Pharmaceutico-analytical study of *Samaguna*, *Dwiguna* and *Shadguna Balijarita Rasa Sindura*

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Abstract

Introduction: *Rasa Sindura* is a *Kupipakva Rasayana* which is very popular and is widely used in therapeutics till date. Therapeutic property of *Rasa Sindura* varies with proportion of *Gandhaka* added to *Parada*. **Aims and Objective:** The present study was carried out to reevaluate and compare differences in the pharmaceutical analytical study of *Rasa Sindura* prepared with the changing proportion of *Gandhaka*. To prepare *Samaguna* (RS), *Dwiguna* (2RS) and *Shadguna* (6RS) *Balijarita Rasa Sindura* and to carry out X-ray diffraction study (XRD) and Fourier-Transform Infrared spectroscopy (FTIR) of the samples. **Material and Methods:** *Samaguna*, *Dwiguna* and *Shadguna Balijarita Rasa Sindura* were prepared as per classical reference and the samples were subjected to XRD and FTIR. **Results:** The duration of trituration for the preparation of *Samaguna, Dwiguna* and *Shadguna Kajjali* was 70, 40 and 24 h respectively. The duration of heating was 16, 16 and 19 h respectively. Average percentage yield of RS, 2RS and 6RS was 54%, 34.44% and 11.35%, respectively XRD showed increased crystallinity of the samples. FTIR spectrum revealed the decrease of unsaturated carbon and exchangeable proton as the proportion of sulfur increased in the preparation. **Conclusion:** XRD and FTIR suggested better stability of the crystals of 6RS than 2RS and RS.

Keywords: *Balijarana, Kupipakva Rasayana, Rasa Sindura*

Introduction

Kupipakva Rasayana are unique pharmaceutical procedures in the field of Rasashastra wherein mercury along with other minerals, metals are sublimed by subjecting it to a gradual increase in temperature for a specified time. *Kupipakva Rasayana* are more potent and quick acting even in a smaller dose.

Rasa Sindura is a *Kupipakva Rasayana* which is very popular and is widely used in therapeutics till date. It is prepared by heating *Kajjali* filled in a glass bottle wrapped with multiple layers of cloth smeared with mud, in a sand bath through *Valuka Yantra* for the specific time period.[1] Its indication ranges from simple ailments such as *Ajirna* and *Aruchi* to chronic ailments such as *Kushtha*, *Rajayakshma* and *Prameha* with disease-specific *Anupana*.[2] Hence, it is widely used in other formulations.

Therapeutic property of *Rasa Sindura* varies with the proportion of *Gandhaka* (sulfur) added to that of *Parada* (mercury) and also the duration of *Gandhaka Jarana.* It is claimed in the text that more the quantity of *Gandhaka* added, more is the potency.[3] However, according to law of definite proportion, eight parts of mercury combines with one part of sulfur. Hence, the present study was carried out to study the structural changes in *Rasa Sindura* prepared with different proportion of sulfur.

Hence, the present study was carried out to provide scientific analytical basis in support of the above concept of variation in therapeutic property of *Rasa Sindura* prepared with change in the proportion of *Gandhaka.*

Aims and objectives

This study aims to re-evaluate and compare differences in the pharmaceutical analytical study of *Rasa Sindura* prepared

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with the changing proportion of *Gandhaka*. (1) To prepare *Samaguna Balijarita Rasa Sindura* (RS), *Dwiguna Balijarita Rasa Sindura* (2RS), (2) To prepare *Shadguna Balijarita Rasa Sindura* (6RS) and to carry out X-ray diffraction (XRD) and Fourier Transform Infra-red spectroscopy (FTIR) study of the samples.

Materials and Methods

Preparation of *Samaguna, Dwiguna* and *Shadguna Balijarita Rasa Sindura*

Parada and *Gandhaka* were obtained from local market. *Vatankura* and *Kumari* were procured from herbal garden of JSSAMC and *Rakta Karpasa Pushpa* was procured from Krishi Vigyana Kendra, Bellary. Raw materials were used for the preparation after assessing its genuinity. *Parada Shodhana* (detoxification of mercury) was carried out by levigating it with equal quantity of *Haridra* (*Curcuma longa* Linn.) powder and sufficient quantity of fresh juice of *Kumari* (*Aloe barbadensis*) in a *Khalva Yantra* (mortar and pestle) till it turns to a homogeneous mixture. Later on *Chakrikas* (flakes) were prepared and dried. Flakes were subjected to *Urdhvapatana* (sublimation) in *Urdhvapatana-Yantra* for 6 h by giving *Kramagni* (sequential heat pattern) namely 2 h of mild heat, 2 h of moderate heat and 2 h of intense heat. Later on, the apparatus was allowed for self-cooling. *Parada* which was adhered to the upper pot was collected, washed thoroughly with hot water, filtered through cloth, dried and was stored.[4]

Gandhaka Shodhana (detoxification of sulfur) was done using *Bhudhara Yantra* wherein a pot was half filled with milk and was buried in the ground up to its neck. A cloth was tied over the mouth of the pot and *Gandhaka* was spread over the cloth. An iron griddle was placed inverted over it on which 8–10 cow dung cakes were placed and ignited.[5] After self-cooling, *Shuddha Gandhaka* in beads shape was found at the bottom of the pot which was washed thoroughly with hot water and dried. Lateron it was powdered and stored.

Thus *Shodhita Parada* and *Gandhaka* (processed mercury and sulfur) were used to prepare *Samaguna, Dwiguna* and *Shadguna Kajjali* as per classical reference which were later used to prepare respective *Rasa Sindura*.[6-8]

Preparation of *Rasa Sindura*

It can be classified under the following three headings:

Preparatory phase

An amber-colored glass bottle was taken and washed thoroughly with hot water and dried. A cloth smeared with *Multani Mitti* (Fuller's earth) was folded into four folds and pasted to the bottom of the bottle. The bottle was covered with seven layers of cloth smeared with *Multani Mitti* and dried. *Kajjali* was given *Bhavana* (levigation) with *Vatankura Swarasa, Rakta Karpasa Pushpa Swarasa* and *Kumari swarasa* for *Samaguna, Dwiguna* and *Shadguna Kajjali* respectively, dried, powdered, filled in the bottle and was placed in *Valuka Yantra*.

Main pharmaceutical operation

The fire was ignited and light heat was given till the appearance of white fumes. Later on mild heat was given till the stage of corking. Bottle was corked with the help of *Gopichandana* covered with cloth smeared with *Multani Mitti* after *Sheeta shalaka* test and copper coin test was found positive. Sand surrounding the neck of the bottle was removed and *Teevragni* (high heat) was given for about 2 h. later on, the addition of fuel was stopped. After complete self cooling to room temperature the *Kupi* was removed from the *Valuka Yantra*.

Post pharmaceutical operation

Covering of the bottle was scraped out, and the bottle was cleaned from outside. Thread dipped in mineral oil was tied around the bottle below the level of deposition of *Rasa Sindura* collected and was ignited. After the thread was burnt, a wet cloth was wrapped around the bottle and pressed to break open the bottle. Later, *Rasa Sindura* was carefully collected such that it was devoid of glass pieces.

Analytical study

X-ray diffraction study

Samples RS, 2RS, and 6RS were subjected to XRD study using BRUKER-binary V2 Advance X-ray diffractometer and was equipped with Cu K α (λ-1.5 406) radiation operated at 40KV/30mA. The sample was well grounded to 200 mesh and air dried. The X-ray diffractometer scans were made on randomly oriented samples form 5–100 2θ with a step size of 0.0190 and 64.7053 s time per step. The 2-theta value and intensity of the peak (counts) are represented on X and Y-axes, respectively. Higher the value of counts represents higher the crystallinity of the phase. For identification of each phase, strong peaks were chosen and compared with standard X-ray Powder Diffraction file (XPDF). XRD pattern of the three samples was compared with each other and data were analyzed using X-pert high score plus.

Fourier transform infrared spectroscopy

The analysis of the samples was done using Agilent Carry 660 with ATR range 400–4000.

Observations

Parada Shodhana

It was found difficult to triturate *Parada* and *Haridra* powder because of mobility of *Parada*. However, after adding *Kumari* juice, it became core easy as the *Parada* began to disintegrate more rapidly.

Gandhaka Shodhana

Particles were found floating over the layer of milk. In one of the trial, it was found that the *Gandhaka* was present over the cloth even after heating. This was because the cloth was thick and contained starch. Hence, the molten *Gandhaka* could not pass through the pores of the cloth.

Preparation of Kajjali

- As the ratio of *Gandhaka* added to that of *Parada* increased, the duration for preparation of *Kajjali* decreased [Table 1]
- Globules of mercury disintegrated more rapidly in the presence of a higher proportion of sulfur.

Preparation of Rasa sindura

Observations during preparation of RS, 2RS and 6RS have been tabulated in Table 2. Temperature graph is shown in Figures 1-3 respectively. As the proportion of Sulfur increased the height of the flame also increased [Figures 4-6].

Results

Pharmaceutical study

Results of preparation of RS, 2RS and 6RS have been tabulated in Table 3.

Analytical study

XRD pattern of all the three samples [Figure 7] showed peaks only due to mercuric sulfide. It has an empirical formula of HgS. The particle size of the samples was calculated using Scherer's Equation Dp = 0.94λ/β½ cos θ, where Dp is the crystal size, λ is the source wavelength, β½ is the peak FWHM, θ is the peak position and is tabulated.

Table 1: Duration of *Kajjali* preparation

Name	Hg:S	Duration (h)
Samaguna Kajjali	1:1	70
Dwiguna Kajjali	1:2	40
Shadguna Kajjali	1:6	24

Table 2: Comparative observation during preparation of *Samaguna Rasa Sindura*, *Dwiguna Rasa Sindura* and *Shadguna Rasa Sindura*

Observations	Duration taken		
	RS	2RS	6RS
Appearance of white fumes	2 h	1 h 40 min	2 h
Appearance of yellow fumes	4 h 15 min	4 h 45 min	4 h 45 min
Appearance of flame	7 h 30 min	7 h 30 min	7 h
Height of flame	3 inch	5 inch	8 inch
Duration of flame	4 h 15 min	3 h 45 min	6 h
Corking	14 h	13 h 40 min	16 h 30 min
Time taken for preparation	16 h	16 h	19 h

RS: *Samaguna Rasa Sindura*, 2RS: *Dwiguna Rasa Sindura*, 6RS: *Shadguna Rasa Sindura*

There was no change in the crystal structure of the three samples as the peak position was the same in XRD pattern of all the three samples. The intensity of peak increased with increase in the proportion of sulfur which implies higher crystallinity.

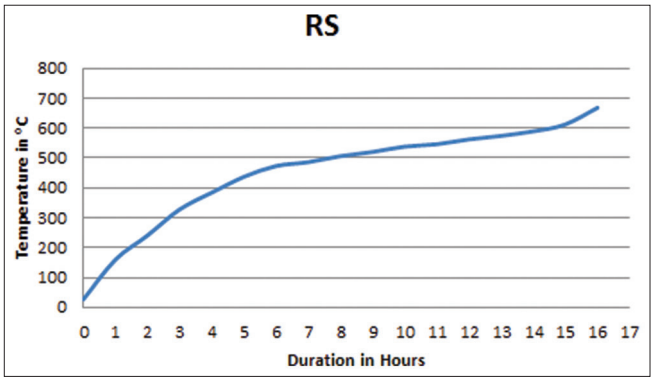

Figure 1: Temperature graph of *Samaguna Rasa Sindura*

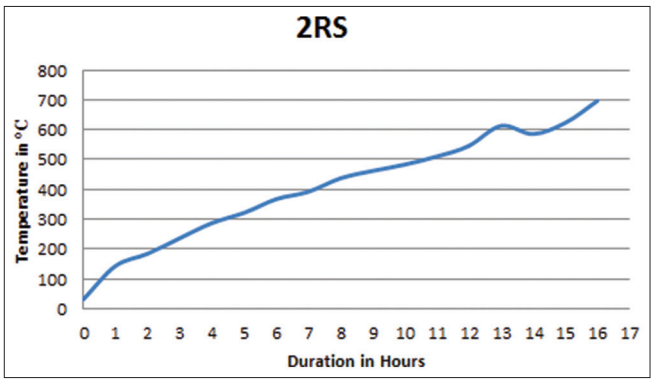

Figure 2: Temperature graph of *Dwiguna Rasa Sindura*

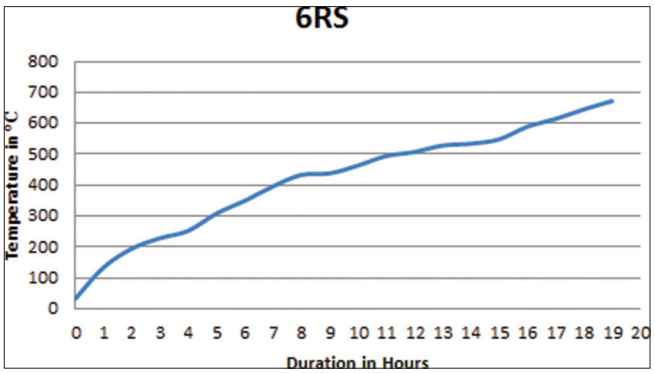

Figure 3: Temperature graph of *Shadguna Rasa Sindura*

Table 3: Results of *Samaguna Rasa Sindura*, *Dwiguna Rasa Sindura* and *Shadguna Rasa Sindura* prepared in two batches

Samples	Batch 1		Batch 2		Average	
	Kajjali in g	Yeild in g	*Kajjali* in g	Yeild in g	Yeild in g	Percentage yeild
RS	200	110	200	106	108	54
2RS	180	60	180	64	62	34.44
6RS	150	16	150	18	17	11.33

RS: *Samaguna Rasa Sindura*, 2RS: *Dwiguna Rasa Sindura*, 6RS: *Shadguna Rasa Sindura*

FTIR spectrum of sample RS, 2RS and 6RS [Figures 8-10] has 8, 5 and 3 peaks denoting different functional groups, respectively. The number of peaks denoting the presence of unsaturated carbon are 6, 7 and 4 in sample RS, 2RS and 6RS, respectively and peaks denoting the presence of exchangeable proton are 3, 2 and 2 respectively [Table 4].

Figure 4: Flame during Preparation of *Samaguna Rasa Sindura*

Figure 6: Flame during preparation of *Shadguna Rasa Sindura*

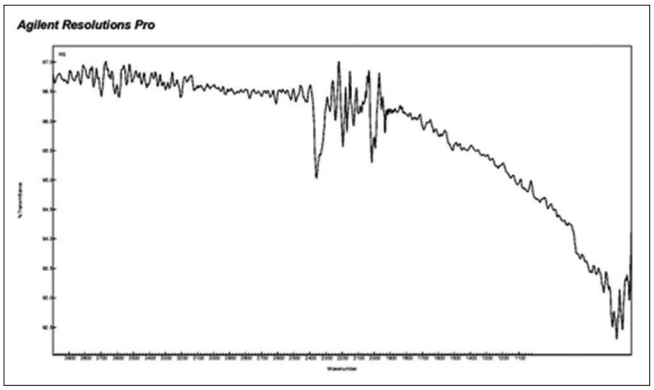

Figure 8: Fourier Transform Infra-red spectroscopy of *Samaguna Rasa Sindura*

Discussion

Mercury has a great affinity to the sulfhydryls or thiols. The mercury atom or molecule will tend to bind with any molecule present that has sulfur or a sulfur-hydrogen combination in its structure. Hence, globules of *Parada*

Figure 5: Flame during preparation of *Dwiguna Rasa Sindura*

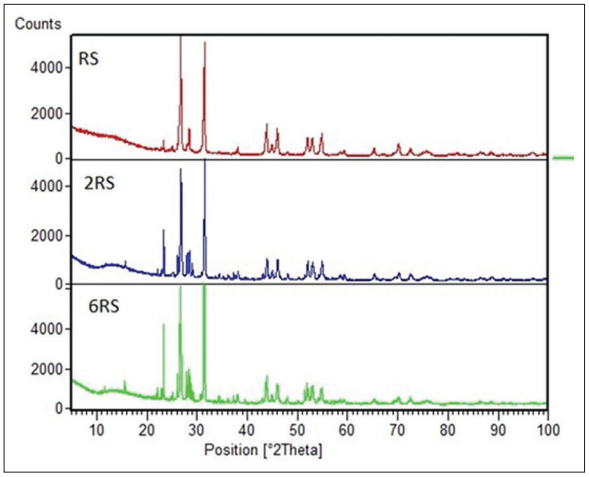

Figure 7: XRD pattern of *Samaguna*, *Dwiguna,* and *Shadguna Rasa Sindura*

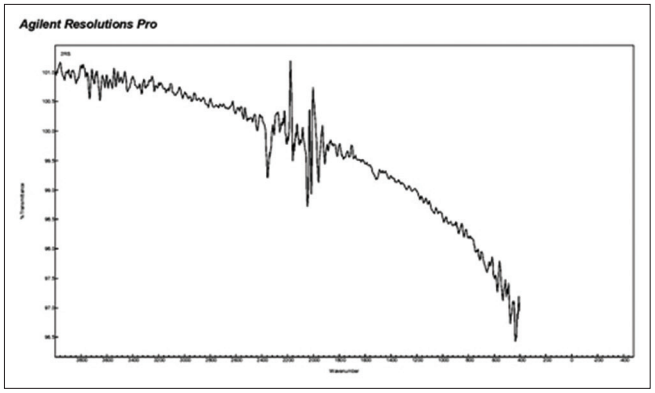

Figure 9: Fourier-Transform Infra-red spectroscopy of *Dwiguna Rasa Sindura*

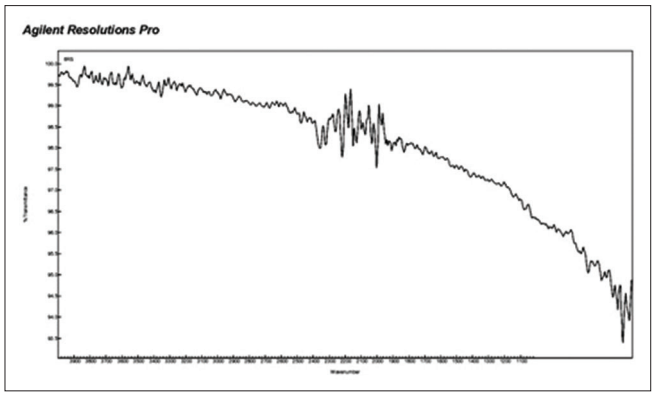

Figure 10: Fourier-Transform Infrared spectroscopy of *Shadguna Rasa Sindura*

molecules disintegrated rapidly in sulfur during the preparation of *Kajjali*.

The duration of fumes and flames increased with the increase in the proportion of sulfur added. The amount of *Kajjali* taken was varied as a precautionary measure to avoid spillage of *Kajjali* during the heating process. Hence, the exact increase in the duration of fumes and flame could not be noted. However, the increase in sulfur odor and size of the flame was observed.

The previous work done on similar concept of increased potency of *Rasa Sindura* with increased proportion of sulfur showed slight variations in quantitative analysis but nothing conclusive on therapeutic efficacy.[9] Hence, in the present study, quantitative analysis was not carried out.

XPD pattern lack in unique suggests compounds like our *Rasoaushadhi* is. Hence, we can just compare our sample with standard one, but precise identification cannot be made. No change in the crystal structure because there is no change in the position of the peaks. Since the atomic number of mercury is 80 and atomic number of sulfur is 16 and due to the addition of sulfur concentration, there is a possibility that sulfur atom can occupy the interstitial sites of mercury. Thus there is a minute change in peak positions due to strain generation. It is also noticed that the intensity of peaks increased with increase in the sulfur concentration which indicates higher crystallinity and stability. Although RS, 2RS and 6RS were identified as cinnabar; there was the difference in D space and intensity which implies definitely there is some difference in the crystallinity.

Through FTIR, it was found that as the proportion of *Gandhaka* to that of *Parada* increased in the preparation of *Rasa Sindura*, the number of functional groups were decreased. The presence of functional group is very much essential in drug absorption as it increases the rate of absorption. However, at the same time, the presence of multiple functional groups may hinder drug absorption as they hinder the action of other functional groups. Hence, it may be postulated that 6RS has better absorption compared to 2RS and RS. FTIR showed the presence of more unsaturated Carbon and exchangeable proton in RS than 2RS and was least in 6RS. This might result in more stability of 6RS compared to 2RS and RS. Although FTIR showed the presence of the functional group, considering the amount and duration of heat given during preparation, the functional group will be disintegrated, hence their significance is unknown.

Table 4: Fourier Transform Infra-red spectroscopy results

Functional Group	Wavelength range	Number of peaks		
		RS	2RS	6RS
Aldehyde C=O stretch	1740-1690	1	-	-
Carboxylic acid C=O stretch	1780-1710	1	-	-
Nitrile C≡N stretch	2260-2220	1	-	-
Carboxylic Acid O-H stretch	3000-2500	-	1	-
Amine N-H stretch	3500-3300	1	1	1
Alcohol/phenol O-H stretch	3550-3200	2	2	1
Amide N-H stretch	3700-3500	2	1	1
Total number of peaks denoting functional group	-	8	5	3
Unsaturated Carbon	>3000	6	7	4
Presence of exchangeable proton	310-3600	3	2	2

RS: *Samaguna Rasa Sindura*, 2RS: *Dwiguna Rasa Sindura*, 6RS: *Shadguna Rasa Sindura*

Conclusion

Advanced analytical techniques can be useful for the characterization of *Rasa* preparations, but they fail to give information regarding its pharmacodynamics. The concept of increased potency of *Kupipakva Rasayana* with the increase in the proportion of *Gandhaka* added could not be proved analytically in spite of using the modern, sophisticated instruments though it has been proved clinically.

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Self.

Conflicts of interest

There are no conflicts of interest.

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