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ORIGINAL RESEARCH ARTICLE- EXPERIMENTAL STUDY

PHARMACEUTICO ANALYTICAL STANDARDIZATION OF *RASA BHASMA* PREPARED AS PER *RASA MANJARI* BY USING *BHUDHARA YANTRA*

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Abstract

Metals and minerals are being used in *Ayurveda* pharmaceutics and therapeutics since a very long period. Herbomineral formulations or *Rasoushadhi* are considered as more potent and quick acting. *Rasa* (mercury) is given prime importance in *Rasashastra* owing to its remarkable therapeutic potential. However, Rasa is to be utilized in any of the three forms i.e; *Mrita Parada*, *Baddha Parada* and *Moorchitha Parada*. *Mruta Parada* refers to *Parada Bhasma* which is claimed to have immense therapeutic potential and also helps to improve longevity. *Rasa Bhasma* or *Parada*(mercury) *Bhasma* is a formulation mentioned in various text books of *Rasashastra* viz., *Rrasa Ratna Smuchchaya, Rasa Raja Sundara, Yoga Ratnakara, Bhavaprakasha, Sharangadhara Samhita, Rasa Prakasha Sudhakara, Vaidya Yoga Tarangini, Brihat Nighantu, Rasa Manjari, Rasa Tarangini* etc. But nowadays practically *Rasa Bhasma* is not being prepared due to the difficulty in the process and heat sensitivity of *Parada* (mercury). Here, an attempt was made to prepare *Rasa Bhasma* by following the reference of *Rasa Manjari* with due importance to Standard Operative Procedure. Three batches were prepared to standardize the process. Prepared *Bhasma* was tested following organoleptic, classical *bhasma pareeksha* and physico-chemical parameters. Advanced instrumental methods like SEM-EDAX, XRD, DLS and ICP-OES etc were followed to develop analytical standards.

Key words: *Ayurveda, Parada, Rasa Bhasma,* standardization

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1. **INTRODUCTION**:

Parada (mercury) is considered to be the most potent drug in ancient Alchemic sciences where it was used for *dhatuvada*. As age advanced the *parada* which was used for *dhatu vada* was tried and successfully used in *Dehavada* also. In this era three types of utility of parada was brought into existence i.e; *mrita parada, baddha parada and moorchitha parada.Parada* when is processed and made *moorchitha* cures diseases, when it is made *baddha* i.e. solidified gives salvation to the person and when made into *Bhasma* form gives the person *amaratva* i.e; longevity to the person. *Mrita Parada* was also considered that the *Parada Bhasma* also brought life to a dying person. The present formulation of *Parada Bhasma* has been tried to be prepared by various scholars but most of the preparations were not upto the specific standards. *Rasa Bhasma* or *Parada Bhasma* is a formulation mentioned in various text books of *Rasashastra* viz.,*Rasa Ratna Samuchchaya, Rasa Raja Sundara, Yoga Ratnakara, Bhavaprakasha, Sharangadhara Samhita, Rasa Prakasha Sudhakara, Vaidya Yoga Tarangini, Brihat Nighantu, Rasa Manjari, Rasa Tarangini* etc. A variety of ingredients have been used by the various ancient scholars in the preparation of *Parada Bhasma.* It is quite possible that various unpublished and unnoticed manuscripts on *Rasashastra* will have some more methods of preparation of Parada Bhasma. In various traditions also, it is possible that various methods of Parada Bhasma preparations will prevail which traditional *Rasa Sadhakas* do not disclose. However, a scientific validation is lacking.

Specification of Color of *Rasa Bhasma*:

1. White coloured *Parada Bhasma*
2. Red coloured *Parada Bhasma*
3. Yellow coloured *Parada Bhasma*
4. Black coloured *Parada Bhasma*
5. *Parada Bhasma* of unspecified colour

In the present study reference from *Rasa Manjari*[1] is been selected where black coloured Bhasma is expected.

1.1 Aims and objectives:

- To prepare the *Rasa Bhasma* according to the reference of *Rasamanjari* in three batches.
- To develop SOP of *Rasa Bhasma* by preparing in three batches with due importance to process and equipment validation
- Analysis of all the batches of *Rasa Bhasma* with relevant pharmaceutico-analytical parameters to develop in house standards.

2. MATERIALS AND METHODS:

Study is divided into two parts:

2.1. Pharmaceutical Study

2.2. Analytical Study

2.1. Pharmaceutical Study:

A. *Shodhana* (purification) **of raw materials:**

i. *Parada*

ii. *Gandhaka*

B. Preparation of *Kajjali*

C. *Bhavana* with *Ghritakumari* juice

D. Preparation of *Samputayantra*(capsule of earthen saucers) and *Bhudhara Yantra*

A. *Shodhana* of raw materials:

i. ***Shodhana*** **(purification) of *Parada***: Purification of *Parada* was carried out according to the reference of *Rasa Tarangini*[2].

Procedure- 99.99% pure mercury was procured from Neelkanth Sales Corporation, New Delhi. Purity of the sample was certified with ICP-OES report. 5 kg of *Parada* was taken with 5 kg of *Churna* (lime powder) in granite *Khalva*(mortar) and trituration was done for 72hrs. *Sudha choorna*(lime powder) was added little by little. The mixture was washed with hot water and filtered through double layered cloth till only *parada* was remained. Thus, the clear 4 Kg and 860 grams of *Parada* was obtained. Obtained *Parada* was taken into a *Khalva yantra* and equal quantity of garlic paste (4.86 kg.) and half the amount of *Saindhava lavana* (2.43 kg.) is added. Trituration was again applied until whole mixture became black colour (paste of garlic). The washing and decanting was applied with the help of hot water 10 times to get pure blemishless *(Shuddha) Parada.* Observations and results are shown in **table 1.**

Table 1 Showing Result of Parada Shodhana:

Drug	**Quantity in kilogram**
Ashuddha Parada	5
Sudha Churna	5
Dehusked garlic	4.8
Saindhava Lavana	2.4

ii. ***Shodhana*** **(Purification) of *Gandhaka*(Sulfur)[3]:**

99.9% pure sulphur powder was procured from authorized suppliers from Ahmedabad. 600 grams of *Gandhaka* (Sulphur) was taken and powdered in a *Khalava Yantra*. In an iron pan equal amount (600 grams) of cow ghee was taken and melted to which powdered *Gandhaka* was added. After complete melting of *Gandhaka*, it is filtered through a clean cloth into stainless steel vessel containing 1.2 litres of cow milk. Milk was then discarded and *Gandhaka* was washed thoroughly in hot water and dried properly. The same procedure was repeated for two more times and dried under shade. After drying *Shuddha Gandhaka*

obtained was powdered. Each time fresh cow milk and cow ghee were taken. Observations and results are shown in Table 2a and 2b. Cow ghee and cow milk were collected locally from the known source and tested for purity before using.

Table 2 a. Showing the observations during Gandhaka Shodhana

Para Meters	Dhalana	Before Dhalana			During Dhalana	After Dhalana	
		Gandhaka	Go Dugdha	Gohritha	Gandhaka	Gandhaka	Go Dugdha
Temperature	1st	27^0C	27^0C	27^0C	116^0C	-	80^0C
	2nd	27^0C	27^0C	27^0C	116^0C	-	94^0C
	3rd	27^0C	27^0C	27^0C	116^0C	-	88^0C
Colour	1st	Crystalline Dull yellow	White	Yellow	Reddish Brown	Bright Yellow	Yellowish white
	2nd	Bright Yellow	White	Yellow	Dark Yellow	Light Yellow	Yellowish white
	3rd	Light Yellow	White	Yellow	Golden Yellow	Yellow	Yellowish white
Odour	1st	Irritant Sulphur smell	Pleasant	Pleasant	Irritant	Slightly less irritant	Of Sulfur with mild aroma of cow ghee
	2nd	Slightly irritant +of Ghrita	Pleasant	Pleasant	Less irritant	Slightly sulphur +Of Ghrita	Of Sulfur with mild aroma of cow ghee
	3rd	Slightly sulphur + Of Ghrita	Pleasant	Pleasant	Non irritant	Of Ghrita +Non irritant	Of Sulfur with mild aroma of cow ghee
Texture	1st	Solid, hard	Liquid	Liquid	Liquid	Solid, Smooth	Curdled

	2nd	Solid, Hard	Liquid	Liquid	Liquid	Solid, Smooth	Liquid
	3rd	Solid	Liquid	Liquid	Liquid	Solid, Smooth	Liquid

Table 2 b. Table showing result of Gandhaka Shodhana

Quantity of Gandhaka (grams)		Quantity of Ghee (grams)	Quantity of Milk (liters)	Quantity of Shuddha Gandhaka (grams)	Loss in grams
1st process	600	600	1.2	592	8
2nd process	592	592	1.2	590	2
3rd process	590	590	1.2	585	5

B. Preparation of *Kajjali*:

The *Kajjali* is prepared by grinding one part (500 grams) of *Shuddha Parada* with half part (250 grams) of *Shuddha Gandhaka*. *Shuddha Parada* was taken in a *Khalava Yantra*, added with *Shuddha Gandhaka Churna* frequently in small quantities and triturated till a lustreless, jet black coloured powder is obtained. Obtained powder was *Rekhapurna* (fills finger creases) and *Varitara* (floats on water) confirming microfinenature of the product. Total 715 grams of *Kajjali* was obtained. [Table 3]

Table 3. Result of Kajjali preparation:

Drug	**Quantity in Grams**
Shuddha Parada	500
Shuddha Gandhaka	250
Kajjali Obtained	715
Loss	35

C. *Bhavana* with *Ghrita Kumari Swarasa* (Levegation with Aloe vera leaf juice):

Prepared *Kajjali* (150 grams per batch, in total three batches viz, **RRMBh 1, RRMBh 2 and RRMBh 3)** was added with required amount of freshly extracted juice of Aloe vera leaf and levegated till a soft mass is obtained with all the *Subhavita Lakshana*(features of perfect levegation). This semisolid mass is made into *chakrika* (discs) using specially designed

mould; maintaining the thickness of 0.5 cm and a diameter of 3.5 cm. Discs are completely dried under sun. *Aloe vera* leaf was collected from the garden of the institute.

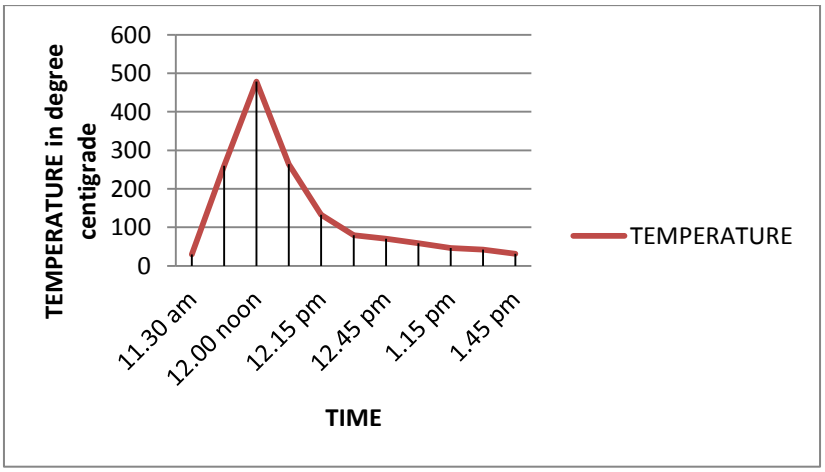
D. Preparation of *Samputayantra* and *Bhudhara Yantra*.

Prepared Chakrika are placed in a single layer in a clean dry earthen saucer (*Sharava*), covered with another similar earthen saucer placed inverted, the joint is sealed with mud plasters and dried to get *Sharava Samputa*. This was subjected to heat in a *Bhudhara Yantra*[4]. For Bhudhara Yantra pit with (3.75x3.75x3.75 cm^3) sand around and total dimension of pit as 20x20x20 cm^3 was used. Eight cowdungs of standard dimensions (9x6 cm with an average volume of 380 cm^3 yielding an average heat energy of 7000J/gm^0C) based on previous study in the institution (Gauthaman et.al.) were placed at the top and lit with fire. *Sharava samputa* was collected after self-cooling and the contents inside was carefully, collected and finely powdered. Such three batches were prepared to develop Standard Operative Procedure. Ingredients are mentioned in Table 4. Temperature pattern observed in three batches are shown in Graphs 1, 2 and 3. Yield of *Rasa Bhasma* obtained is shown in Table 5.

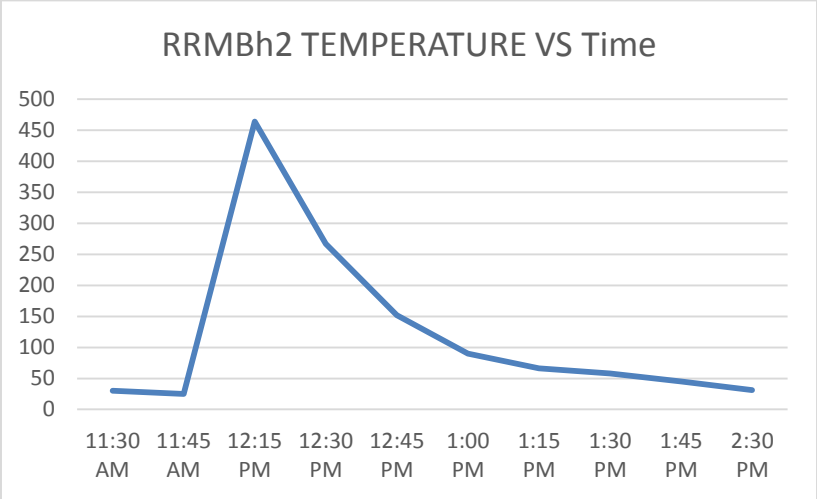
Table 4. Showing the Ingredients of Rasa Bhasma:

Sl.No	Sanskrit Name	Part used	Latin/English name	Quantity	Textual reference	Batch No.
1.	Kajjali	Compound	Black sulphide of mercury	160 grams	Rasa Tarangini	KRKJL01
2.	Kumari	Fresh leaf juice	Aloe vera	85 ml	API, Bhavaprakasha	RMT/FKMR01

Graph 1.Temperature pattern observed in Batch RRMBh 1



Graph 2.Temperature pattern observed in Batch RRMBh 2



Graph 3.Temperature pattern observed in Batch RRMBh 3

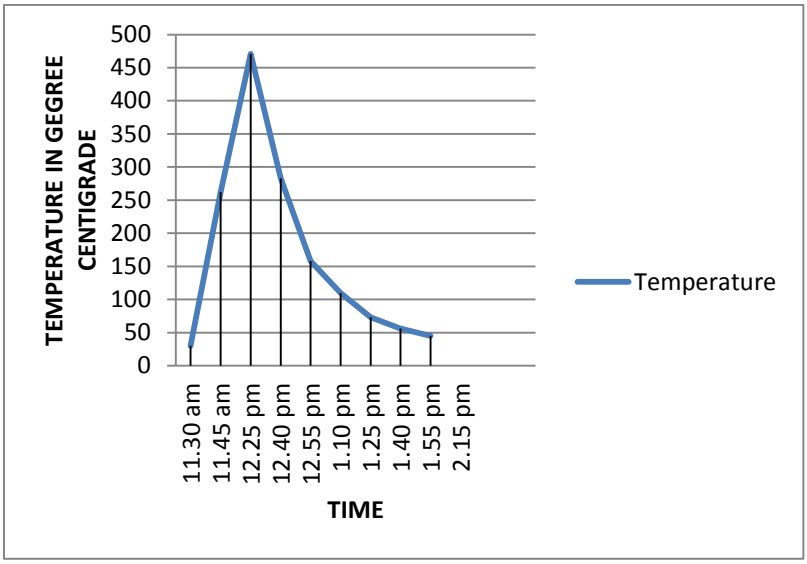


Table 5. Table showing the yield of Rasa Bhasma :

Batch	**Quantity of Kajjali in grams**	**Quantity of Rasa Bhasma in grams**
Batch 1	160	135
Batch 2	160	99
Batch 3	160	105
Total	**480**	**339 grams**

2.2. Analytical study:

All the three batches of prepared *Rasa Bhasma* and *Kajjal*i were subjected to analysis using basic organoleptic parameters and classical *Bhasma Pareeksha* followed by instrumental methods of analysis. All the results of *Rasa Bhasma* were compared with

those of *Kajjali*. Parameters used are as follows:

2.21. Organoleptic parameters like colour, touch, odour and taste

2.22. *Bhasma Pareeksha* like *Nishchandra, Rekha poornata, Varitara* etc.

2.23. Particle size estimation by Scanning Electron Microscope and Laser Diffraction method

2.24. Semiquantitative analysis of elements by EDAX

2.25. Quantitative estimation of elements by ICP-AES method

2.26. Phase analysis by X-Ray Diffraction method

2.21. Organoleptic parameters: Bhasma samples were dark black in colour without any shining particles. They were in powder form, smooth to touch; tasteless and odourless (Table no. 6).

Table 6. Organoleptic parameters of the samples:

S.No.	Sample	Colour	Lustre	Appearance	Touch	Odour	Taste	Feel of weight
1	Kajjali	Dark Black	Absent	Fine powder	Smooth	Absent	Tasteless	Heavy
2	RRMBh1	Dark Black	Absent	Fine powder	Smooth	Mild burnt odour	Tasteless	Comparatively lighter
3	RRMBh2	Dark Black	Absent	Fine powder	Smooth	Mild burnt odour	Tasteless	Comparatively lighter
4	RRMBh3	Dark Black	Absent	Fine powder	Smooth	Mild burnt odour	Tasteless	Comparatively lighter

2.22. *Bhasma Pareeksha*: All the samples were observed against sunlight and found lustreless. *Bhasma* samples were *Rekha Poorna* (filled finger creases), *Varitara* (floated on still water) and also passed *Unama* test (grain placed on floating layer of *Bhasma* also remained floating). When sprinkled on fire found *Nirdhuma* (smokeless) an important test for *Rasa Bhasma*[5]. (Table No.7).

Table 7. Results of Bhasma Pareeksha of the samples:

S.No.	Sample	Rekhapurnata	Varitara	Unama	Nirdhuma

1	Kajjali	+ ve	+ ve	+ ve	-ve
2	RRMBh1	+ ve	+ ve	+ ve	+ ve
3	RRMBh2	+ ve	+ ve	+ ve	+ ve
4	RRMBh3	+ ve	+ ve	+ ve	+ ve

2.23. Particle size estimation:

a. Scanning Electron Microscopy: SEM photomicrograph of *Kajjali* and *Rasa Bhasma* samples show the appearance of particles of 10 µ and less than 5 µ sized particles in all the samples. SEM images of the drug samples show cubic shape like structure with the particle size lying in the micro range. Particles with Rhombohedral features are also observed. From the image it is clear that several crystallites are agglomerated in a particle giving rise to microcrystalline structure as shown in Fig. 1a-4b.

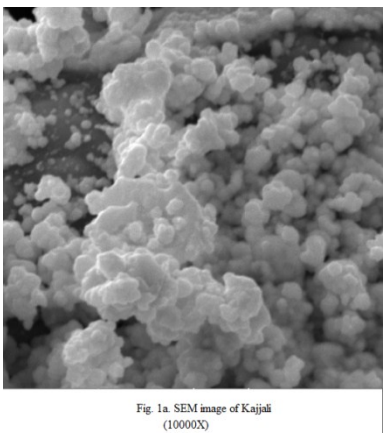


Fig.1a.SEM image of Kajjali (10000X)

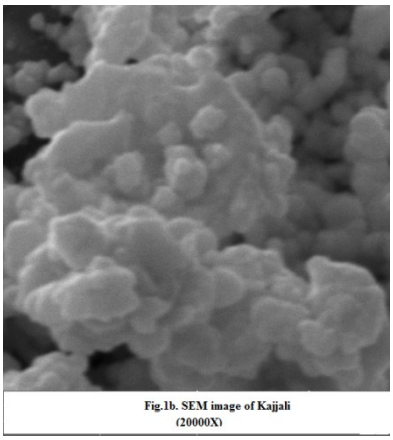


Fig.1b.SEM image of Kajjali (20000X)

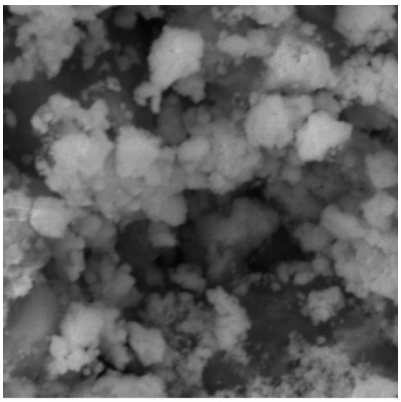


Fig2a. SEM image of Rasa Bhasma sample RRMBh1(10000X)

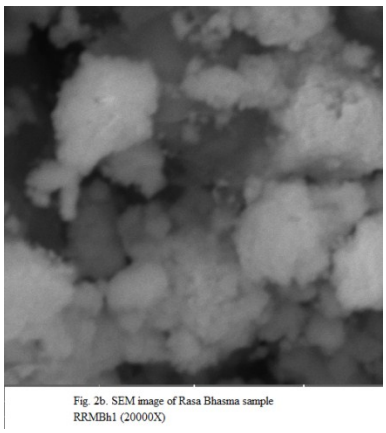


Fig2b. SEM image of Rasa Bhasma sample RRMBh1 (20000X)

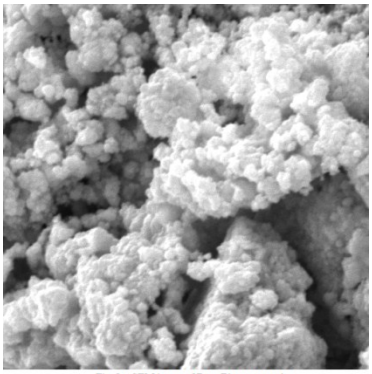


Fig. 3a. SEM image of Rasa Bhasma sample RRMBh2 (10000X)

Fig3a. SEM image of Rasa Bhasma sample RRMBh2 (10000X)

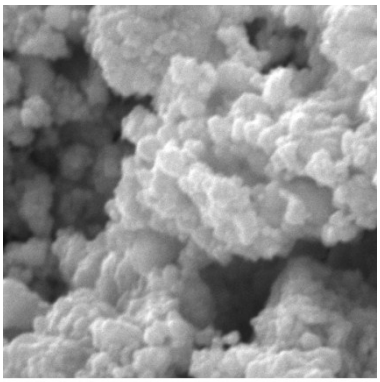


Fig. 3b. SEM image of Rasa Bhasma sample RRMBh2 (20000X)

Fig3b. SEM image of Rasa Bhasma sample RRMBh2 (20000X)

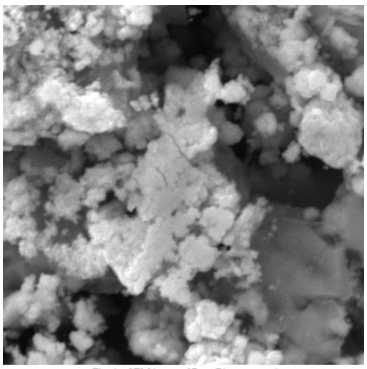


Fig. 4a. SEM image of Rasa Bhasma sample RRMBh3 (10000X)

Fig4a. SEM image of Rasa Bhasma sample RRMBh3 (10000X)

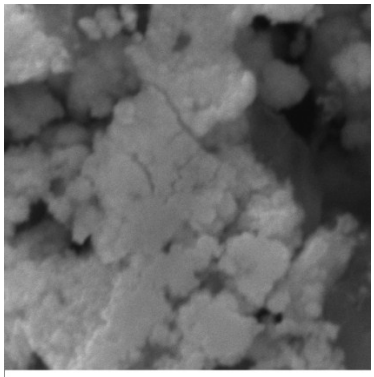


Fig. 4b. SEM image of Rasa Bhasma sample RRMBh3 (20000X)

Fig4b. SEM image of Rasa Bhasma sample RRMBh3 (20000X)

Laser diffraction method: Particle size analysis was done following laser diffraction method. Laser diffraction measures particle size distributions by measuring the angular variation in intensity of light scattered as a laser beam passes through a dispersed particulate sample. All the samples had particles less than 200µ in size. Result is displayed in Table no.8

Table 8. Average particle size of Kajjali and Rasa Bhasma samples (Malvern Laser Diffraction Method)

S.No.	Sample	Average particle size
1	Kajjali	29.34 µ
2	RRMBh1	197.01 µ
3	RRMBh2	138.68 µ
4	RRMBh3	138.68 µ

2.24. EDAX analysis:

This is to evaluate complete material balance. The samples in the form of powder were prepared as pellet in a boric acid matrix and subjected to XRF analysis. Each characteristic peak of the element compared with the standard energy levels and the elements were identified. Elemental content present

in the drug sample is reported in Table no. 9

Table9. EDAX report of the samples:

S.No.	Elements in %	Kajjali	RRMBh1	RRMBh2	RRMBh3
1	Mercury	70.89	49.12	77.97	63.66
2	Sulphur	24.27	44.59	18.41	32.67
3	Carbon	4.84	5.79	3.62	3.68
4	Oxygen	-	0.50	-	-

2.25. ICP-OES:

ICP-OES (Inductively coupled plasma - optical emission spectrometry) is a technique in which the composition of elements in samples can be determined using plasma and a spectrometer. Calcium, Iron and Zinc were detected in the samples, Lead and Arsenic were below detection limits. Results are displayed in Table No.10

Table 10.ICP-OES report of the samples:

S.No.	Elements in ppm	Kajjali	RRMBh1	RRMBh2	RRMBh3
1	Calcium	133.38	189.76	195.32	183.51
2	Iron	43.47	37.06	54.14	41.39
3	Zinc	21.04	3.31	1.36	1.16
4	Lead	BDL	BDL	BDL	BDL
5	Arsenic	BDL	BDL	BDL	BDL

2.26. X Ray Diffraction Study:

In XRD analysis, for sample of *Kajjali* maximum intensity was at 2θ angle of 26.499 and for *Rasa Bhasma* samples the maximum intensity was at 2θ angle were at 26.309, 26.394, 26.382 for the samples RRMBh1, RRMBh 2and RRMBh 3 respectively. Kajjali sample showed 8 peaks, sample RRMBh1 showed 10 peaks and other samples showed identical six peaks. (Table no. 11, 12, 13, 14 and Figures 5, 6a, 6b and 6c).

Table11. Representing XRD peak list of Kajjali

No.	2-Theta(deg)	D(ang.)	Height (counts)	FWHM(deg.)	Int.I(counts deg)	Int.W(deg)	Asym.factor

1	23.183(8)	3.8335(5)	193(14)	0.129(10)	39.0(16)	0.20(2)	0.81(14)
2	**26.499(5)**	**3.3609(6)**	**1197(35)**	**0.310(8)**	**639(4)**	**0.534(19)**	**1.38(12)**
3	27.85(2)	3.200(3)	64(8)	0.11(2)	7.6(13)	0.12(4)	1.9(17)
4	30.10(16)	2.967(16)	27(5)	0.5(3)	14(16)	0.5(7)	1.1(4)
5	30.64(2)	2.9154(19)	174(13)	0.52(6)	102(15)	0.59(13)	1.1(4)
6	31.37(3)	2.850(3)	45(7)	0.48(8)	24(4)	0.54(18)	1.1(4)
7	43.818(13)	2.0644(6)	348(19)	0.429(15)	230(3)	0.66(4)	0.68(10)
8	47.85(11)	1.900(4)	15(4)	0.35(8)	5.8(13)	0.39(19)	1.2(16)

Table12. Representing XRD peak list of *Rasa Bhasma* sample RRMBh1

No.	**2-Theta(deg)**	**D(ang.)**	**Height (counts)**	**FWHM(deg.)**	**Int.I(counts deg)**	**Int.W(deg)**	**Asym.factor**
1	15.38(6)	5.76(2)	12(3)	0.25(5)	3.3(7)	0.28(14)	1.9(18)
2	23.032(5)	3.8584(8)	123(11)	0.138(19)	27.5(10)	0.22(3)	3.2(15)
3	**26.309(9)**	**3.3847(12)**	**882(30)**	**0.255(9)**	**341(4)**	**0.387(17)**	**1.01(17)**
4	27.63(6)	3.226(7)	35(6)	0.17(5)	7.2(15)	0.20(8)	0.9(14)
5	30.434(8)	2.9348(8)	181(13)	0.269(13)	75(2)	0.42(4)	0.60(9)
6	31.098(9)	2.8736(8)	98(10)	0.29(2)	43(2)	0.44(7)	0.60(9)
7	42.62(3)	2.1198(14)	13(4)	0.19(10)	3.6(8)	0.28(14)	0.8(17)
8	43.694(8)	2.0699(4)	322(18)	0.281(13)	131.7(18)	0.41(3)	2.0(4)
9	45.717(15)	1.9830(6)	27(5)	0.15(4)	5.0(9)	0.18(7)	5(7)
10	47.70(2)	1.9049(9)	16(4)	0.27(11)	6.2(10)	0.40(17)	1.0(19)

Table13. Representing XRD peak list of *Rasa Bhasma* sample RRMBh2

No.	**2-Theta(deg)**	**D(ang.)**	**Height (counts)**	**FWHM(deg.)**	**Int.I(counts deg)**	**Int.W(deg)**	**Asym.factor**
1	23.11(3)	3.846(5)	27(5)	0.14(7)	6.6(7)	0.24(7)	2(3)
2	23.83(3)	3.731(5)	17(4)	0.15(8)	4.5(7)	0.27(11)	2(3)
3	**26.394(5)**	**3.3740(6)**	**1683(41)**	**0.211(4)**	**542(3)**	**0.322(10)**	**1.21(13)**
4	30.570(11)	2.9220(10)	400(20)	0.241(15)	161(2)	0.40(3)	1.5(4)
5	31.263(13)	2.8588(11)	36(6)	0.19(5)	10.1(15)	0.28(9)	3(4)

6	43.751(7)	2.0674(3)	711(27)	0.222(11)	254(2)	0.357(17)	1.05(16)

Table14. Representing XRD peak list of *Rasa Bhasma* sample RRMBh2

No.	2-Theta(deg)	D(ang.)	Height (counts)	FWHM(deg.)	Int.I(counts deg)	Int.W(deg)	Asym.factor
1	23.11(3)	3.846(4)	69(8)	0.14(3)	15.4(9)	0.22(4)	2(2)
2	**26.382(5)**	**3.3756(7)**	**1336(37)**	**0.208(6)**	**442(3)**	**0.331(11)**	**1.17(14)**
3	30.524(13)	2.9263(12)	289(17)	0.266(15)	120(3)	0.42(4)	0.9(3)
4	31.186(18)	2.8857(16)	61(8)	0.29(4)	28(3)	0.46(10)	0.9(3)
5	34.22(6)	2.618(5)	13(4)	0.14(12)	2.7(8)	0.22(12)	1(3)
6	43.736(9)	2.0681(4)	542(23)	0.232(13)	202(2)	0.37(2)	0.97(18)

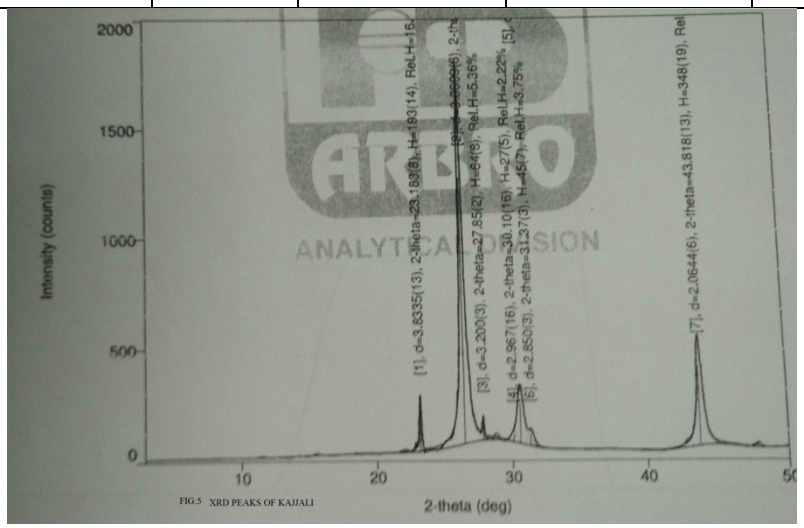


Fig.5 XRD peaks of Kajjali

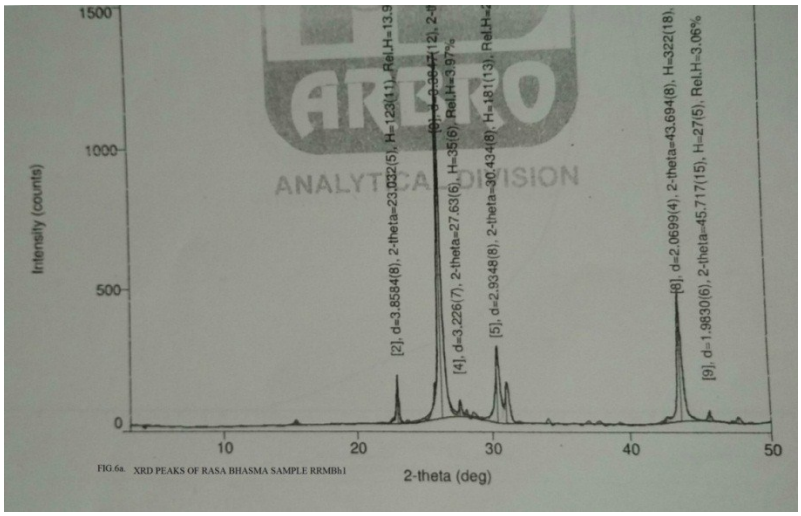


Fig. 6a. XRD peaks of Rasa Bhasma sample RRMBh1

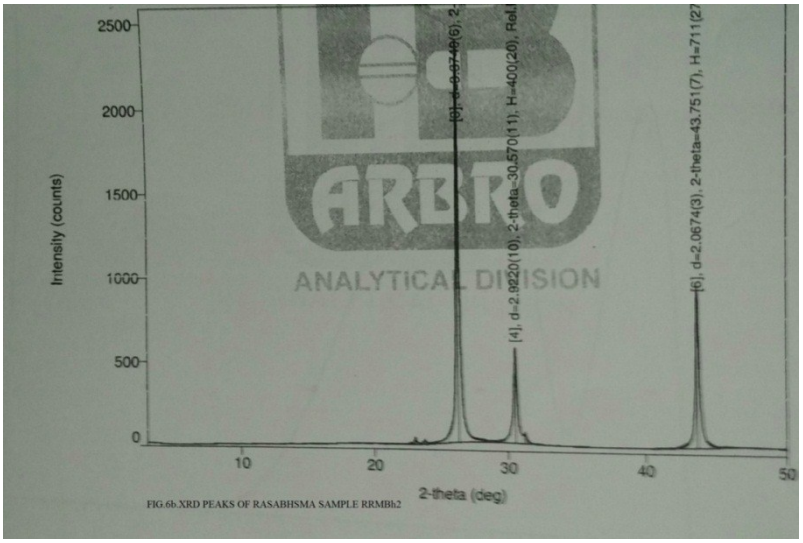


Fig.6b. XRD peaks of Rasa Bhasma sample RRMBh2

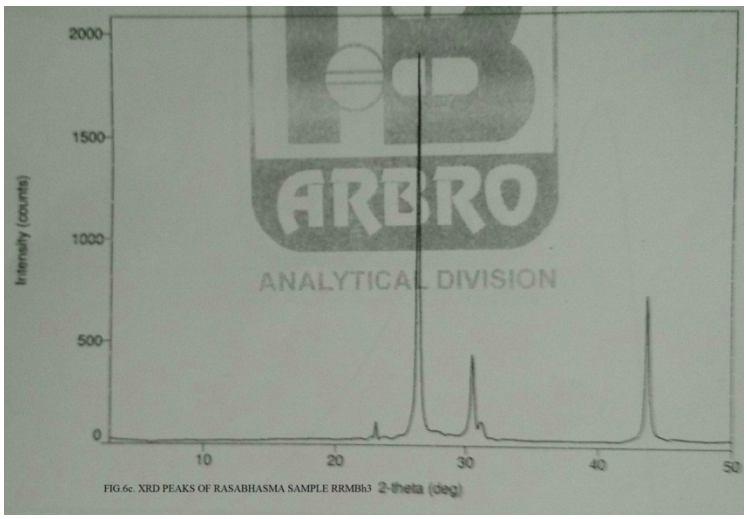


Fig.6c. XRD peaks of Rasa Bhasma sample RRMBh3

3. DISCUSSION

Parada Shodhana was carried out using *Sudha churna* and *Lashuna*. Garlic plays an important role in the detoxification of mercury. When garlic bulb is crushed, Alliin is converted into Ajoene which reacts with mercury to form mercuric sulphur oxide. Garlic and Hg reaction is a redox process where there is a reactant undergoing oxidation and one undergoing reduction[6].

Three batches of *Rasa Bhasma* were prepared as per the reference of *Rasa Manjari* using *Bhudhara Yantra*. For the *Puta* to reach *Swanga Sheeta* it took 2 hour 15 minutes to 3 hours, maximum temperature reached was between 460^0C to 480^0 among the samples.

Bhasma samples were dark black due to the presence of black sulphide of mercury, smooth to touch because of the micro fineness of the particles. All the samples were lustreless

indicating the absence of free mercury. Samples were *Rekapoorna* i.e. filled the space between the ridges of finger tips. According to a study the mean ridge to ridge distance for the male subjects was 0.46mm and for female subjects was 0.41mm.Only the particles finer than this can enter the finger creases[7]. *Bhasma* and *Kajjali* samples floated on water and also the grain placed on the floating samples remained floating. For particles with density lower than that of water, floating is observed (buoyant force greater than the gravity) while denser particles sink. The position of the particle in a liquid is not influenced by the sequence of events i.e. independent of whether powder is added to liquid or liquid is added to the powder. However the floatability test for *bhasma* is to observe the floatability of a powder sprinkled on the surface of water and this is expected to involve interfacial forces that act at the three interfaces (gas-liquid, liquid-solid and gas-solid). When a powder of higher true density like *Rasa bhasma* is sprinkled on surface of water, its ability to float on the surface depends on the surface energy of the powder. When the adhesive force between the powder and a liquid is lower than the cohesive forces between the molecules of liquid, the powder surface is not wetted by the liquid. Hence, the particles with lower surface energies are associated with increased contact angle with water, implying hydrophobicity and non-wetting character. For such nonwetting solids, there exists a critical contact angle for the surface, above which the material floats[8]. This happens when the weight of the solid is overcome by the surface tension forces[9]. As the weight of the particle decreases with particle size, the critical contact angle also decreases with particle size[10]. Also, the reduction in surface free energy with decrease in particle size has been demonstrated[11]. Hence, for a properly prepared *bhasma* the contact angle with water would be greater than the critical contact angle owing to extremely smaller size of *bhasma* particles, making them float on water[12]

Scanning Electron microscopy is found to be useful in getting the topographic image of the samples and also revealed the presence of micro particles. As the resolution beyond 20000X could not be achieved nano particles were not characterized.

Laser particle analyser showed the presence of particles ranging from 138.68µ to 197.01µ in *Rasa Bhasma* samples but particles in *Kajjali* were finer (average 29.34µ).

EDAX in association with SEM is a non-destructive technique for the semiquantitative estimation of elements in the samples. Mercury was present in maximum quantity in *Rasa Bhasma* samples (with an average of 63.58%). *Kajjali* had a higher percentage of

mercury (70.89%). Sulphur was next major element in *Rasa Bhasma* samples (with an average of 31.89%). Presence of carbon and oxygen indicate the presence of organic materials in the samples.

ICP-OES is highly sensitive and capable of determination of a range of metals and several non- metals at concentrations below 1 part in 10^{12}. All the samples had calcium, iron and zinc in small quantities. However percentage of zinc was lower in *Rasa Bhasma* samples when compared with *Kajjali*

XRD pattern shows metacinnabar HgS along with free sulphur in all the *Rasa Bhasma* samples including *Kajjali*. HgS is present in cubic form, 2θ position at 26.499, 26.309, 26.394, and 26.382 with d-spacing of 3.3609, 3.3847, 3.3740, 3.3756 Aº respectively in samples *Kajjali, Rasa bhasma* RRMBh1, 2 and 3.Free sulphur is present in ortho-rhombic form. Absence of free mercury is confirmed in XRD analysis.

4. CONCLUSION

Bhudhara Yantra method mentioned in *Rasa Manjari* is found to be practically a successful method to prepare black coloured *Rasa Bhasma*. However, whether it fulfils all the characters of *Rasa Bhasma* is debatable. Sophisticated analytical techniques like SEM-EDAX, ICP-OES, XRD etc are found to be very useful for the standardization of *Rasa Bhasma*. Previous study carried out by Y. Prasad et.al[13] has established that *Rasa Bhasma* prepared by this method is non- toxic. Hence, this *Rasa Bhasma* sample can be further utilized in clinical practice to establish its therapeutic potential.

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