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Standard Operative Procedure of *Rasa Bhasma* Following Poling Method and its Analytical Standardization

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Abstract:
Use of metallic preparations in healthcare is a unique feature Ayurveda. *Rasashastra* deals with the processing of metals including Mercury, Gold, Silver, Lead, Zinc, Copper etc. in such a way that they can be safely administered and found to be therapeutically effective. *Parada* i.e. mercury is most important among them and it is in different disease conditions with great authority. *Bhasma* is one of the forms in which Parada is administered internally which is claimed to be therapeutically highly effective. But being a metal of high sensitivity to heat, preparation of *ParadaBhasma* is found to be extremely difficult and only a few such attempts are proved to be successful. Here, an attempt was made to prepare *Rasa Bhasma* by following the reference of *BharataBhaishajyaRatnakara* with due importance to Standard Operative Procedure. Three batches were prepared to standardize the process. Prepared *Bhasma* was tested following organoleptic, classical *bhasmapareeksha* 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, poling

1. Introduction

Parada(mercury) is being used in Ayurveda for therapeutic purpose since a long time. During the period of RasashastraParada was advised to be used in three different forms. They are*mritaparada, baddhaparada and moorchithaparada.*It is claimed that *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. *MritaParada* was also considered that the *ParadaBhasma* also brought life to a dying person. The present formulation of *ParadaBhasma* has been tried to be prepared by various scholars but most of the preparations were not upto the specific standards. *Rasa Bhasma* or *ParadaBhasma* is a formulation mentioned in various text books of *Rasashastra*viz., *RasaRatna Samuchchaya, Rasa Raja Sundara, Yoga Ratnakara, Bhavaprakasha, SharangadharaSamhita, Rasa Prakasha Sudhakara, Vaidya Yoga Tarangini, BrihatNighantu, Rasa Manjari, Rasa Tarangini* etc. Only a few attempts were successful in preparing Rasa Bhasma[1,2]. In the present study reference from *Bharata Bhaishajya Ratnakara*[3] is been selected where black colouredBhasma is expected.

1.1 Aims and objectives:

- To prepare the *Rasa Bhasma* according to the reference of *BharataBhaishajyaRatnakara* 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**

B. Preparation of *Kajjali*

C. Poling i.e. heating the Kajjali in open iron stirring with Nyagrodhadanda (twig of *Ficusbengalensis*)

A. *Shodhana* of raw materials:

Shodhana* (purification) of *Parada: Purification of *Parada* was carried out according to the reference of *Rasa Tarangini*[4].

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. *Sudhachoorna*(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 *Khalvayantra* and equal quantity of garlic paste (4.86 kg.) and half the amount of *Saindhavalavana* (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.**

***Shodhana* (Purification) of *Gandhaka*(Sulfur)[5]:**

99.9% pure sulphur powder was procured from authorized suppliers from Ahmedabad. 600 grams of *Gandhaka* (Sulphur) was taken and powdered in a *KhalavaYantra*. 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 *ShuddhaGandhaka* 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.

B. Preparation of *Kajjali*:

The *Kajjali* is prepared by grinding one part (500 grams) of *ShuddhaParada* with half part (250 grams) of *ShuddhaGandhaka*. *ShuddhaParada* was taken in a *KhalavaYantra*, added with *ShuddhaGandhakaChurna* 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.(**Table3**)

C. Poling i.e. Heating the Kajjali in open iron stirring with Nyagrodhadanda (twig of *Ficusbengalensis*):

Prepared *Kajjali* (100 grams per batch, in total three batches viz, **RBBBh1, RBBBh 2 and RBBBh 3)** was taken in an iron pan and heated at a temperature between 200 and 250^0 C with continuous stirring using

ArdraNyagrodhadanda (fresh twig of *Ficusbengalensis*). Procedure is continued till smoke completely ceases and black powder is obtained.

Precautions :

- Throughout the procedure heat is maintained between 200^0C and 250^0C
- Continuous stirring is maintained
- Protective masks are used to prevent the inhalation of yellowish sulphur fumes
- Fire is turned off immediately when white fumes started to appear

Ingredients are mentioned in **Table 4**. Temperature pattern and observations in three batches are shown in **tables 5, 6 and 7** and **Graphs 1, 2 and 3**. Yield of *Rasa Bhasma*obtained is shown in **Table 8**.

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 *BhasmaPareeksha* 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.*BhasmaPareeksha* like *Nishchandra, Rekhapoornata, 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. 9**).

2.22.*BhasmaPareeksha*: All the samples were observed against sunlight and tested for classical BhasmaPareeksha like Rekhapoornata, Varitara, Unama and Nirdhuma.

2.23.Particle size estimation:

a. Scanning Electron Microscopy: The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. An electron microscope is a type of microscope that uses a particle beam of electrons to illuminate a specimen and create a highly-magnified image. Electron microscopes have much greater resolving power than light microscopes that use electromagnetic radiation and can obtain much higher magnifications of up to 2 million times, while the best light microscopes are limited to magnifications of 2000 times. The samples were mounted rigidly on a specimen holder called a specimen stub and subjected to analysis.

b. 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.

2.24.EDAX analysis:

Energy Dispersive X-ray Spectroscopy (abbreviated EDS, EDX, or EDAX) is an analysis tool used to determine the elemental composition of a sample. EDAX works by analyzing the spectrum of emitted X-rays from a sample as a beam of high energy electrons is incident upon its surface. By comparing the emitted X-ray photon energies to expected values from various elements one may determine which elements are present in a particular sample, and in what ratios. In the present study ZAF Method Standardless Quantitative Analysis was done. Instrument used was 6380 (LA) at a voltage 20.0kV, probe current of 1.00000 nA and PHA mode: T3. Each characteristic peak of the element compared with the standard energy levels and the elements were identified.

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. It is a type of emission spectroscopy that uses the inductively **c**oupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelength characteristic of a particular element. Here Kajjali and Bhasma samples were subjected to ICP-OES analysis to analyse selected elements like calcium, iron, zinc, arsenic and lead.

2.26.X Ray Diffraction Study:

To determine the different crystalline phases present in the samples XRD studies was done. X-ray diffraction (XRD) patterns were obtained using a Shimadzu XRD-6000 diffractometer with Cu - Kα as target with 40 KV voltages and 30 MA current. The X-ray diffraction of the sample was matched against the standard reference spectra library of software for phase identification.

3. Results and discussion

Paradashodhana yielded 4.8 kgs of ShuddhaParada with a loss of 4% (**Table 1**). *ParadaShodhana* was carried out using *Sudhachurna* 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]. Detailed observations of *GandhakaShodhana* are shown in **Table 2a**. After *Shodhana* 585 grams of *ShuddhaGandhaka* was obtained with a loss of 2.5% (**Table2b**). At around 110 ^{0} C *Gandhaka* started melting and around 116^0 C it got completely molten. On pouring this molten *Gandhaka* with *Ghrita*into milk impurity like stone etc. were observed when it was pored through the cloth. The time taken for second and third time was little more when compared to first time. This may be due to the moisture content, milk protein and partial transformation of sulphur. Fresh milk contains Calcium and Enzymes inhabiting Vitamin C, Vitamin B12, Xanthine and Lactoferin etc. Here the protein and fat are in a Coagulated form. During *Dhalana*, fat-soluble impurities may be removed. Organic sulphur that is present in milk may have a role in improving the bioavailability of inorganic sulphur. After *Dhalana*, color of sulphur turned to yellowish green, due to the dissociation of fat-soluble sulphur and it became instantly hot. On all the three repetitions of *Dhalana*, the temperature of *Gandhaka* remained stable at 116^0 C and that of milk after *Dhalana* was ranged from 80-94^0 C. In all the three processes, initially sulphur had irritating smell which, after *Dhalana* was relatively less.

ArdhaGunaGandhakaKajjali (one part of *Parada* and Half part of *Gandhaka*) was prepared as per the reference. It took around 45 hours to get lustreless jet black Kajjali. Total 715 grams of *Kajjali* was obtained (**Table 3**) with a loss of about 4.7%.

Three batches of *Rasa Bhasma* were prepared as per the reference of *BharataBhaishajyaRatnakara.* An attempt was made to keep the temperature between 200^{0}C and 250^{0}C. There was difficulty in maintaining the temperature especially while preparing second and third batch. Hence, sand bath was used in second batch. In third batch Kajjali caught fire during the process which was put off immediately by closing the vessel to remove the contact with air (Observations are mentioned in **tables 5, 6 and 7**). Average yield of Rasa Bhasma obtained was 55.67% (**Table8**).

Bhasma samples were dark black, smooth, fine and tasteless. Organoleptic characters are mentioned in **Table 9**. Rasa Bhasma Samples were found lustreless. *Bhasma* samples were *RekhaPoorna* (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*[7] (**Table No.10**).*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[8]*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[9]. This happens when the weight of the solid is overcome by the surface tension forces[10]. As the weight of the particle decreases with particle size, the critical contact angle also decreases with particle size[11]. Also, the reduction in surface free energy with decrease in particle size has been demonstrated[12]. 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[13]

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. SEM photomicrograph of *Kajjali* and *RasaBhasma* samples show the appearance of particles of submicron level in all the samples. SEM images of the drug samples show cubic shape like structure with the particle size lying in the microrange. 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**.

Particle size analysis was done using Laser particle analyser. Kajjali showed the average particle size of 29.34µ. Rasa Bhasma samples RBBBh1, RBBh2 and RBBh3 showed average particle size of 10.92µ, 29.29µ and 17.64µ respectively. Lowest particle size observed in Rasa Bhasma sample was 146 nm (in sample RBBBh1). All the samples had particles less than 100µ in size. Result is displayed in **Table no.11**

EDAX in association with SEM is a non-destructive technique for the semi quantitative estimation of elements in the samples. Mercury was the major component followed by sulphur. Presence of carbon and oxygen in Bhasma samples in small quantities indicate the presence of organic materials. Elemental content present in the drug sample is reported in **Table no. 12**

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}. Calcium, Iron and Zinc were detected in the samples; Lead and Arsenic were below detection limits. Results are displayed in **Table No.13**and **Graphs 4, 5, 6, and 7**

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 26.504, 26.473, 26.325 for the samples RBBBh1, RBBBh 2and RBBBh 3 respectively. Kajjali sample showed 8 peaks, sample RBBBh1 showed 8 peaks and RBBh2 and RBBBh3 samples showed 5 and 4 peaks respectively. (**Table no. 14, 15, 16, 17**). **XRD** pattern shows metacinnabarHgS along with free sulphur in all the *Rasa Bhasma* samples including *Kajjali*. In the preparation of Kajjali, HgS, metacinnabar is produced from elemental mercury and pure sulphur, in non-stoichiometric conditions, by means of mechanical energy provided by the process of trituration.HgS is present in cubic form, 2θ position at 26.499, 26.504, 26.473, and 26.325with d-spacing of 3.3609, 3.3603,

3.3642, 3.3827A^{o} respectively in samples *Kajjali, Rasa bhasma* RBBBh1, 2 and 3.Free sulphur is present in ortho-rhombic form. Absence of free mercury is confirmed in XRD analysis.

4. Conclusion

Preparation of *Rasa Bhasma*following poling method using *NyagrodhaDanda*as described in BharataBhaishajyaRatnakara 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, and XRDetc are found to be very useful for the standardization of *Rasa Bhasma*. It is essential to subject this product to extensive toxicity study so that its safety is established and therapeutic application becomes feasible. Hence, this *Rasa Bhasma* sample can be further utilized in clinical practice to establish its therapeutic potential.

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7. Conflicts of interest: None declared

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TABLES

Table1 Showing Result ofParadaShodhana:

Drug	**Quantity in kilogram**
AshuddhaParada	5
SudhaChurna	5
Parada Obtained after grinding with Sudhachurna	4.86
Dehusked garlic	4.86
SaindhavaLavana	2.43
ShuddhaParada Obtained	**4.8**

Table 2 a. Showing the observations during GandhakaShodhana

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 GandhakaShodhana

Quantity of Gandhaka (grams)		Quantity of Ghee (grams)	Quantity of Milk (liters)	Quantity of ShuddhaGandhaka (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
Final yield				**585**	**15 grams (total loss)**

Table 3. Result of Kajjali preparation:

Drug	Quantity in Grams
ShuddhaParada	500
ShuddhaGandhaka	250
Kajjali Obtained	715
Loss	35

Table 4. Showing the Ingredients of Rasa Bhasma (similar for all the three batches):

Sl.No	Sanskrit Name	Part used	Latin/English name	Quantity	Textual reference	Batch No.
1.	Kajjali	Compound	Black sulphid of mercury	100 grams	Rasa Tarangini	KRKJL02
2.	Nyagrodhadanda	Fresh branch	Ficusbengalensis	1 (approximately 3 feet)	Bhavaprakasha	FSNG01, 02 and 03

Table 5.Observations with temperature pattern in Temperature pattern observed in Batch RBBBh 1

Time	Temperature	Observations
10:20 AM	128^0C	Powdery form, faint sulphurodour
10:30 AM	131^0C	Kajjali started melting
10:40 AM	203^0C	Yellow fumes emitted from slimy, molten, shiny Kajjali, strong smell of sulphur fumes
10:45 AM	189^0C	Molten appearance, with continuous stirring temperature varying between 180-200^0C
10:50 AM	176^0C	Molten Kajjali started sticking to the pan. Irritant yellow fumes increased making the stirring difficult.
11:00 AM	209^0C	Bolus formation of Kajjali
11:10 AM	189^0C	Bolus gets thickened
11:20 AM	148^0C	No visible yellow fumes. Thick mass/bolus formed.
11:30 AM	210^0C	Thick mass/bolus not sticking to the pan
11:45 AM	192^0C	Small tiny pellets, non-sticky formed with yellow fumes (seen on reflected light)
11:55 AM	181^0C	Slight yellow fumes (seen on reflected light), smell of Gandhaka reduced, dry pellets, non-sticky
12 Noon	172^0C	Dry pellets seen with yellow fumes
12:10 PM	173^0C	Dry powder with yellow fumes
12:20 PM	169^0C	Dry powder with increased yellow fumes
12:30 PM	197^0C	Dry powder with reduced yellow fumes
12:40 PM	199^0C	Dry powder with increased yellow fumes
1:00 PM	129^0C	Complete powder with yellow fumes
1:20 PM	126^0C	Complete powder, no yellow fumes, white fumes appeared, fire turned off.

Table 6.Observations with temperature pattern in Temperature pattern observed in Batch RBBBh 2

Time	Temperature	Observations
10:08 AM	62^0C	Powdery form, faint sulphurodour
10:12 AM	307^0C	Yellow coloured powder and caught fire on pan, sulphur started burning with offensive odour
10:15 AM	315^0C	Sulphur continues to burn forming yellowish powder and sticking to the pan. Even when fire was turned off material continued to burn, with continuous stirring fire got extinguished and temperature fell down to 79^0C
As it was difficult to maintain the temperature, direct heating was avoided, instead ValukaYantra was used		
10:40 AM	57^0C	ValukaYantra is heated at 57^0C, iron pan kept on ValukaYantra
10:48 AM	61^0C	Black coloured powder in pan, no fumes seen, no yellowish colour,and no smell.
10:55 AM	68^0C	Black coloured powder in pan, no fumes seen, no yellowish colour, and no smell.
11:05 AM	83^0C	Slight fumes are observed
11:20 AM	93^0C	Yellowish fumes not seen, as white fumes begin to evolve fire is put off. Black coloured powder remained in pan.

Table 7.Observations with temperature pattern in Temperature pattern observed in Batch RBBBh 3

Time	Temperature	Observations
10:20 AM	134^0C	Powdery form, faint sulphurodour
10:25 AM	210^0C	Fumes appear
10:30 AM	300^0C	Kajjali caught fire with strong fumes and offensive odour of sulphur
10:35 AM	357^0C	Even after switching of the fire Kajjali remained burning which gradually got extinguished leaving black powder behind

Table 8. Table showing the yield of Rasa Bhasma :

Batch	Quantity of Kajjali in grams	Quantity of Rasa Bhasmain grams
Batch 1	100	63
Batch 2	100	50
Batch 3	100	54
Total	300	**167**

Table 9. 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	RBBBh1	Dark Black	Absent	Fine powder	Smooth	Mild burnt odour	Tasteless	Comparatively lighter
3	RBBBh2	Dark Black	Absent	Fine powder	Smooth	Mild burnt odour	Tasteless	Comparatively lighter
4	RBBBh3	Dark Black	Absent	Fine powder	Smooth	Mild burnt odour	Tasteless	Comparatively lighter

Table 10. Results of BhasmaPareeksha of the samples:

S.No.	Sample	Rekhapurnata	Varitara	Unama	Nirdhuma
1	Kajjali	+ ve	+ ve	+ ve	-ve
2	RBBBh1	+ ve	+ ve	+ ve	+ ve
3	RBBBh2	+ ve	+ ve	+ ve	+ ve
4	RBBBh3	+ ve	+ ve	+ ve	+ ve

Table 11.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	RBBBh1	10.92 µ
3	RBBBh2	29.29 µ
4	RBBBh3	17.64 µ

Table12. EDAX report of the samples:

S.No.	Elements in %	Kajjali	RBBBh1	RBBBh2	RBBBh3
1	Mercury	70.89	77.41	64.63	75.81
2	Sulphur	24.27	15.86	30.56	20.13
3	Carbon	4.84	5.75	4.30	4.06
4	Oxygen	-	0.98	0.51	-

Table 13.ICP-OES report of the samples:

S.No.	Elements in ppm	Kajjali	RBBBh1	RBBBh2	RBBBh3
1	Calcium	133.38	208.78	139.88	141.73
2	Iron	43.47	506.59	52.48	62.92
3	Zinc	21.04	2.85	2.64	2.75
4	Lead	BDL	BDL	BDL	BDL
5	Arsenic	BDL	BDL	BDL	BDL

Table14. 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)

Table15. Representing XRD peak list of *Rasa Bhasma* sample RBBBh1

No.	2-Theta(deg)	D(ang.)	Height (counts)	FWHM(deg.)	Int.I(counts deg)	Int.W(deg)	Asym.factor
1	23.93(2)	3.715(4)	11(3)	0.17(7)	2.0(9)	0.19(14)	1(3)
2	**26.504(5)**	**3.3603(6)**	**1389(37)**	**0.295(5)**	**606(3)**	**0.436(14)**	**0.78(6)**
3	28.32(3)	3.149(3)	65(8)	0.21(2)	15.9(13)	0.24(5)	1.5(8)
4	30.648(12)	2.9148(12)	248(16)	0.381(15)	144(3)	0.58(5)	0.95(15)
5	31.317(6)	2.8540(5)	339(18)	0.177(10)	92(2)	0.27(2)	0.95(15)
6	43.866(11)	2.0623(5)	542(23)	0.330(11)	285(3)	0.52(3)	1.15(19)
7	44.799(15)	2.0215(6)	37(6)	0.22(4)	13.0(17)	0.36(10)	1.15(19)
8	45.876(6)	1.9765(2)	98(10)	0.17(2)	28.1(11)	0.29(4)	0.8(4)

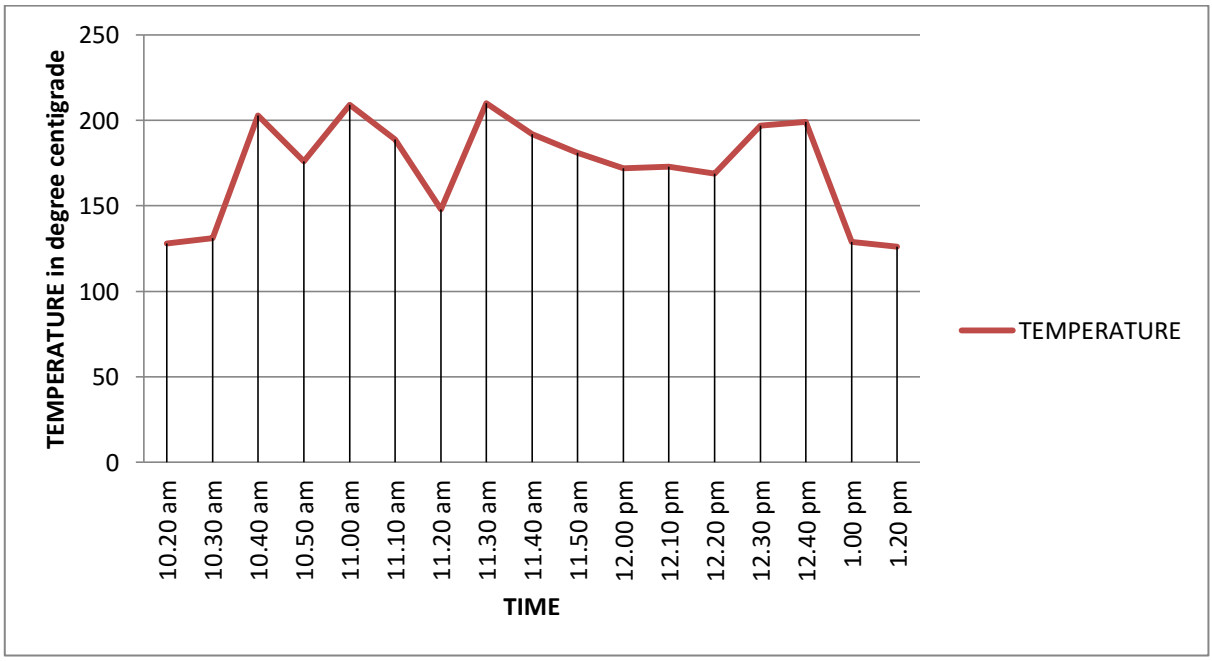
Table16. Representing XRD peak list of *Rasa Bhasma* sample RBBBh2

No.	2-Theta(deg)	D(ang.)	Height (counts)	FWHM(deg.)	Int.I(counts deg)	Int.W(deg)	Asym.factor
1	23.20(3)	3.830(5)	41(6)	0.12(5)	8.2(8)	0.20(5)	2(3)
2	**26.473(6)**	**3.3642(7)**	**1239(35)**	**0.247(5)**	**470(3)**	**0.379(13)**	**1.14(13)**
3	30.610(10)	2.9183(9)	260(16)	0.319(14)	121(3)	0.47(4)	0.75(11)
4	31.244(16)	2.8605(14)	51(7)	0.31(4)	23(2)	0.45(11)	0.75(11)
5	43.820(5)	2.0643(2)	537(23)	0.229(11)	212(2)	0.40(2)	0.63(7)

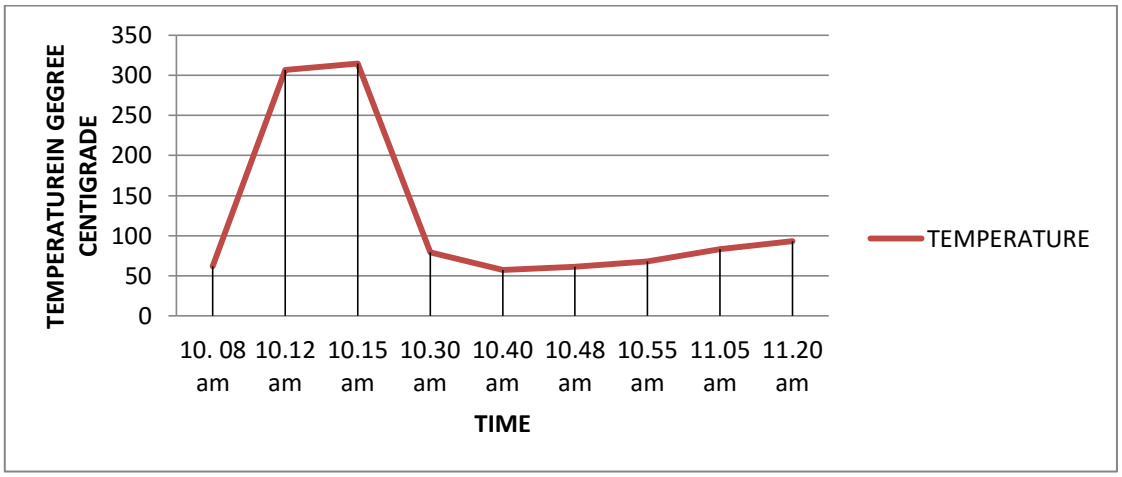
Table17. Representing XRD peak list of *Rasa Bhasma* sample RBBBh3

No.	2-Theta(deg)	D(ang.)	Height (counts)	FWHM(deg.)	Int.I(counts deg)	Int.W(deg)	Asym.factor
1	**26.325(5)**	**3.3827(6)**	**1353(37)**	**0.234(5)**	**490(3)**	**0.362(12)**	**1.16(12)**
2	30.464(11)	2.9319(10)	270(16)	0.317(14)	127(3)	0.47(4)	0.82(14)
3	31.104(14)	2.8730(12)	61(8)	0.27(3)	25(2)	0.40(9)	0.82(14)
4	43.682(8)	2.0705(3)	576(24)	0.249(13)	237(2)	0.41(2)	1.01(15)

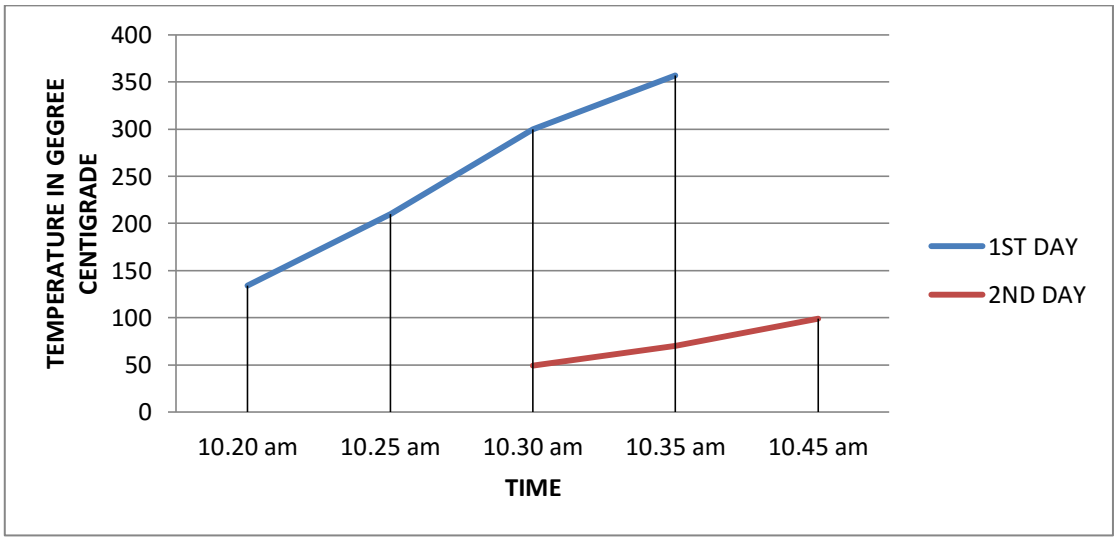
GRAPHS



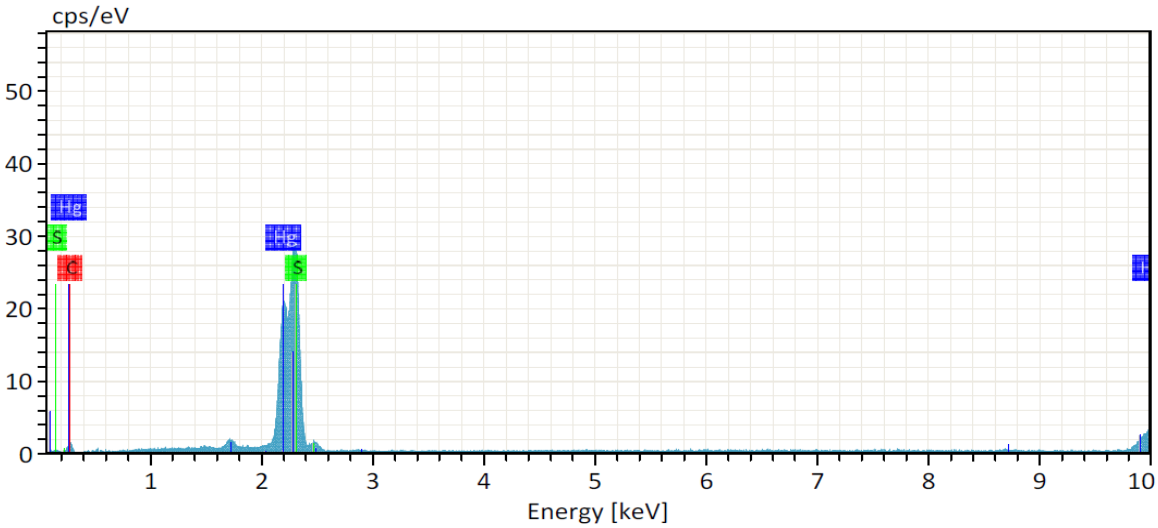
Graph 1.Temperature pattern observed in Batch RBBBh 1



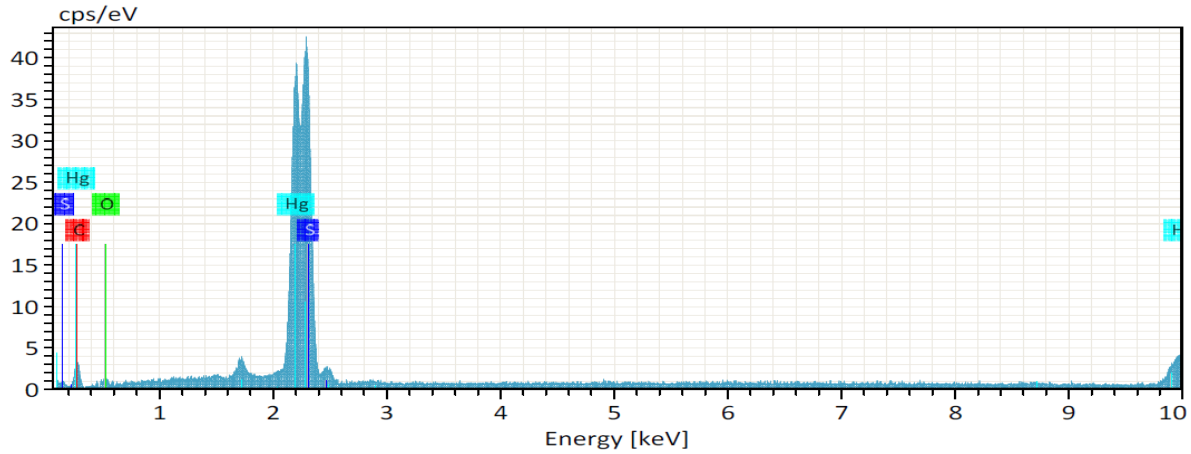
Graph 2.Temperature pattern observed in Batch RBBBh 2



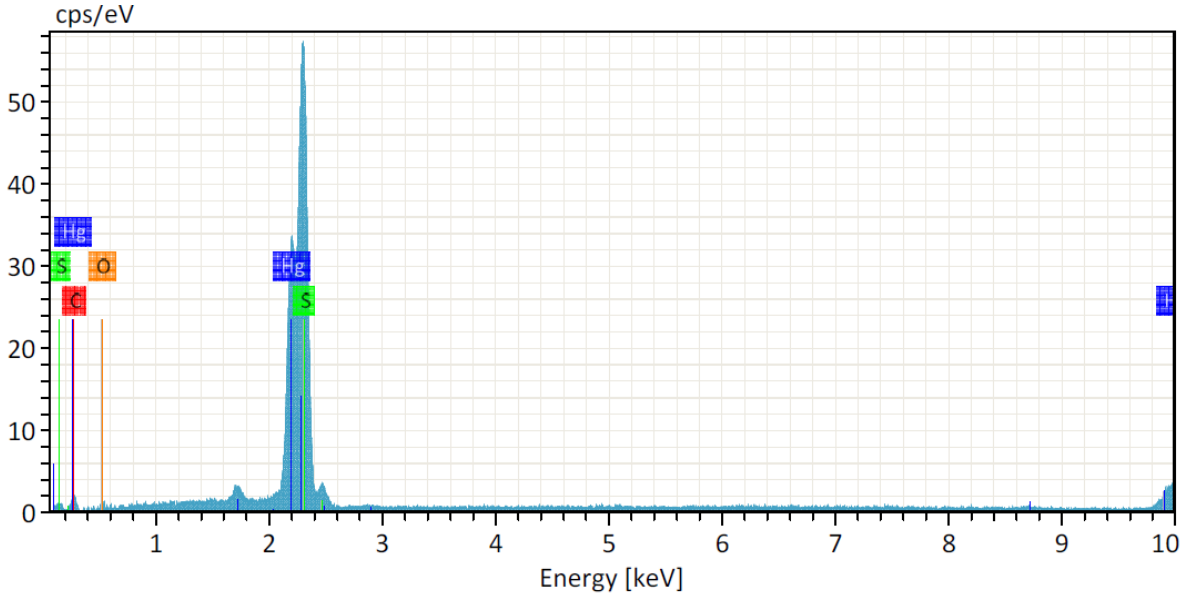
Graph 3.Temperature pattern observed in Batch RBBBh 3



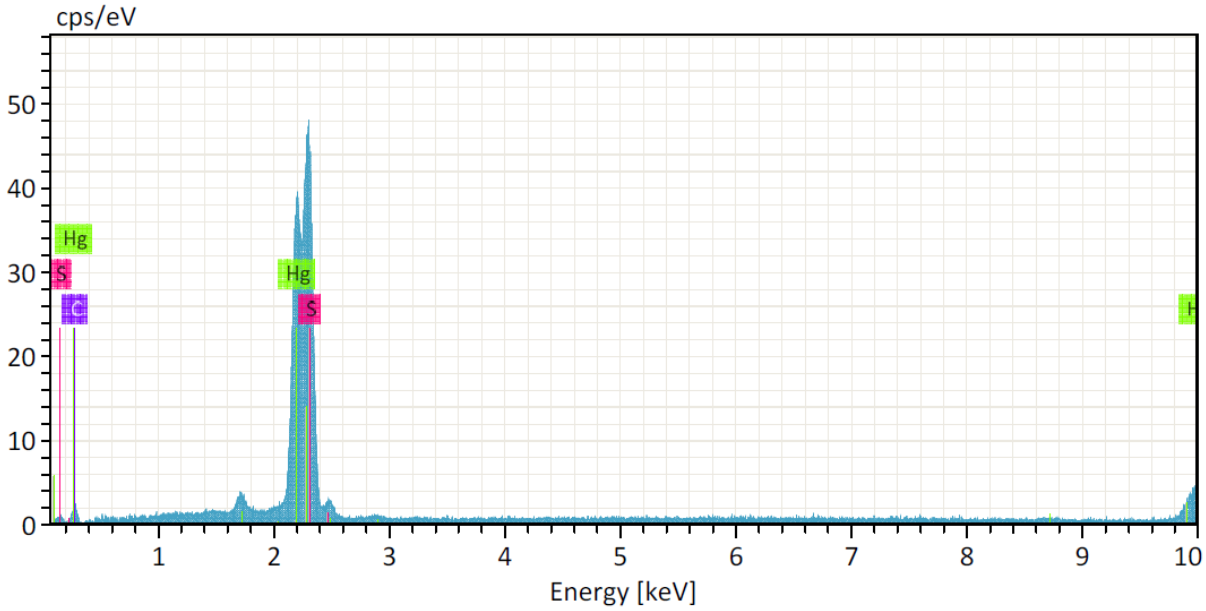
Graph4. EDAX peaks for Kajjalihowing the elemental composition



Graph5. EDAX peaks for Rasa Bhasma sample RBBBh1showing the elemental composition



Graph6. EDAX peaks for Rasa Bhasma sample RBBBh2showing the elemental composition



Graph7. EDAX peaks for Rasa Bhasma sample RBBBh2showing the elemental composition

FIGURES

SEM PHOTOMICROGRAPHS

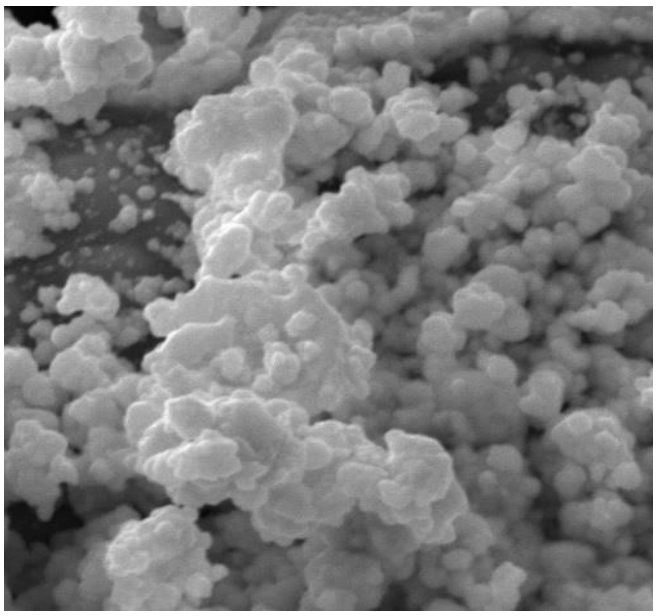


Fig.1a.SEM image of Kajjali (10000X)

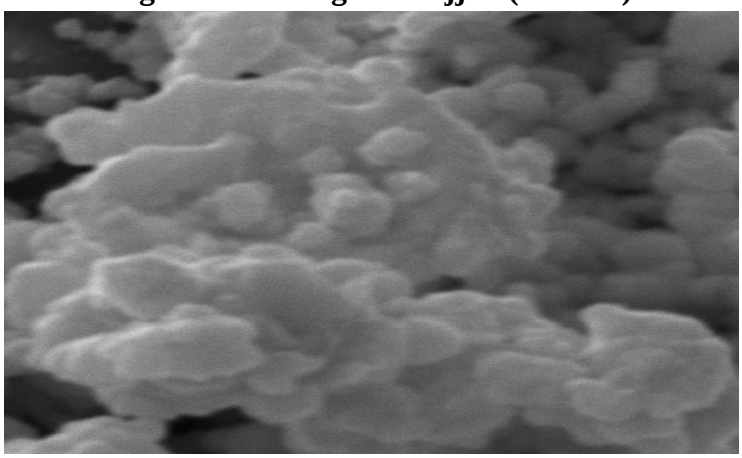


Fig.1b.SEM image of Kajjali (20000X)

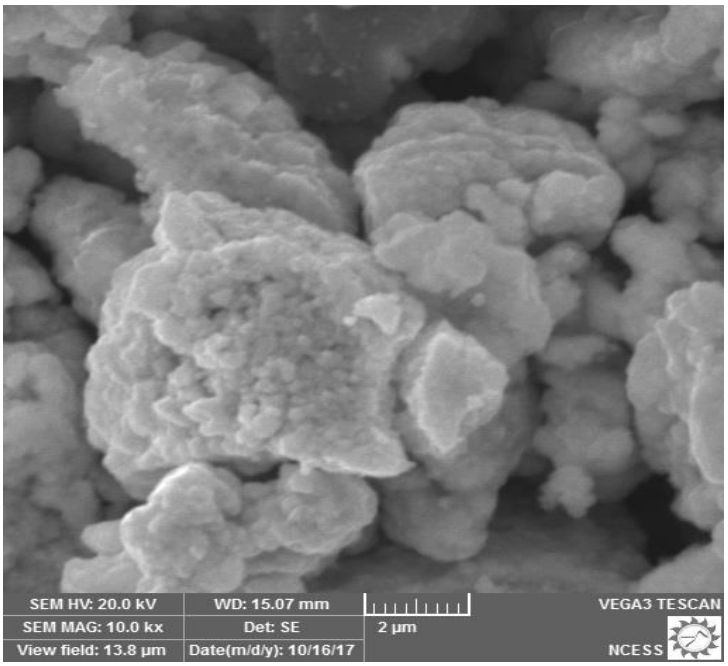


Fig2a. SEM image of Rasa Bhasma sample RBBBh1(10000X)

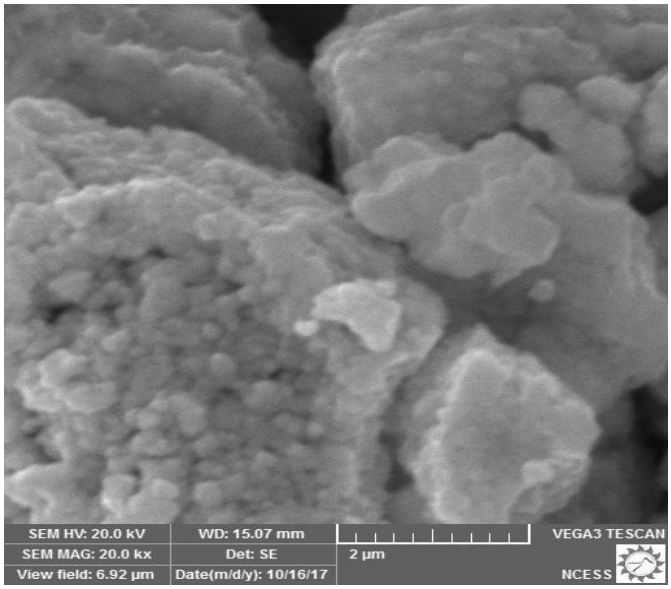


Fig2b. SEM image of Rasa Bhasma sample RBBBh1 (20000X)

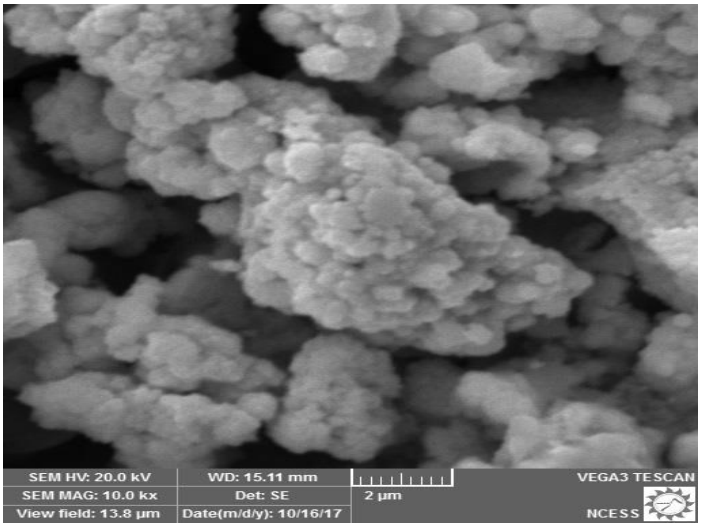


Fig3a. SEM image of Rasa Bhasma sample RBBBh2 (10000X)

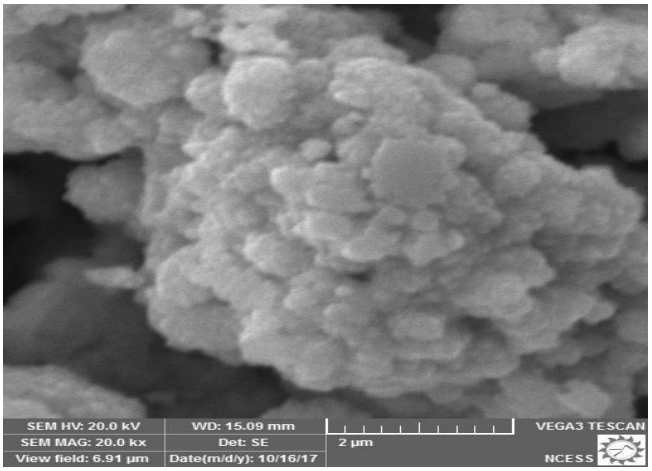


Fig3b. SEM image of Rasa Bhasma sample RBBBh2 (20000X)

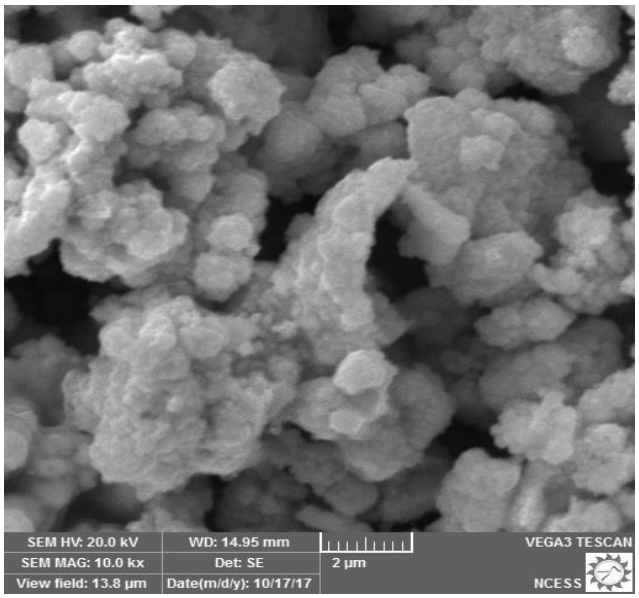


Fig4a. SEM image of Rasa Bhasma sample RBBBh3 (10000X)

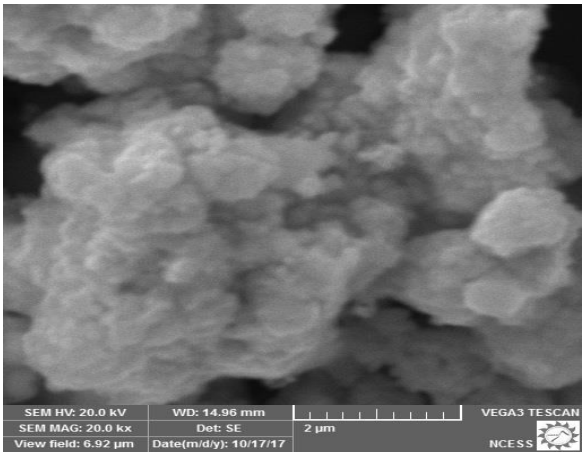


Fig4b. SEM image of Rasa Bhasma sample RBBBh3 (20000X)