ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

ANALYTICAL EVALUATION OF *ABHRAKA BHASMA* SAMPLES AFTER *MARANA*, *AMRITIKARANA* AND *LOHITIKARANA*

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Article info

Article History:
Received: 18-12-2023
Accepted: 14-01-2024
Published: 04-02-2024

KEYWORDS:
Abhraka bhasma, *Amritikarana*, analysis, *Marana*, Particle size, SEM.

ABSTRACT

Rasaushadhies or herbo-mineral formulations undergo rigorous processes to convert into nano size. The particle size does matters a lot in terms of absorption, assimilation and proper excretion from the body. The pharmaceutical procedures should be strictly adhered so as to ensure safety and efficacy. *Abhraka bhasma* is a popularly used formulation in Ayurvedic practice. *Abhraka* or black mica is a bad conductor of heat. So the process of *Bhasmikarana* requires high quantum of heat in order to render into *Bhasma* form. *Amritikarana* is a specific process included in the preparation of *Abhraka bhasma*. The current study evaluates the essentiality of this process in *Bhasmikarana*. A detailed analytical study is been carried out to find the differences in the samples before and after *Amritikarana.* The present study reveals that the particle size has reduced much and the iron content was at maximum concentration in the samples after the process of *Amritikarana* and *Lohitikarana*.

INTRODUCTION

Abhraka bhasma (incinerated mica) is a widely used formulation in Ayurvedic practice. It is used either alone or as an ingredient of several herbo-mineral formulations. Considering the wide applicability and therapeutic benefits, studies are being planned on various aspects of *Abhraka bhasma.* As a necessary step to eliminate any possible residual toxic effect and make *Abhraka bhasma* therapeutically safe and effective. This is a step indicated after a prolonged process on *Abhraka*. After *Amritikarana*, an additional step of *Lohitikarana* is indicated with the intention of regaining the lost colour during *Amritikarana*.

A scientific curiosity always exists regarding the necessity of *Amritikarana* as it is specially mentioned in selected types of *Bhasma* like *Abhraka* and *Tamra* (copper). Pharmaceutical, chemical and biological aspects of *Amritikarana* are found to be essentially investigated.

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https://doi.org/10.47070/ijapr.v12i1.3046

The study aims at revealing the difference between the different samples prepared after *Marana*, *Amritikarana* and *Lohitikarana* through analytical parameters.

Description of *Marana* was followed by the concept of *Amritikarana*. As defined in *Rasa Tarangini*, *Amritikarana* is a process carried out to eliminate the residual toxicity of certain *Bhasma*[1]. This process is popularly used in the context of *Tamra* and *Abhraka bhasma* even though it is also referred in the context of *Loha bhasma*. As the name indicates this process is to bring into *Amrita* (nectar) like properties in the *Bhasma*. *Amritikarana* is considered to have its effect not only in improving safety but also efficacy. It is considered that *Amritikarana* is aimed at removing the residual impurities and also *Rukshata* (dryness) that is produced by repeated heating. In addition to increase the potency commonest method followed in the context of *Amritikarana* is to heat *Abhraka bhasma* with *Triphala kashaya* and cow's ghee in an iron pan till the complete loss of moisture[2]. Mild heat is applied until the medicinal fluids are completely evaporated. *Bhasma* that remains at the end of the process is safer and possesses higher therapeutic efficacy. *Goghrita* (cow's ghee) alone, *Goghrita* along with *Kumari swarasa* (*Aloe vera* juice) are also indicated[3].

By the process of *Amritikarana, Bhasma* will attain enhancement of properties along with loss of brick-red colour. The main aim of this process is to remove the remaining impurities from *Bhasma*, reduce the *Rukshata* (dryness) caused due to *Agni Samskara* (repeated exposure to heat) and to increase the potency of *Bhasma*. After the process of *Amritikarana* of *Abhraka bhasma*, it loses its brick red colour[4,5]. To regain the lost colour during the process, *Lohitikarana* is done. In this process *Bhasma* is triturated with *Rakta varga dravya's* and subjected to 2-3 *Gajaputas* (quantum of heat).[6]

MATERIALS AND METHODS

Shodhana

Abhraka shodhana was done by *Nirvapa* (quenching) in cow's milk for seven times[7].

Marana

Abhraka bhasma prepared as per the reference of Ayuvedic Formulary of India Part I, 18/1. *Shodita abhraka* is triturated with *Ravi moola kwatha* (decoction of *Calotropis gigantea*) for seven times and subjected to seven *Gajaputa*. Then it is triturated with *Nyagrodha moola kvatha* and three *Gaja puta's* are given. Later seven *Bhavanas* with *Kadalikanda swarasa* and subjected to seven more *Putas*[8].

Amritikarana

The *Abhraka bhasma* was mixed with *Triphala kwatha* and cow's ghee and *Amritikarana* was carried out. *Abhraka bhasma* became black in colour after the process.

Lohitikarana

Lohitikarana was done with *Manjistadi kwatha* (decoction of *Rubia cordifolia*) and after three *Putas* the brick-red color was regained[5].

The samples were subjected for analysis by employing two different kinds of parameters.

Evaluation on Classical Parameters

i. Organoleptic characters
ii. *Nishchandrata* test
iii. *Rekhapurnata* test
iv. *Varitara* test
v. *Unam* test

Evaluation on Modern Analytical Parameters

i. Loss on drying
ii. Ash value
iii. Acid Insoluble Ash
iv. Ethanol soluble extractive
v. Water soluble extractive
vi. Qualitative test for iron
vii. pH value
viii. Namburi Phased Spot test
ix. X-ray Diffraction
x. SEM-EDAX (Scanning electron microscope and Energy Dispersive X-ray Spectroscopy)
xi. AAS (Atomic Absorption Spectroscopy)

Organoleptic Characters

Three samples of *Abhraka bhasma* were procured, one after *Marana* (AB-M), after *Amritikarana* (AB-A) and *Lohitikarana* (AB-L).

Table 1: Showing the details of organoleptic characters

Physical characters	AB - M	AB -A	AB - L
Sound	Absent	Absent	Absent
Appearance	Fine powder	Fine powder	Fine powder
Colour	Light brick - red	Jet black	Dark brick - red
Nichandrata	+ve	+ve	+ve
Varitara	+ve	+ve	+ve
Unama	+ve	+ve	+ve
Touch	Soft	Soft	Soft
Rekhapurnata	+ve	+ve	+ve
Taste	Tasteless	Tasteless	Tasteless
Odor	Odorless	Burned odor	Odorless

Chemical Analysis

All the three samples i.e., *Abhraka bhasma* after *Marana*, *Abhraka bhasma* after *Amritikarana*, *Abhraka bhasma* after *Lohitikarana* were analyzed for L.O.D, ash value and acid insoluble ash content. It was analyzed for the content of iron and silica qualitatively.

Loss on drying

Table 2: Showing chemical data regarding Loss on Drying of the three samples of *Abhraka Bhasma*

Name of the sample	L.O.D
A.B - M	4.96%
A.B - A	8.96%
A.B - L	11.16%

(A.B – *Abhraka Bhasma*, M- *marana*, A – *Amritikarana*, L – *Lohitikarana*)

Ash Value, Acid Insoluble Ash & Extractive Values

Table 3: Showing Comparative chemical data of *Bhasma* obtained after *Marana*, *Amritikarana*, *Lohitikarana*

S.No	Parameters	AB - M	AB - A	AB - L
1	Ash value %w/w	93.75%	82.4%	99.05%
2	Acid insoluble ash % w/w	47.5%	45%	43.5%
3	Water soluble extractive	21.25%	7.5%	12.5%
4	Ethanol soluble extractive	17.3%	10.05%	19.5%
5	pH value	8.24	7.25	7.40

Qualitative Analysis

Table 4: Showing the details of qualitative analysis

Tests	AB - M	AB – A	AB -L
Ferric iron	Present	Present	Present
Silica	Present	Present	Present

N.P.S.T[6]

Table 5: Showing N.P.S.Test- Results on potassium iodide paper

Sample	Region analysed	Phases (min)		
		Phase I (0-5min)	Phase II (0-20)	Phase III (0-48hrs)
AB-M	Central spot	Dark brown	Light yellow	Light yellow
	Middle segment	Light ring	Light yellow colored ring with dark brown margin	Light yellow centre with brown ring
	Peripheral segment	Light brown halation	Dark brown margin with brown halation	Brown shade
AB - A	Central spot	Brown	Brown	Light brown
	Middle segment	Light brown color ring	White ring with irregular margin	Dark brown shade
	Peripheral segment	Light brown shade	Dense brown shade	Dark brown shade
AB-L	Central Spot	Dark brown	Yellow colored ring	Light yellow colored ring
	Middle segment	Light yellow ring	Light yellow ring with irregular brown margin	White color ring
	Peripheral segment	Brown shade	Light yellow ring	Light brown shade

X- Ray Diffraction Results

In the sample, A.B. – M, major phase is of the compound having maximum absorption (Ref I 100) at an angle 26.767° (at 38 counts and D space 3.33)

In the sample, A.B. – L, major phase is of the compound having maximum absorption (Ref I 100) at an angle 26.782° (at 151 counts and D space 3.329).

In the sample, A.B. – A, major phase is of the compound having maximum absorption (Ref I 100) at an angle 26.84° (at 40 counts and D space 3.322).

SEM- Results

Scanning electron microscope image of selected fields of *Abhraka Bhasma* samples have shown the surface topography (images 1, 2, and 3).

The particle size range was also observed which is depicted in the following table:

Table 6: Showing the details of particle size range

Sample	Magnification	Particle size in µ	
		Minimum	Maximum
A.B.-M	750X	3.73	7.93
A.B. –A	750 X	3.22	7.47
A.B.- L	2000 X	1.9	6.08

EDAX (Scanning Electron Microscope and Energy Dispersive X-Ray Spectroscopy)

Table 7: Showing the details of EDAX

Metals	AB - M	AB - A	AB - L
Magnesium	7.18	7.88	8.34
Aluminium	21.84	21.52	22.16
Silica	40.51	41.13	41.73
Calcium	1.396	0.87	0.81
Iron	29.06	28.596	56.935
Phosphorus	Nil	Nil	1.01

AAS (Atomic Absorption Spectroscopy)

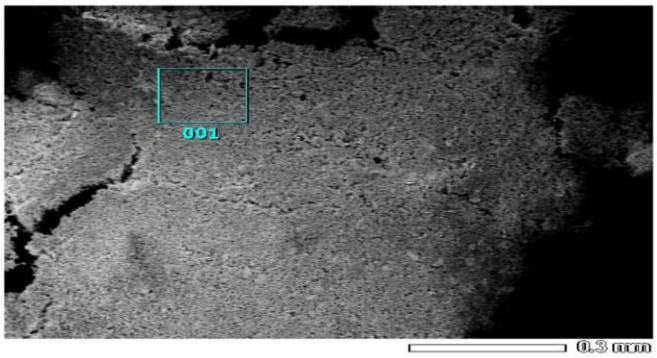
Table 8: Showing the details of AAS

Metals	Concentration in mg/kg		
	AB-M	AB – A	AB - L
Magnesium	13123	13850	16352
Aluminium	206500	230400	239500
Calcium	970	840	1410
Iron	35830	42280	42840

SEM-EDAX OF AB-M

View000

1/1



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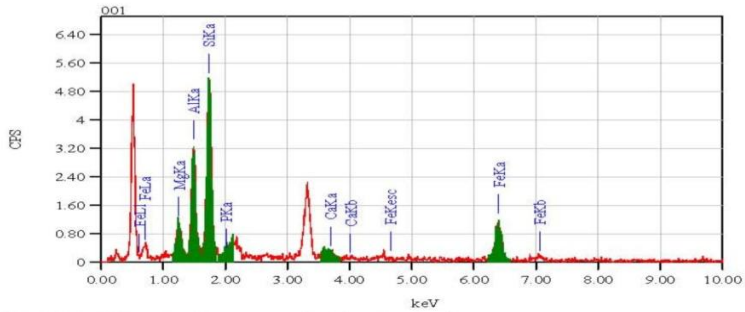
Volt : 20.00 kV
Mag : x 100
Date : 2010/11/03
Pixel : 640 x 480



Acquisition Parameter
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Probe Current: 1.00000 nA
PHA mode : T3
Real Time : 50.92 sec
Live Time : 50.00 sec
Dead Time : 1 %
Counting Rate: 327 cps
Energy Range : 0 - 20 keV

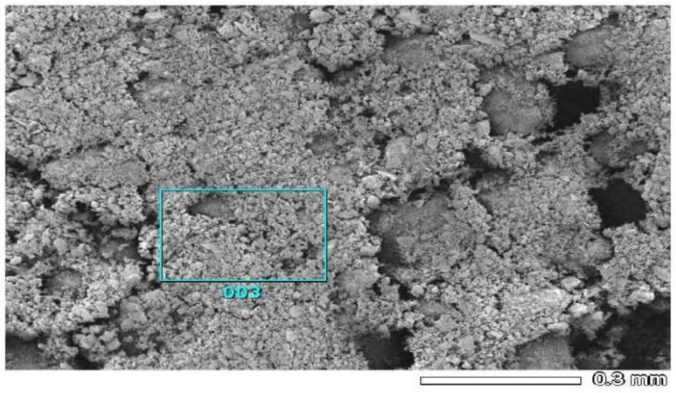
ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.5934

Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
Mg K	1.253	6.21	0.83	8.24				5.1760
Al K	1.486	22.31	0.82	26.66				20.3945
Si K	1.739	41.50	0.99	47.63				35.9173
P K								
Ca K	3.690	0.79	1.17	0.64				1.0964
Fe K	6.398	29.19	2.39	16.85				37.4156
Total		100.00		100.00				

SEM- EDAX OF AB-A

View000

1/1



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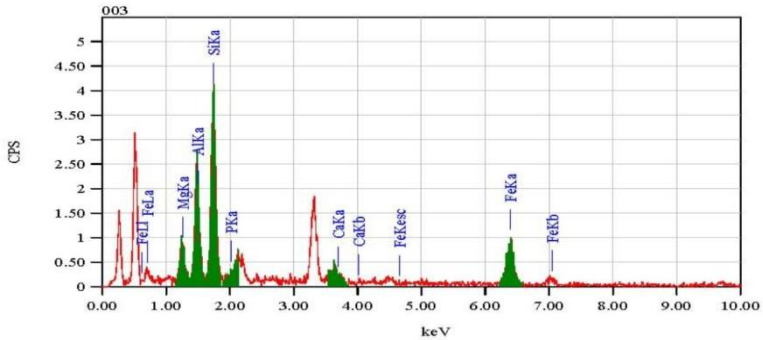
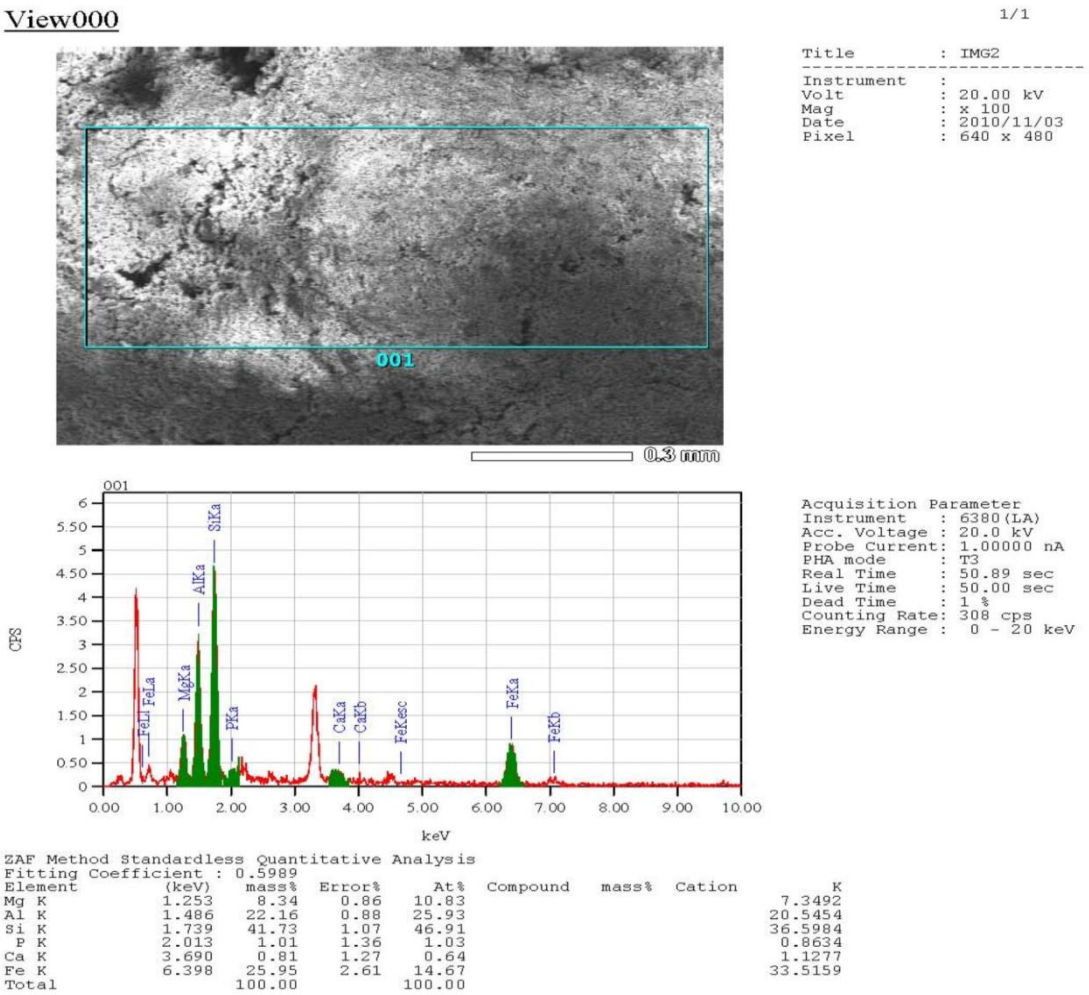


Acquisition Parameter
Instrument : 6380(LA)
Acc. Voltage : 20.0 kV
Probe Current: 1.00000 nA
PHA mode : T3
Real Time : 50.78 sec
Live Time : 50.00 sec
Dead Time : 1 %
Counting Rate: 271 cps
Energy Range : 0 - 20 keV

ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.6602

Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
Mg K	1.253	7.39	0.83	9.79				6.1769
Al K	1.486	20.88	0.83	24.90				18.8382
Si K	1.739	41.90	0.99	48.02				36.5377
P K								
Ca K	3.690	0.40	1.17	0.32				0.5618
Fe K	6.398	29.43	2.40	16.96				37.8853
Total		100.00		100.00				

SEM- EDAX OF AB-L

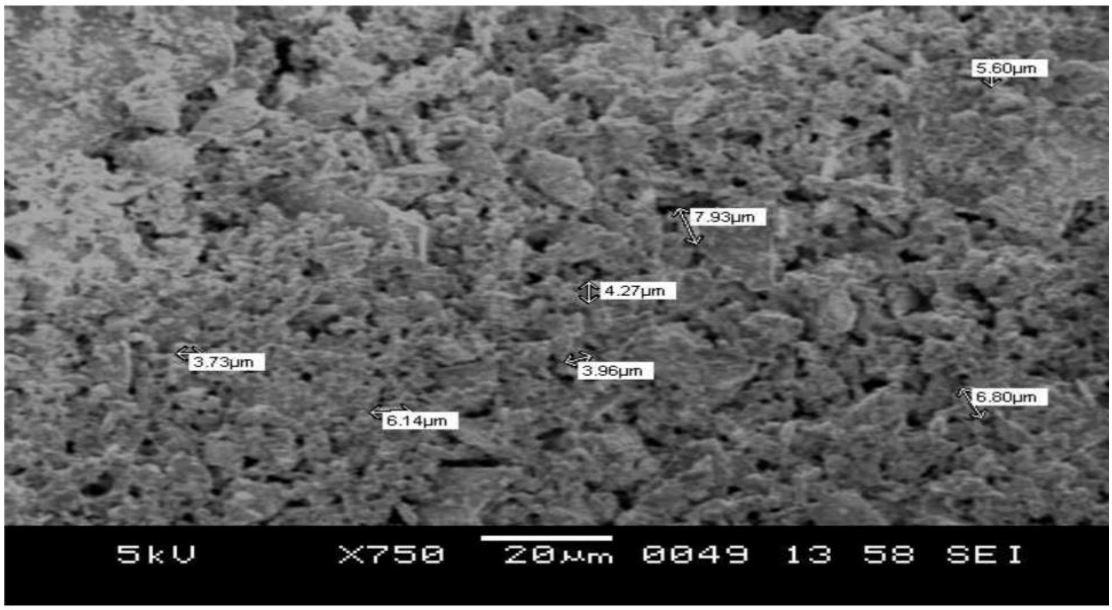


ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.5989

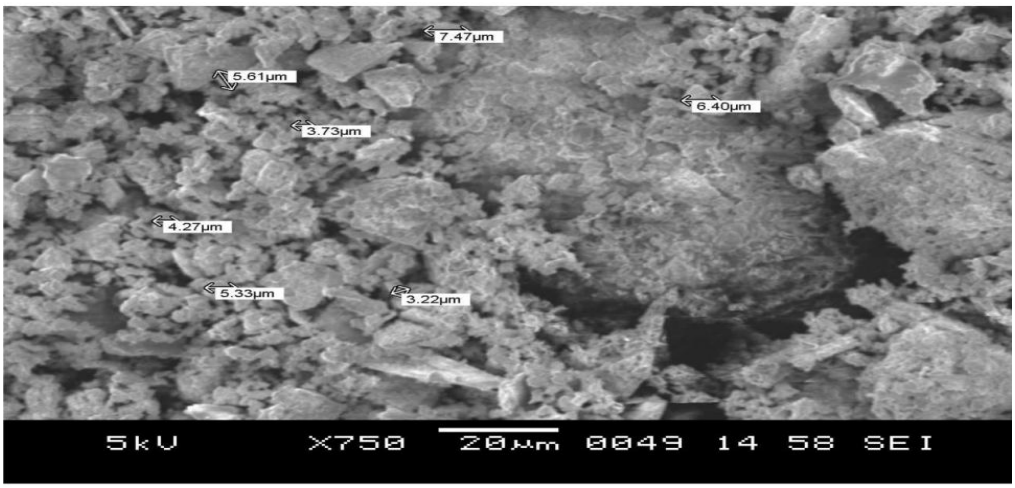
Element	(keV)	mass%	Error%	At%	Compound	mass%	Cation	K
Mg K	1.253	8.34	0.86	10.83				7.3492
Al K	1.486	22.16	0.88	25.93				20.5454
Si K	1.739	41.73	1.07	46.91				36.5984
P K	2.013	1.01	1.36	1.03				0.8634
Ca K	3.690	0.81	1.27	0.64				1.1277
Fe K	6.398	25.95	2.61	14.67				33.5159
Total		100.00		100.00				

Figure 1: SEM-EDAX AB-M,SEM-EDAX AB-A,SEM-EDAX AB-L

SEM OF AB-M



SEM OF AB-A



SEM OF AB-L

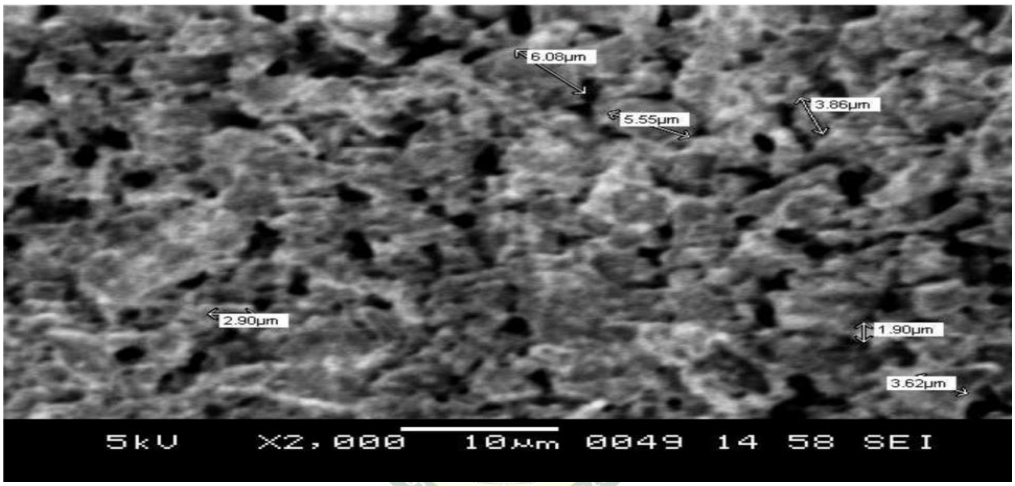
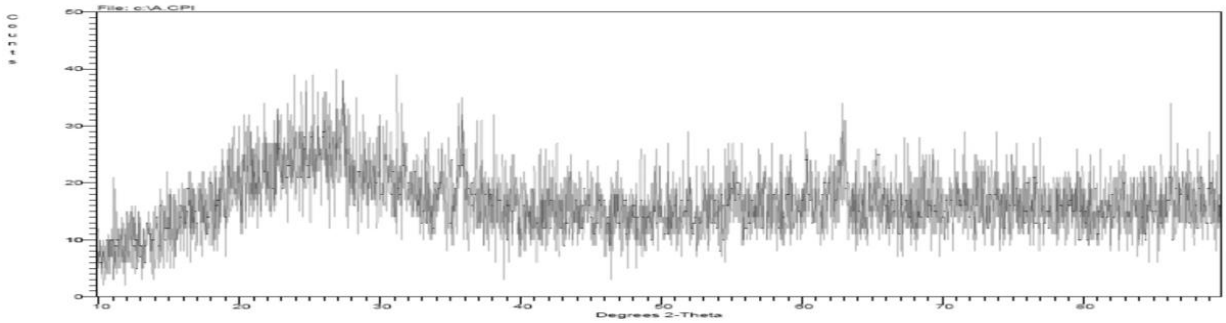


FIGURE 2: SEM OF AB-M, SEM OF AB-A, SEM OF AB-L

XRD OF AB – M



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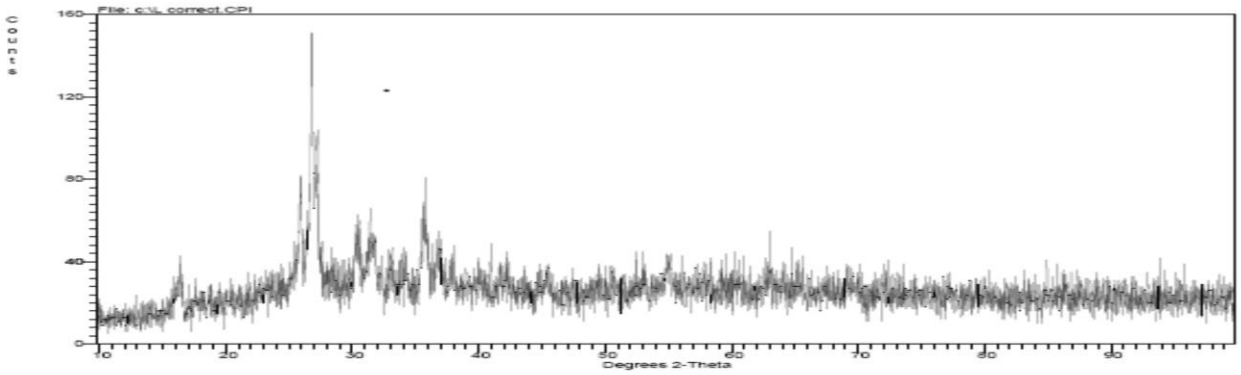
3	26.84	40	3.322	100
4	28.317	35	3.152	88
5	28.6	29	3.121	72
6	34.9	28	2.571	70
7	35.837	35	2.506	88
8	37.2	31	2.417	78
9	43.62	25	2.075	62

XRD OF AB – A



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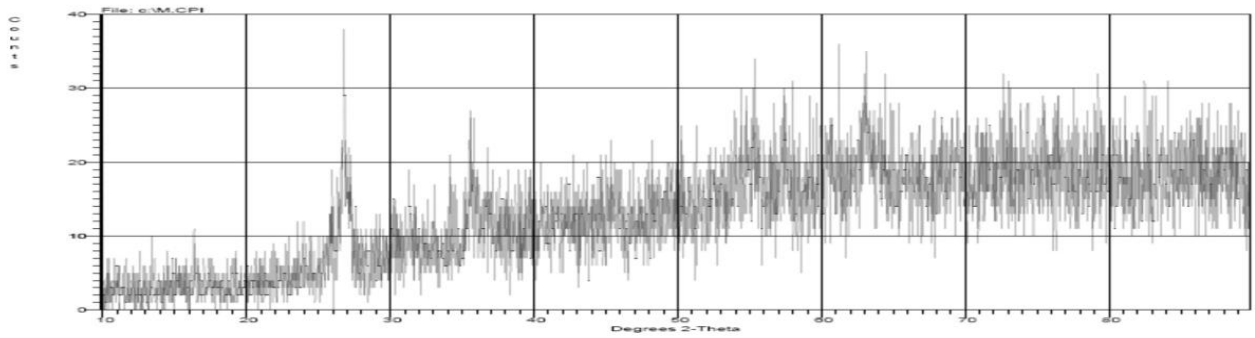
No	Angle	Counts	Dspace	Rel I
1	16.46	39	5.385	26
2	22.314	34	3.984	23
3	25.95	82	3.434	54
4	26.782	151	3.329	100
5	27.299	104	3.267	69
6	31.4	66	2.849	44
7	36.36	49	2.471	32
8	36.98	51	2.431	34
9	40.97	49	2.203	32
10	51.4	34	1.778	22
11	57.2	37	1.61	25
12	76.74	33	1.242	22
13	92.9	35	1.064	19

XRD OF AB – L



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No	Angle	Counts	Dspace	Rel I
1	26.767	38	3.33	100
2	54.4	30	1.687	79
3	59.94	25	1.543	61
4	82.48	31	1.169	82
5	83.	29	1.163	76

FIGURE 3: XRD OF AB –M, XRD OF AB – A, XRD OF AB-L

DISCUSSION

The study included testing of *Abhraka bhasma* obtained immediately after *Marana* (sample AB-M), after *Amritikarana* (sample AB-A) and *Amritikarana* and *Lohitikarana* (sample AB-L). Samples were evaluated with basic organoleptic characters and classical parameters of *Bhasma pariksha*.

Difference in color was noted among the samples. Sample AB-M was light brick-red in colour where AB –L was dark brick-red, sample AB-A was jet black. All the samples were tasteless indicating the absence of metallic nature due to the formation of compounds. All the samples were *Nischandra*

(lusterless) which is possibly because of the conversion of insoluble silicates into final soluble amorphous form. All samples were soft and smooth to touch indicating the fineness of particles. All samples were odorless except for a faint odor in sample AB-A which is probably due to the burning of organic materials in *Triphala* and cow's ghee. The samples satisfied all the other *Bhasma parikshas*.

During the process of *Amritikarana* the colour of *Abhraka bhasma* which was brick-red immediately after *Marana* turns blackish. It is estimated that the ferric oxide (Iron III oxide) formed by repeated *Puta* (quantum of heat) during *Marana* is responsible for the colour. On heating *Abhraka bhasma* with *Triphala kashaya* and *Goghrita* a part of the iron III oxide may change to ferrous (iron II oxide) which is black. Addition of *Triphala kashaya* also may be a factor that contributes in darkening the colour towards brownish or blackish[7].

The secret behind this can be revealed by referring to the periodic table. Iron which is a major constituent in *Abhrak bhasma* is a transition element (4^{th} period), forms 3 and 2 types of oxides respectively. These oxides of an element may transform to one another type on heating. Three types of oxides are formed by iron.

- Iron (II) ferrous oxide – FeO (black powder produced by heating iron oxalate in the absence of air)
- Iron (III) Ferric oxide – Fe_2O_3 (red powder prepared by heating iron hydroxide in strong heat)
- Iron (II, III) Ferroso ferric oxide – Fe_3O_4 (Bluish black prepared by heating in air or steam to redness).

  Iron (III) oxide is the basic oxide readily reacting with dilute acids to form corresponding iron (ii) salta, this makes it the most absorptive form of iron, which will be digested in stomach. Due to the process of *Amritikarana*, iron III changes to iron II, III in the first step and iron III changes to iron II as earthen *Sarava* is covered causing the absence of air. So the red colour which was due to iron III is lost and *Bhasma* become brownish black in colour.

After the process of *Amritikarana*, *Lohitikarana* is advised. Main purpose of *Lohitikarana* is to regain the lost colour. *Bhavana* with *Rakta varga dravya* and *Gaja puta* are advised for *Lohitikarana*. Most probably during this process, a part of ferric oxide that was converted into ferrous form forming ferresso ferric oxide is rapidly converted into Fe_2O_3 to regain brick-red colour. Usage of *Rakta varga dravya kashaya* like *Manjista kashaya* may have its own contribution in the process.

Chemical analysis data regarding loss on drying (L.O.D) was observed minimum in case of sample AB-M and gradually increasing after *Amritikarana* and *Lohitikarana*. This may be a parameter that supports its textual description of *Rukshata* (dryness) as a disadvantage of *Abhraka bhasma* that is not subjected to *Amritikarana*. A higher amount of ash value observed in all the samples corresponds to the standards prescribed. Substantially higher amount of acid insoluble ash formed in the samples indicate good amount of inorganic compounds that are soluble in weak acids. These materials may have therapeutic values.

The qualitative analysis for the presence of iron was confirmed by the appearance of Prussian blue colour appeared in all the three samples.

The colour change observed across 3 different phases of *Abhraka bhasma* on potassium iodide and potassium ferro cyanide treated papers were characteristic and corresponded to the standard description of NPST findings.

X-Ray diffraction method was followed to determine the different crystalline phases present in the samples of *Abhraka bhasma*. In all the three samples maximum absorption was observed at an angle between 26.7 & 26.85 with a D space of 3.32-3.33. The major phase of crystallite in all the samples look to be hexagonal ferric oxide.

In Scanning Electron Microscope, it is observed that particle size in reducing after successive heating i.e. particle size in *Bhasma* after *Amritikarana* alone was lesser than sample AB-M. Particle size was still smaller in sample AB-L.

Energy Dispersive X-ray spectroscopy i.e., EDAX couples with SEM was used for the quantitative estimation of selected elements in the samples. The results indicate that iron content was maximum in the sample AB-L (56.785%) when compared with sample AB-M (29.06%) and sample AB-A (28.596%). It was seen that calcium content was gradually reducing from stage to stage in the samples. There was no apparent change in the values of aluminum, magnesium and silica content among the sample. Phosphorus appeared in traces only in sample after *Lohitikarana*, may be the *Bhavana dravya* (*Manjista kashaya*).

As EDAX is semi quantitative more sensitive method for the estimation of elements given at ppm levels was planned with the help of AAS. The metallic elements iron, calcium, aluminum, magnesium were estimated in all the three samples. All these elements found to be at maximum concentrations in the sample AB-L.

CONCLUSION

Amritikarana is a special procedure required to be done after *Marana* of *Abhraka*. The procedure ensures optimum quality and efficacy of *Abhraka bhasma* undergone *Amritikarana* and *Lohitikarana* processes. From the above observations, the relevance of each procedure mentioned specifically for the incineration of metals and minerals is validated. The particle size, iron content and presence of trace elements are seen in maximum concentration in the sample AB-L. This study throws light on the mechanism behind *Amrtikarana* and *Lohitikarana* of *Abhraka bhasma*. This validates the significance of *Samskara's* mentioned for each material used in *Rasashastra*.

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Cite this article as:
Arya S Varma, Satyanarayana Bhat, Dinesh Nayak. Analytical Evaluation of Abhraka Bhasma Samples after Marana, Amritikarana and Lohitikarana. International Journal of Ayurveda and Pharma Research. 2024;12(1):46-55.
https://doi.org/10.47070/ijapr.v12i1.3046
Source of support: Nil, Conflict of interest: None Declared

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