardized data for future works. The quality control tests including both preliminary and sophisticated analysis performed for all the inhouse and marketed samples also varies signifying the variations in their raw material authentication, pharmaceutical processing and quality.

**Table 1: Ingredients of Shirashooladivajra Rasa**

S.No.	Name of ingredients	Part used	Botanical Name/English Name	Proportion
1.	<i>Shuddha Parada</i>	-	Processed mercury	48gm
2.	<i>Shuddha Gandhaka</i>	-	Processed sulphur	48gm
3.	<i>Tamra bhasma</i>	-	Incinerated copper	48gm
4.	<i>Lauha bhasma</i>	-	Incinerated iron	48gm
5.	<i>Shuddha guggulu</i>	Exudate	<i>Commiphora wightii</i> (A.) BHANDARI	192gm
6.	<i>Haritaki</i>	Fruit	<i>Terminalia chebula</i> RETZ.	32gm
7.	<i>Bibhitaki</i>	Fruit	<i>Terminalia bellirica</i> (GAERTN.) ROXB.	32gm
8.	<i>Amalaki</i>	Fruit	<i>Emblica officinalis</i> GAERTN.	32gm
9.	<i>Yastimadhu</i>	Root	<i>Glycyrrhiza glabra</i> L. var. violace	12gm
10.	<i>Pippali</i>	Fruit	<i>Piper longum</i> L.	12gm
11.	<i>Shunthi</i>	Rhizome	<i>Zingiber officinale</i> ROSC.	12gm
12.	<i>Vidanga</i>	Fruit	<i>Embelia ribes</i> BURM.F.	12gm
13.	<i>Bilva</i>	Whole plant	<i>Aegle marmelos</i> (L.) CORREA EX. SCHULTZ	12gm
14.	<i>Agnimantha</i>	Whole plant	<i>Premna serratifolia</i> L	12gm
15.	<i>Shyonaka</i>	Whole plant	<i>Oroxylum indicum</i> (L.) VENT.	12gm
16.	<i>Patala</i>	Whole plant	<i>Stereospermum chelonoides</i> (L.F.) DC.	12gm
17.	<i>Gambhari</i>	Whole plant	<i>Gmelina arborea</i> L.	12gm
18.	<i>Gokshura</i>	Whole plant	<i>Tribulus terrestris</i> L.	12gm
19.	<i>Brihati</i>	Whole plant	<i>Solanum virginianum</i> L.	12gm
20.	<i>Kanthakari</i>	Whole plant	<i>Solanum xanthocarpum</i> SCHRAD & WENDL	12gm
21.	<i>Shalaparni</i>	Whole plant	<i>Desmodium gangeticum</i> (L.) DC.	12gm
22.	<i>Prushnaparni</i>	Whole plant	<i>Uraria picta</i> (Jacq.) DC.	12gm

**Table 2: Yield Obtained of Three Inhouse Batches**

S.no.		Batch 1	Batch 2	Batch 3
1.	Raw drug taken (gm)	684	684	684
2.	<i>Kwatha</i> obtained (ml)	684	684	684
3.	<i>Kwatha</i> after addition of <i>Guggulu</i> (ml)	200	210	208
4.	Total mass obtained (gm)	840	845	837
5.	Total weight after drying (gm)	672	676	669.5
6.	Yield (in %)	99.1	99.4	98.9

**Table 3: Organoleptic Characteristics**

Samples → Characters ↓	IH1	IH2	IH3	MS1	MS2	MS3	MS4	MS5	MS6
<b>Colour</b>	Black	Black	Black	Black	Grey	Grey	Dark brown	Brown	Black
<b>Appearance</b>	Round	Round	Round	Round	Compressed	Compressed	Compressed	Compressed	Round
<b>Touch</b>	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
<b>Taste</b>	Astringent ( <i>Kshaya</i> ), Sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), Sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), , Sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), Sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), Sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), Sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), Sour ( <i>Amla</i> )	Astringent ( <i>Kshaya</i> ), , Sour ( <i>Amla</i> )

IH: Inhouse Sample MS: Marketed Sample

**Table 4: Time Required for Different Processes**

S.No.	Process	Time required		
		1	2	3
1.	<i>Kajjali</i> preparation	30 hr	32 hr	31 hr
2.	Trituration after adding <i>Tamra</i> and <i>Lauha bhasma</i>	15 min	14 min	14 min
3.	Trituration after adding herbal ingredients	15 min	12 min	14 min
4.	Levigation till desired consistency is obtained	3 hr	2.50 hr	3 hr

**Table 5: Average Analytical Data of all Samples**

Analysis	IH1	IH2	IH3	MS1	MS2	MS3	MS4	MS5	MS6	Mean
<b>pH</b>	3.80	3.79	3.81	4.53	4.72	4.54	4.10	4.02	3.92	4.13±2.3
<b>Hardness</b>	9	9	9	4	9	6	10	6	10	8±1.5
<b>Friability</b>	0.2%	0.3%	0.2%	4.92%	0.3%	0.2%	0.2%	0.8%	0.002%	0.79 ±0.91
<b>Weight variation</b>				311.49 ± 7.77	294.11 ± 8.57					
<b>Disintegration time (in mins)</b>	>60	>60	>60	25	20	53	40	31	>60	45.44±13.03
<b>Loss on drying</b>	0.7%	0.7%	0.6%	0.4%	0.9%	1.17%	1.33%	1.08%	0.8%	0.85±0.31
<b>Water soluble extractive</b>	5.24%	5.476%	5.342%	6.224%	5.8224%	9.286%	7.13%	8.19%	6.496%	6.57±1.06
<b>Alcohol soluble extractive</b>	5.52%	5.586%	5.2%	7.568%	3.488%	5.076%	4.328%	4.028%	3.968%	4.96±1.28
<b>Ash value</b>	14.738	14.296	14.23	15.606	15.38	16.24 2	12.966	14.90	14.56	14.76±0.67
<b>Acid insoluble ash</b>	4.716	4.686	4.825	3.008	6.082	4.068	4.458	4.67	4.79	4.58±0.44

IH- Inhouse sample, MS- Marketed sample

Picture 1: HPTLC data of all samples at 254nm &amp; 366nm



List of Compared References:							
Sample Name	Description	Correlation	C	Factor	Correlation C	Discri	Pass / Fail
C:\pel_data\spectra\Batch 1.sp	Batch 1	1.0000		1.0000	0.9800		Pass
C:\pel_data\spectra\Batch 2.sp	Batch 2	0.9967		1.3251	0.9800		Pass
C:\pel_data\spectra\Batch 3.sp	Batch 3	0.9962		1.1777	0.9800		Pass
C:\pel_data\spectra\MS 1.sp	MS 1	0.9838		0.9229	0.9800		Pass
C:\pel_data\spectra\MS 3.sp	MS 3	0.9442		1.3830	0.9800		Fail
C:\pel_data\spectra\MS 5.sp	MS 5	0.9369		0.9721	0.9800		Fail
C:\pel_data\spectra\MS 2.sp	MS 2	0.9119		1.8883	0.9800		Fail
C:\pel_data\spectra\MS 4.sp	MS 4	0.8327		1.6037	0.9800		Fail
C:\pel_data\spectra\MS 6.sp	MS 6	0.7087		1.6502	0.9800		Fail

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