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Nanoparticles of biotite mica as *Krishna Vajra Abhraka Bhasma*: synthesis and characterizationAsmita Wele^{a,*}, Sourav De^{b,1}, Madhuri Dalvi^a, Nandini Devi^c, Vijaya Pandit^d^a BVDU College of Ayurved, Pune-Satara Road, Pune, 411043, India^b University of Debrecen, Department of Physical Chemistry, Debrecen, H-4002, Hungary^c National Chemical Laboratory, Pashan, Pune, 411008, India^d BVDU Medical College, Pune-Satara Road, Pune, 411043, India

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ABSTRACT

Background: Bio-inorganic nanoparticles or metal nanoparticles are used in medicine for diagnostic and treatment purposes. The nanomedicines from traditional Ayurvedic system are termed as bhasma. Rasashastra, the branch of inorganic medicines of Ayurveda, has documented monographs of metal-mineral bhasmas as potent drugs. However there is lack of scientific analytical data of the end products. **Objectives:** Present study was aimed at finding out the morphological, structural, elemental and chemical composition of the Krishna vajra abhraka bhasma (KVB).

Materials and methods: Bhasma of KVB (Biotite Mica) was prepared in our laboratory using biotite mica sheets befitting selection criteria and carrying out further processes with strict SOPs as per AFI.

Results: The bhasma complied with the confirmatory tests from Rasashastra. The physical and physicochemical tests correlate with the results obtained by instrumental analytical methods. SEM revealed square shaped nanoparticles of mean size of 92.3 nm. EDAX showed presence of Si, Mg, O, Fe, Ca, Na, C, K and Al. XRD revealed the crystalline nature of bhasma with mixture of various individual oxides and spinel shape of the crystal. DLS showed that the nanoparticles are unimodal in nature. FTIR and NMR showed the organic functional groups obtained from cow milk and selected herbs, indicating unique bio-inorganic nature of the KVB.

Conclusion: The therapeutic potential imparted to the formulation could be due to the cow milk and specific herbs utilized during the manufacturing process.

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1. Introduction

Bio-inorganic nanoparticles or metal nanoparticles are used in medicine for diagnostic and treatment purposes [1]. *Rasashastra*, a prominent branch of Ayurveda, has documented about 80 inorganic substances out of which 35 are converted into *Bhasma*. It is the powder form of an element, which is obtained by processing the particular metal-mineral with plants and animal produce using various synthesis methods, leading to formation of compounds

with different physicochemical properties than parent material and thereby imparting therapeutic potential to the final product [2–4]. *Bhasma* is a mixture of micro and nano particles [5,6]. Metal *Bhasmas* i. e. Ayurvedic nanomedicines and also nanoparticles (NPs) of Gold, Silver, Iron, and Zinc are widely studied in past decade with promising results in cancer, diabetes, immunomodulation etc. [7–9]. *Bhasma* of Biotite Mica, an iron rich mineral, is a drug of choice for chronic diseases, works as *Rasayana* and also is an important ingredient of many herbo-mineral formulations. It has attracted scientist's attention over past few years due to its wide applicability. *Abhraka* and its *bhasma* is included in Ayurvedic Pharmacopeia of India (API) and Ayurvedic Formulary of India (AFI) [10,11]. It is used to treat many of the complex diseases viz. *Shwas* (Bronchial Asthma), *Pandu* (Anaemic disorders), *Kshaya* (Pulmonary tuberculosis), *Jeernajvara* (Chronic low grade fever), *Prameha* (Diabetes syndrome), *Udara* (ascitis), *Kleibhya* (impotency),

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Pleehavridhhi (splenomegaly), *Urdhvaswasa* (shallow and rapid respiration), and many more. The term '*Sarvavyadhi*' indicates its application in all sorts of diseases and conditions indicating that it probably works by modulating immune system [12]. Three varieties of *Abhraka bhasma* available in the market are *dashaputi*, *shataputi*, and *sahasraputi* which are used for all the above mentioned conditions.

Plants used in the preparation of *Abhraka bhasma* vary according to the indication for which the *bhasma* is to be used is the general principle of Ayurvedic drug development. In order to prepare the *bhasma* for use in respiratory diseases like bronchial asthma, we chose the preparation method number 9 from the text *Rasatarangini* [13]. The plants advised in the selected method; aerial roots of *Vata* (*Ficus benghalensis*, L.), whole plant of *Kantakari* (*Solanum xanthocarpum*, S. and W.), leaves of *Vasa* (*Adhatoda vasica*, L.), dry pericarp of *Bibhitaka* (*Terminalia bellirica*, Gaertn.) and leaves of *Erand* (*Ricinus communis*, L.) are reported to have action in respiratory system as indicated in Table 1. Using these herbs Krishna vajra *abhraka bhasma* (KVB) was prepared in our laboratory adhering to the traditional *bhasma* making technology (Figs. 1 and 2) [13]. We systematically examined morphological, structural, elemental and chemical composition of the KVB and the results are reported in this paper.

2. Materials and methods

2.1. Manufacture of the Bhasma

2.1.1. Sampling and identification of Krishna vajra *abhraka*

In 2004 when this study was initiated, pharmacopoeia standards for raw KVB did not exist. It got published in 2008. Traditionally many varieties were used by pharmacies. To ascertain the quality KVB; 15 samples sold as biotite were collected from five regions of India viz. Nellore, Gundur- Andhra Pradesh, Mumbai-Maharashtra, Jaipur and Bhilwada - Rajasthan, Giridih- Jharkhand. These samples were analysed using Rasashastra parameters and 4 samples were discarded. Task was to identify KVB from remaining 11 samples which were coded. These were examined by a panel of 12 expert Ayurvedic manufacturers. The two raw material samples which complied with Rasashastra parameters, tested and agreed upon as best by ayurvedic group were authenticated by geologists as Biotite variety of Mica upon characterization using sophisticated

analytical techniques like ICP-MS, ICP-AES, XRD etc. Thus KVB identified as 'Biotite mica from pegmatite rock', of formula $[K(Mg,Fe)_3 (AlSi_3O_{10})(OH_2)]$ was used for the present study (Fig. 2a).

2.1.2. Procurement and standardization of biotite and other processing herbal drugs

The starting material biotite mica was procured from Kalyan Rama mica mines in Kalichedu Village, Nellore Andhra Pradesh. The *Rasashastra* tests and physicochemical analyses were performed on the material which complied with prior data. Voucher specimen was deposited with the central laboratory of Central Council for Research in Ayurvedic Sciences (CCRAS), New Delhi.

Similarly, herbal drugs used for processing were handpicked following Ayurvedic guidelines for type, part, maturity of specimens and then dried in pharmacy in shade. The same samples were authenticated and deposited as voucher specimens at Agharkar Research Institute, Govt. of India, Pune. Each sample was tested as per methods in Ayurvedic pharmacopoeia and Herbal pharmacopoeia including HPTLC profile, for standardization (See Table 1 for plants used.).

Fresh cow milk (quality ascertained by standard physicochemical analysis like pH, specific gravity, total solids, fat, protein etc.) was procured from the dairy on the day of *shodhan* process. Husked brown rice was procured from rice mill and distilled water was prepared in our lab using the Labconco Water Pro®RO Stations models 90750-00 and 90750-02. Mature leaves of *R. communis* L. were harvested for each heating cycle, from the Institution's herbal garden.

2.1.3. Processing of selected biotite mica into Bhasma

The biotite books were separated to remove adulterants like soil, small rocks and other associated minerals including other varieties of mica (Fig. 2a). Selected biotite sheets were processed in 3 stages, each stage has multiple steps (Fig. 3).

2.1.3.1. Stage I – Shodhan. Raw biotite sheets were heated on flame till red hot (653 °C) and quenched into fresh raw cow milk and were allowed to cool (Fig. 2b). The process was repeated for 6 more times (making total 7) in fresh cow milk every time [14]. This was *shud-dha* biotite in flex form (Fig. 2c).

Table 1

Plants used for making KVB showing the major phytochemical compounds and activities for pulmonary disorders.

| Sr. no. | Plant name | Part and product used | Phytochemicals | Actions on Respiratory System |
|---------|--|---|--|--|
| 1. | <i>Ficus benghalensis</i> , Linn | Aerial root decoction (aqueous extract) | Eicosane, Hydrocarbons, various acids, flavonoids, amino acids [16]. | 1. Immunomodulatory 2. Antiinflammatory 3. Sustained in vitro antimicrobial activity against <i>Klebsiella pneumoniae</i> , <i>S. aureus</i> and <i>E. coli</i> [17]. |
| 2. | <i>Solanum virginianum</i> Linn. (<i>S. xanthocarpum</i> , Schrad. and Wendel (<i>S. surattense</i> , Burm.) | Whole plant decoction (aqueous extract) | Solasodine R.S. Solamargine, alpha solamargine, Solasonone, sterols, Cycloartenol [18]. Cholesterol, solasonine alkaloid, glycosides [19]. | 1. Clinical trial for antitussive action. 2. Clinical trial of powder, quath for asthma and cough. 3. Clinical trial for respiratory conditions, chronic asthma, bronchitis [19]. |
| 3. | <i>Adhatoda vasica</i> , Nees (<i>A. zeylanica</i> , Medik; <i>Justicia adhatoda</i> , Linn) | Leaf decoction | Vasicine, vasicinone, kaempferol, quercetin [20]. | 1. Vasicine protects lung damage caused by ovalbumin, aluminium hydroxide. 2. Antibacterial [21]. |
| 4. | <i>Terminalia bellirica</i> (Gaertn), Roxb. | Fruit pericarp decoction | Gallic acid, tannic acid and glycosides, [20]. Ellagic acid and chebulagic acid | 1. Isolated lignans show antifungal activities [22]. 2. Antibacterial activity [23,24]. |
| 5. | <i>Ricinus communis</i> , Linn | Fresh leaf | Ricinine, quercetin, 3-O-beta rutinoid (bio marker -rutin), Alkenes, primary alcohols, aldehydes, various acids- stearic, fatty, kaempferol, [25]. | 1. Leaf extract cytotoxic activity on human tumor cell line, apoptosis effect on human melanoma cells 2. Methanol:water extract in vitro antioxidant activity against DPPH 3. N-Demethylricinine anticholestatic, choleric, and hepatoprotective activities in paracetamol induced hepatic injury in rats [25] |



Fig. 1. The ingredients used to convert Biotite into KVB. **a.** Cows milk; **b.** Rice grains with husk; **c.** Vatajata (*Ficus benghalensis*); **d.** *Solanum xanthocarpum*; **e.** *Vasa patra* (*Justicia adhatoda*); **f.** *Bibhitak* (*Terminalia bellirica*); **g.** *Eranda Patra* (*Ricinus*).

2.1.3.2. Stage II – Dhanyabhraka (size reduction). *Shuddha Abhraka* flex mixed with husked rice grains (paddy husk) were filled in gunny bags and the mouth of the bag was tied firmly. Bag was immersed in distilled water for 24 h. Thereafter, the closed bag was macerated till all the fine particles of KVB were filtered through the pores of the bag. This was *Dhanyabhraka* (Fig. 2d) [14].

2.1.3.3. Stage III – Bhasmikaran. It includes *bhavana* (trituration with liquid) followed by *maran* (incineration). *Dhanyabhraka* was triturated with decoction of the herb till a thick paste was formed (Fig. 2e). Flat round uniform tablets (pellets) of size 4×1 cm, weight average 35 gm each, were made and dried in shade (Fig. 2f). A single layer of 10 dried pellets was wrapped in *R. communis* leaves and placed in an earthen *sharavsamputa* (Fig. 2g). The heating was done in the cubical pit of 2 ft using 100 cow-dung cakes each time, which is known as *Gajaputa* (Fig. 2h). The temperature was monitored and recorded by using Ni–Cd thermocouple pyrometer attached with digital data logger (model DAS -001) having pro-real 1.0 pro-hist 1.0 software. Recorded data was transferred to computer and accordingly graphs were plotted. The inside temperature of the central representative *sharavsamputa* was also recorded. Each cycle from setting the *puta* to taking out of *sharavas* was of 24 h, for practical purpose.

The cycle of trituration followed by incineration was repeated for 10 times for each herbal drug in the following sequence: aerial roots of *Vata* (*F. benghalensis*, L.), whole plant of *Kantakari* (*S. xanthocarpum*, S. and W), leaves of *Vasa* (*Adhatoda vasica*, L.), and dry pericarp of *Bibhitaka* (*T. bellirica*, Gaertn.). Each time the pellets were tied in leaves of *Eranda* (*R. communis*, L.) before incineration [14].

For each *bhavana*, 4 L of decoction was used, which was prepared by boiling 2 kg of the coarse powder of the herb with 16 L of water. The boiling was discontinued when 4 L decoction was remained [15]. Each *bhavana* was of average 9 h when the mixture was thick to get converted into pellets. In this way total 40 cycles were completed to prepare KVB.

Following standard operating procedures, KVB was prepared three times and statistical mean values of the measurements like amount of decoctions used, time for one cycle of trituration, time and temperature of one *puta* were calculated.

2.2. Tests of KVB

2.2.1. Rasashastra

The *bhasma* was analysed on organoleptic parameters of sound (absence of grittiness), touch (soft), appearance (colour brick red, opaque/nonlustrous), taste (*nirasatva*-no taste), odour (non-specific), *Laghutva* and *Sukshmatva* (*bhasma* is very light and fine in appearance) as preliminary tests. It was further tested in laboratory by methods adopted in *Rasashastra* [26]. The tests were; *Rekhaupmatva* (easy lodging of *bhasma* particles in the furrows), *Varitaratva* (sprinkled *bhasma* floats on water), *Uttama/Unam* (floating *bhasma* carries a grain of rice or wheat), *Niruttha* (KVB when heated with silver at high temperature in a furnace should remain unchanged), *Apunarbhava* [KVB should not regain the original state of metal after heating with *mitrapanchaka* (a specific catalyst - mixture of jaggery, fine powder of *Abrus precatorious*, L., honey, ghee and borax) at high temperature], and the most significant test for *Abhraka bhasma* i.e. *Nishchandratva* (lustreless under bright light) [27].



Fig. 2. Photographic presentation of manufacturing. **a.** Separation of Biotite Mica from Schists; **b.** Quenching in milk; **c.** Shodita Abhraka; **d.** Dhanyabhraka; **e.** Trituration of Dhanyabhraka **f.** Pellet formation; **g.** Wrapping of dried pellets in Erandapatra; **h.** Arrangement of Puta; **i.** Ignition of Puta.

2.2.2. Simple laboratory tests

Volumetric analysis using standard laboratory methods such as limit tests for heavy metals; and pH in aqueous solution using PHOENIX RSM-10B pH-meter were conducted on the bhasma [20].

2.2.3. Physicochemical analytical tests

To complement the *Rasashastra* tests and to know the morphology and elemental composition, particle size, and nature; the samples of *shuddha Abhraka*, *dhanyabhraka* and KVB were analysed using SEM–EDAX and XRD. The DLS, FTIR and NMR were performed only on KVB.

Scanning Electron Microscope model PHILIPS XL-30 SERIES equipped with Energy dispersive X-rays analysis was used to study morphology and elements. Sample was sprinkled on carbon tapes, which were placed in vacuum sputter coater and exposed to shower of Gold and Palladium ions for 6 min and then mounted under microscope to take images and readings.

X-ray diffraction patterns were collected on a PANalytical X'Pert Pro dual goniometer diffractometer equipped with X'celerator detector. The wavelength was 1.5416 Å (Cu K α) and step scan of 0.008° with scan time 50.15 s at each step were used. The samples were powdered thoroughly and packed into flat sample holders and the measurements carried out in a Bragg-Brentano reflection geometry. The phase analysis was carried out using the PDF database incorporated in X'pert Pro program suite.

To perform Dynamic Light Scattering, DLS instrument model BI-9000AT of Brookhaven Instruments Corporation was used. 50 mg of all the samples were measured and mixed with 5 mL of water to get a final concentration of 10 mg/mL. The samples were vigorously shaken to get a uniform suspension. The suspensions were then filtered through 0.22 μ filter to get a clear solution. These solutions were ultrasonicated for 6 min on an ultrasonicator and the DLS analysis was immediately performed.

The FTIR spectrum of the bhasma was measured using Perkin Elmer Paragon 1000 PC FT-IR Spectrometer and studied in the region of 4000 to 400 cm^{-1} .

To perform NMR, bhasma was first mixed with deuterated-water (D_2O) and then shaken vigorously to make a homogeneous uniform solution. Then the solution was filtered, and the filtrate was taken in NMR tube for the measurements.

3. Results

3.1. Process of shodhan and maran

During *shodhan*, due to quenching in cow-milk the sheets gradually turned into flex. After 7th quenching, more or less similar size flex were obtained. Traditional *gajaputa* process reveals uniform heating and cooling phases as shown in the Fig. 4 in different ambient temperatures. Average maximum temperature attained was 910°C. Active heating phase was of 390 min followed by annealing phase. The maximum temperature attained was 910°C in 120 min. The time taken from 600°C to 40°C was 180 min. The average time for which the *abhraka* was being heated in the range of 600°C to 900°C was 90 min.

The yield of KVB in the selected process of trituration with 4 herbs and 40 puta was 86.43 percent.

3.2. Rasashastra tests

After 40 puta the bhasma complied with the aforementioned *Rasashastra* tests indicating formation of expected quality finished product (Fig. 5). It withstood all and specifically the *nishchandravta* test satisfactorily though there is no objective parameter to measure the luster.

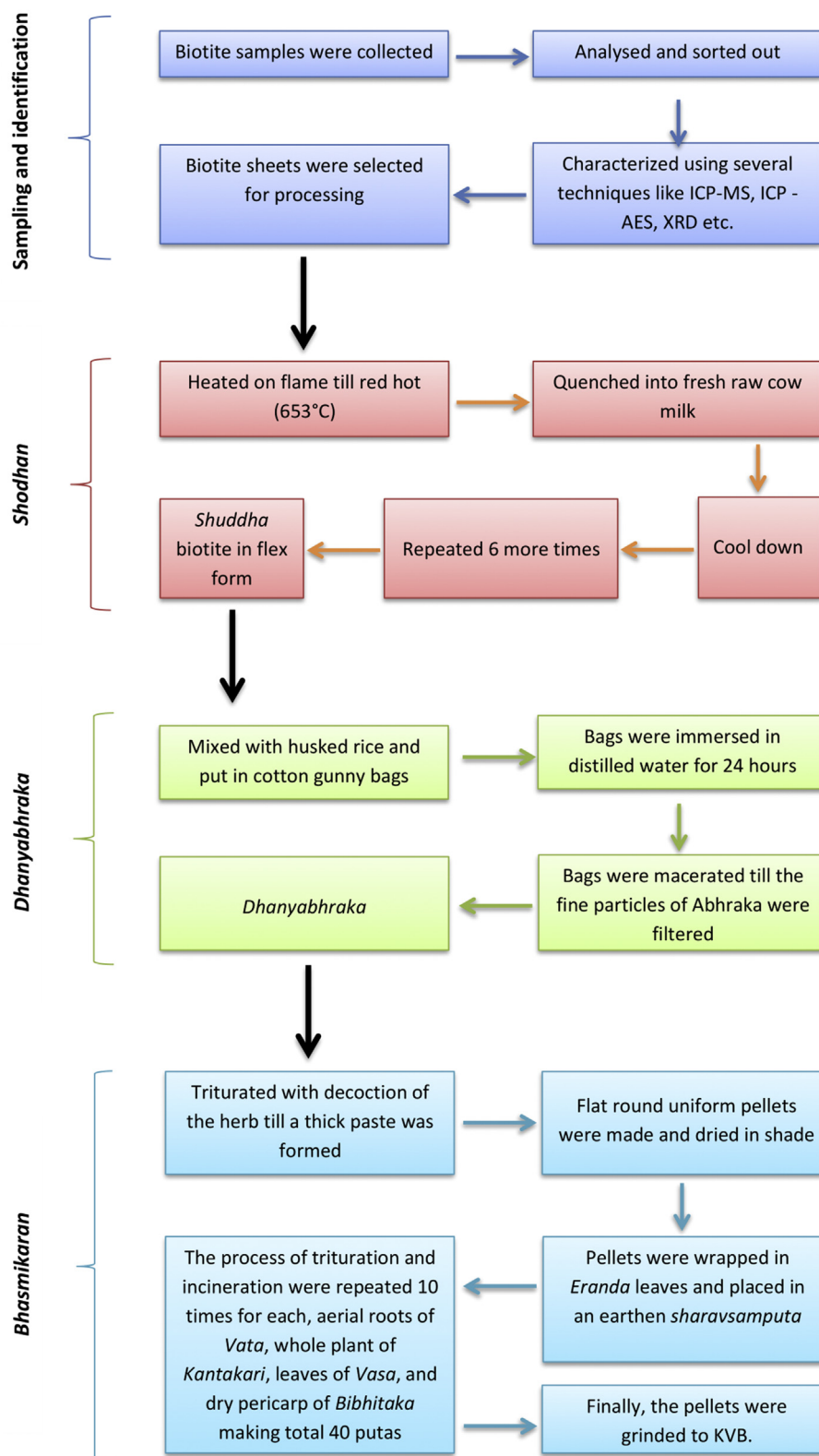


Fig. 3. Flowchart of various processes of preparation of KVB.

3.3. Simple chemical analysis

Limit tests confirmed the presence of Si (36.66%), Mg (3.0%), Al (13.57%) and Fe (7.7%). The pH of the bhasma was 6.5 indicative of weak acidic nature.

3.4. Tests for characterization

3.4.1. SEM

Size and morphology of unprocessed KV, shuddha KV, Dhanyabhraka and KVB obtained using SEM is shown in Fig. 6 (a, b, c, d).

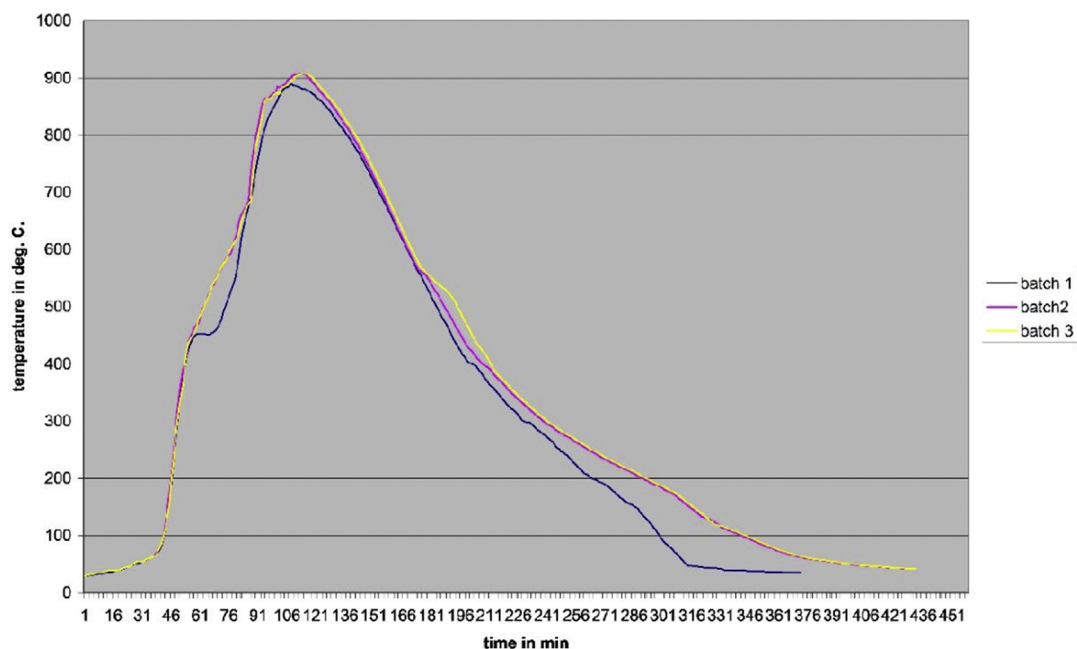


Fig. 4. Temperature graph for Gajaputa.

Magnifications used for samples are 2000X for raw, 5000X for *shuddha* and *dhanyabhraka* and 10000 X for *bhasma*. The size of agglomerated nanoparticles was 2 μm . Within the examined area the smallest size spotted under the field was 87.1 nm (Fig. 6d). The particle size gradually reduces from 174 nm to 87.1 nm from *shuddha* to *bhasma* stage.

3.4.2. EDAX

The elemental composition obtained from EDAX is presented in Table 2. The major elements present in KVB at all the stages are Si, Fe, Ca, and minor are Mg, Na, K and Ti. Phosphorous is present in the *bhasma* which is absent in all other stages.

3.4.3. XRD

The XRD graph of raw *Krishna vajra abhraka* (Biotite mica) matches with standard Biotite mica (Fig. 7a). To study the effect of temperature without any other treatments, the raw mica was

ground well and heated in a laboratory furnace at 500 and 900 °C. However, no change in the layered structure of mica was observed (Fig. 7b). Powder XRD of the 6 samples in the sequence of *shuddha*, *dhanyabhraka*, 10th, 20th, 30th cycles and final KVB gives information of change in accordance with the phases of the process (Fig. 7c). Intensity of the peaks becomes sharper in the successive phases from *shodhan* to each stage of *maran*. *Shuddha Abhrak* (Fig. 7c-1a) shows layered structure which it starts losing after 10th puta (Figs. 6c-1c), completely loses it at the 40th puta (Fig. 7c-1f) and becomes dense phase mixture of oxides based on Al, Si, Mg and Fe. The individual oxides of possible formulae could be (a) $\text{Mg}(\text{AlFe})_2\text{O}_4$ with spinel structure and (b) $\text{KAlSiO}_4/\text{Si}_2\text{O}_6$.

3.4.4. DLS

The DLS graphs reveal cumulative unimodal distribution of the particles in all 3 batches (Fig. 8). At number 61.14, the percent ratio



Fig. 5. Final product (a) and Ayurvedic tests (b).

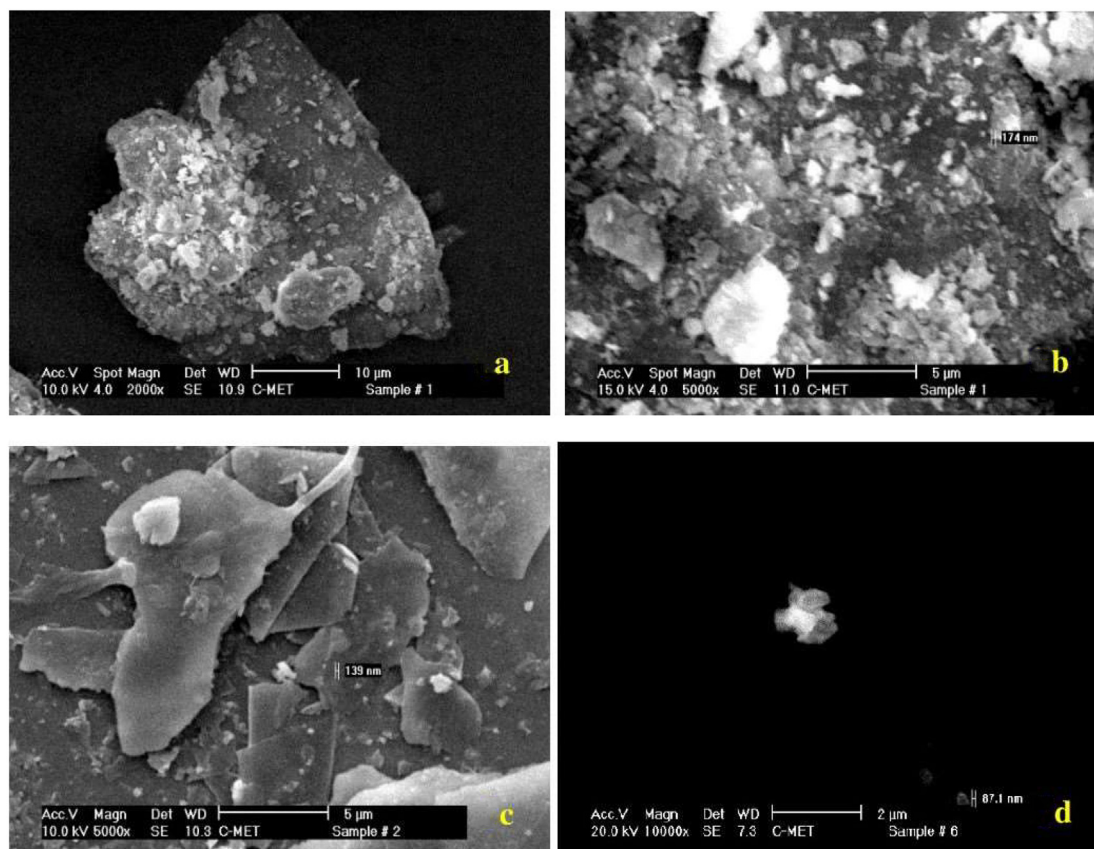


Fig. 6. SEM analysis of KVB. a. Unprocessed powder of KV; b. Shuddha KV; c. Dhanyabhakra; d. KVB particle.

is zero and at 120.65 it is 98; which means 98% of the particles fall in between this range with a mean particle size 92.9 nm.

3.4.5. FTIR

The band (Fig. 9a) in the region of 3700–2700 cm^{-1} represents the hydroxyl group (O–H). The sharp absorption band in the region 3700–3600 cm^{-1} tells about the presence of free alcohol group whereas the broad absorption band in the range 3150–3000 cm^{-1} proves the existence of intra-molecular bonded alcohol groups. Sharp absorption band in the region 2350–2340 cm^{-1} represents stretching bond frequency of O=C=O indicating the presence of carbon dioxide and carbonate group. Strong absorption at the region of 1550–1500 cm^{-1} indicates the presence of nitro compound (N–O bond stretching). Strong and broad absorption in the region 1045–980 cm^{-1} shows the presence of acid anhydride and mono-substituted alkene group. The other absorption bands are in the region 900–400 cm^{-1} , which prove the existence of some benzene derivative (713–694 cm^{-1}), Silicate group (750–470 cm^{-1}), Si–O–Si bond (466–456 cm^{-1}), C–Br bond (685–500 cm^{-1}).

3.4.6. NMR

The ^1H NMR of the bhasma was measured in 360 MHz instrument and the peaks (Fig. 9b) were found at $\delta = 4.04, 3.63, 3.57, 3.55, 3.28, 2.16, 2.13, 1.25, 1.10, 1.02$ ppm. Compared with the standard proton NMR chemical shifts, these findings reveal a possibility of the presence of following protons: alcohol, amine, aniline, alkyl, allylic, α to carbonyl, benzylic, α to nitrogen, α to halogen, α to nitrogen. ^{13}C $\{^1\text{H}\}$ NMR of the solution was also checked in 90 MHz instrument. There were no presence of NMR active carbon signals in the range of $\delta = -20$ to 250 ppm. This may be because of very less water solubility of bhasma in water.

4. Discussion

Mica mineral (*Abhrak*) has 16 varieties, out of which only one, *krishna* (black), *vajra* (does not split or swell upon heating), *abhraka* (sheets/layers) is used for making bhasma [14]. Black coloured biotite mica is rich in essential macro and micronutrients like Mg, K, Fe, Si, Zn, Cu, Se, Cr etc amongst the others [28]. Simple heat test of *Rasashastra* to exclude the sedimentary clays and chlorite from good quality biotite proves valuable to identify 'vajra' characteristic. It separates the natural mica having high layer charge and very limited capacity to exchange ions; from clay minerals like vermiculites bearing hydrated cations in a ratio of 2:1, that expand upon heating [29,30]. Due to the layered structure of mica the interlayer cations such as K^+ , Na^+ , Ca^{2+} can be exchanged only under specific conditions and into specific solutions which assures the desired development of final bhasma product. This explanation also unveiled the logic of the name and use of *krishnavajrabraka* for medicinal purpose.

A detailed review of the texts like *Rasaratnasamucchaya*, *Ayurveda Prakash*, *Rasatarangini* and compendia like *Bharat Bhaishajya Ratnakar* unfold that *Abhraka bhasma* can be prepared using about 68 methods, has been attributed with 17 pharmacological actions, and is indicated in 32 diseases. It may either prove to be a repository of different molecules originating from one starting material *Abhraka* or these variations may be insignificant. Comparison of characterization data of the bhasma prepared using different methods may throw light in this direction. Generally three types of *Abhraka bhasmas* (10, 100 and 1000 puta) are available in the market and 25 herbo-mineral formulations containing *Abhrak Bhasma* are sold [11,31].

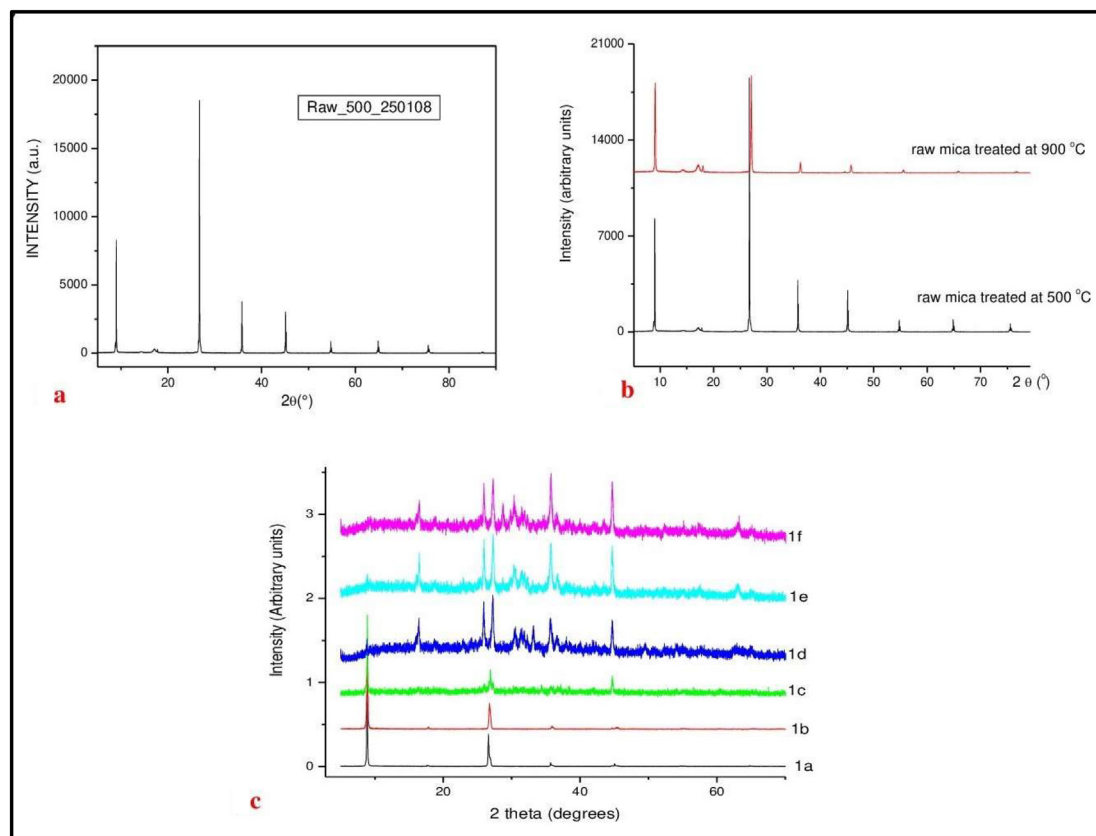


Fig. 7. XRD of Raw KV, and KVB showing phase changes. **a.** Raw KVB biotite mica; **b.** Raw mica treated at two different temperature; **c.** Sequential phase change from shuddha to KVB.

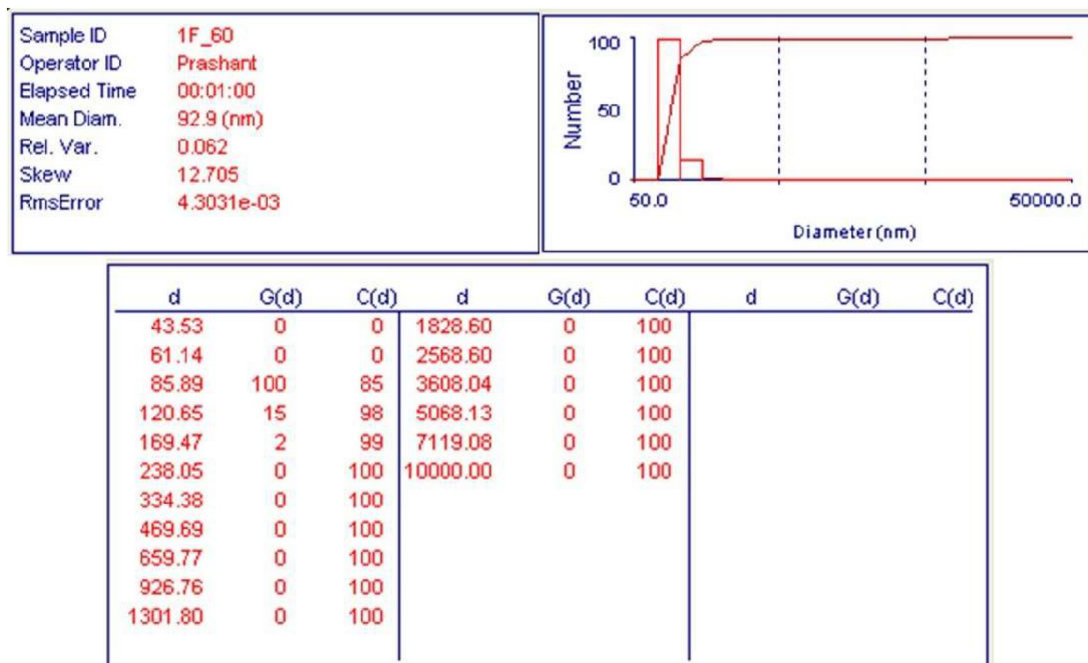


Fig. 8. DLS analysis for particle distribution.

Table 3 shows summary of the therapeutic activities of *Abhraka bhasma* compiled from representative texts of *Rasashastra*.

Though *Abhraka* is iron magnesium predominant drug, it is presumed to be toxic because it is a *bhasma*. '*Bhasma*' is a generic

term for the first level dosage form of mineral-metal category just like powder is the first level dosage form of any dry herb. Not all *bhasmas* are toxic. Another reason of its assumed toxicity could be presence of the radioactive elements Barium, Radium, Thorium,

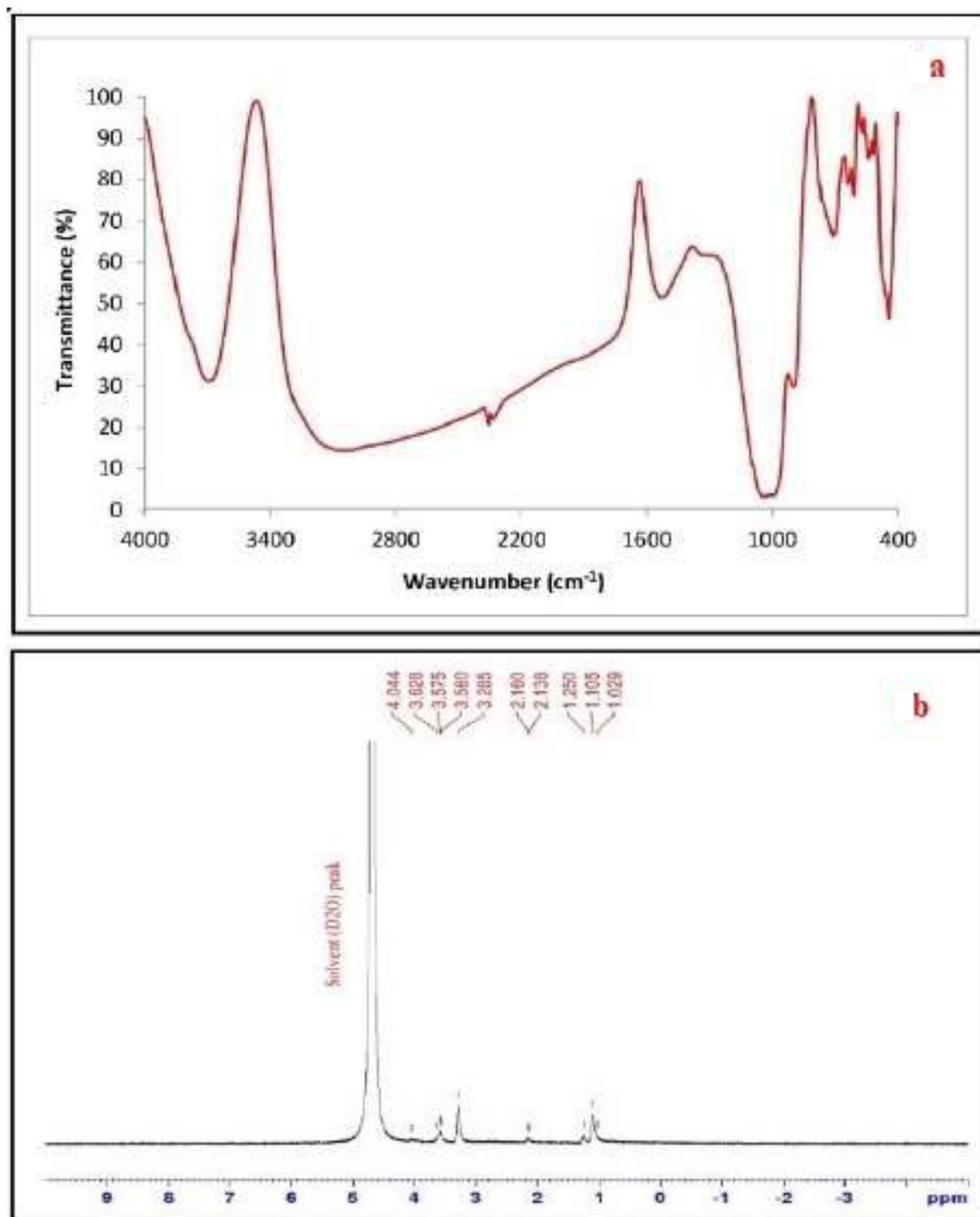


Fig. 9. FTIR and NMR spectra of KVB. a. FTIR spectrum of KVB b. ^1H -NMR of KVB in D_2O .

Table 2

Elemental composition (atomic weight %) at various stages of manufacture of KVB.

| | Si | Al | Fe | Mg | K | Na | Ca | O | C | Ti | P |
|---------|-------|------|-------|------|------|------|------|-------|-------|------|------|
| Raw | 15.19 | 8.59 | 11.76 | 5.49 | 6.25 | 0.50 | 0.22 | 37.78 | 13.91 | 0.79 | 0 |
| Shuddha | 16.10 | 9.14 | 11.74 | 5.67 | 7.17 | 0 | 0 | 35.69 | 11.49 | 0 | 0 |
| Dhanya | 13.29 | 7.04 | 13.17 | 3.96 | 5.90 | 0 | 1.24 | 36.88 | 17.17 | 1.34 | 0 |
| Bhasma | 14.18 | 7.58 | 12.64 | 5.03 | 8.36 | 1.39 | 3.64 | 35.69 | 9.57 | 1.16 | 0.76 |

Uranium, Iodine and Thallium in unprocessed or raw biotite in ppb/ppt amounts. As per the guidelines of *Rasashastra*, only improperly prepared and administered *bhasmas* are not safe and effective [32]. Characterization by *Rasashastra* methods supplemented by

physicochemical methods reveal the nature of the finished product and render preliminary judgement of its safety and efficacy. It was hypothesized that the KVB prepared for the study may differ from known standard *dashaputi*, *shataputi* and *sahasraputi abhraka*

bhasma owing to the change of processing ingredients and number (40) of heating cycles. Except these 2 variations the particular manufacturing process mentioned in Ayurvedic Formulary of India (AFI) was followed for making the KVB.

4.1. Process

4.1.1. Stage I – Shodhan

Shodhan term is translated as detoxification or purification. It is the umbrella term for a group of processes which eliminate toxic material or impurities from the drug and make the product nontoxic for human use. Since this paper discusses chemical changes to use the term detoxification is appropriate, because biotite mica did not get converted into a pure chemical moiety.

During heating at more than 900°C oxidation and dehydration of biotite happens as reported by Rauxhet et al. wherein dissociation of Abhraka was brought about in vacuum and oxygen [33]. It may not be compared with our study because here the biotite was heated in open air, till it changes to red hot with mean temperature of 653°C and was immediately quenched into cow milk which brings about distortion of platy structure with limited oxidation. Mica sheets become brittle with each successive cycle of heating and quenching into milk due to expansion of the interlayer spaces with subsequent loss of the inherent elasticity of mica sheets and decrease in tension and increase in compression force [27]. Previously, Malmstrom and Banwart have shown that the stoichiometry and rate of dissolution of biotite vary greatly with pH [34]. Cow milk being an acidic media, ions like K⁺ are replaced by Ca⁺⁺, Mg⁺ etc. from milk on the cracked surfaces of the layers. Also there is a

possibility of adsorption of metal binding proteins, soluble enzymes and immunoglobulins from milk within the mica layers [35]. The weight gain of 24% (5 kg became 6.18 kg) after *shodhan* can be attributed to acquired moisture and adsorbed solids from cow milk. During further process of heating the moisture is lost.

It has been repeatedly said that during *shodhan* the toxicity of *abhraka* is reduced. In our studies the toxic elements of Abhraka which can be labelled as impurities could not be asserted. Similarly, authors did not come across any study that has reported or identified particular elements from KV (Krishna Vajra Abhraka) getting bound to the milk (or in other cases to cow urine and *Triphala* decoction) which can be termed as impurities. Various studies reporting the displacement of K initially from surface of biotite plates and later from flex, clarify that K is the primary exchangeable ion at low temperature and acidic to neutral pH followed by Fe, Mg, and Si [36,37]. All these are also known essential nutrient elements except Al which is labelled as a toxic element which gets depleted from mica though not completely.

We may say that heating followed by quenching initiates decomposition of biotite into irreversible form (*apunarbhavavta*). The Ayurvedic theory that any inorganic material proves toxic to humans and hence it should be processed to make it *shuddha*, partially organic and thereby bio assimilable sounds befitting [38].

4.1.2. Stage II dhanyabhraka

This peculiar process practised for *Abhraka* causes mechanical reduction of particle size of *shuddha* flex by the simple biological grinder paddy husk. Paddy husk is rich in silica and also has oxides of Ca, Mg, K, Hematite and Titanium; which may get impregnated

Table 3

Ayurvedic Pharmacotherapeutics of *Abhraka bhasma* from representative texts of *Rasashastra*.

| Sr. Rasa No | Virya | Guna | Pharmacological activity | Action on Doshas | Action on Diseases | Reference |
|-------------------------|----------------|----------------------|---|--|---|---|
| 1. Shadrasatmak Shita | Laghu, Snigdha | | Shukravardhak (fertility promoting) Ayushyavardhan (longevity enhancing) Balya (strengthening) Varnya (complexion enhancing) Rasayana (immunomodulatory) Vrishya (aphrodisiac) Ruchikara (appetizer) Dipan (digestive) Grahi (antidiarrheals) | Tridoshnashak (mitigation of deranged tridoshas) | Cures all diseases | Anandkanda ((13th century) [47] Rasaratnasamucchay (13th century) [12] |
| 2. Kashay Madhur | Shita | Snigdha | Dhatuwardhan (tissue building) Shukravardhan Apatyasantankarma (fertility promotion of progeny) Yogvahi (enhancing the activity of the associated substance) | Tridoshnashak | Cures Prameha (Diabetes) Kushtha (skin diseases) Kshaya (Koch's disease) Pandu (anaemia), Grahani (IBD/IBS), Shula (Pain in GIT), Urdhva Shwas (apnea, dyspnoea) Agnimandya (loss of appetite) Udarroga (Ascites) | Ayurved Prakash (18th century) [48] |
| 3. Madhura | Shita | Snigdha | Keshya (hair qualityenhancing), Varnya Netrya (enhances visual acuity), Balya Ruchikara Vrishya Stanyajanan (galactagogue) | Not mentioned. | Cures Shwas-Kaas Kshaya (Bronchial Asthma, COPD, Koch's disease) | Rasatarangini (1st half of 20th century) [13] |
| 4. Presumed as previous | Shita | Presumed as previous | Excellent Rasayana, balya, netrya, Vrishya, Raktaprasaaka (qualitative enhancement of blood tissue), Stanyajanan | Pittaghna (mitigation of deranged pitta) | Cures Pandu, Kamala (Jaundice), Halimak (obstructive jaundice) Agnimandya, Netravikar (diseases of eyesight) | Bharatiya Rasashastra-Dwiwedi (1978) [49] |
| 5. Presumed as previous | Shita | Snigdha | Shukravardhak Ayushyavardhan Balya Varnya Rasayana Vrishya Ruchikara Dipan, Grahi | Tridoshnashak | Cures kshaya & all other diseases when administered with suitable Anupana | Ayurvediya Rasashastra-S. Mishra (1981) [50] |

Vipaka ~ post digestive or metabolic effect of *Abhraka* is not mentioned in the texts. According to the guideline generally *madhura* rasa, *shita* virya are associated with *madhur* vipaka.

upon mica during the 24 h immersion process in water and subsequent vigorous pitting and rolling on the gunny bag [39].

4.1.3. Stage III: bhavana and maran

Bhavana is levigation by the juices or decoctions of the herbs and usually drying in open air of the resultant mixture [40]. The levigation of *dhanyabhraka* with herbal decoctions fractures the mica particles and further facilitates exchange of cations, anions and phytochemicals in the presence of water and heat of constant friction. However, it is difficult to decipher the method of exchange between the pellets wrapped by leaves of *Ricinus communis*, L. The only possibility seems that the leaves turn into ashes during heat cycle and in next levigation the ash becomes part of the pellets. Each of the 4 herbs is levigated for 10 times as against *R. communis*, which gets added into the mixture for 40 times. In the *dashputi abhrak bhasma* also, juice of *R. communis* L. is the only herbal ingredient [11] used for levigation. Known and prominent phytochemical groups from the plants and their pharmacological actions support the proposed activity profile of KVB (Table 1).

Maran process in *gajaputa* exposes the impregnated *Abhraka* in a closed *sharavamsputa* in limited amount of gases. The known oxidation temperature of biotite mica 1000°C. In maran process the recombination of *Abhraka* structure takes place in between 600°C to 900°C which is lower than the required oxidation temperature. The size and temperature range achieved in *gajaputa* during our experiment corresponds with that of standard [41].

The temperature graphs show a similar pattern under bivariate function confirming to standard operating procedure yet the apparent minor variation in temperature in each puta for the 3 batches as seen in Fig. 4 is due to manual error and seasonal variation in ambient temperature. The difference in mean particle size between the 3 batches points towards this manual method of heating which is the only chance of error. Trituration was mechanical and standardized. Though it may not have significant impact on the quality of *Abhraka bhasma*; it confirms that the quantum of heat is instrumental in reducing particle size in desired order [41]. Pre-calibrated electric muffle furnace with earthen sharavas can be a better option for process control for large scale production as well [42]. The average energy (calorific value) of 1 cow dung cake of average weight 450 gm, used in this study, was 10.5 kJ/mol and total 1800 kg cow dung cakes were used for 40 putas. The amount of heat required to convert 5 kg of KV into its bhasma was 18,900 kJ/mol.

4.2. Rasashastra and simple chemical tests

4.2.1. Rasashastra tests

Objective of bhasma making is to bring about complete change in its physical and chemical properties yet retain and add into its biological properties. Compliance of KVB with all Rasashastra tests confirmed that the bhasma is of desired quality. Results of most relevant tests are explained below.

4.2.1.1. Nishchandra test. Any metal invariably bears a shine, which is its physical property. Loss of luster upon testing under bright sunlight using naked eye and also magnifying glass, confirms the change in this property and is indication of completion of *puta* process.

4.2.1.2. Rekhapurnatva test. Since the rate of absorption of drug in the body is directly proportional to the particle size; the finer the particle quicker the absorption (Fig. 5) [43]. This characteristic is quantified by particle size analysis using SEM and DLS (see Figs. 6 and 8).

4.2.1.3. Waritara test. This test shows that the density of the bhasma has reduced than that of water as a result of particle size reduction which also means that the mass of each particle is reduced, and volume increased. Relative density of raw and intermediate particle of *Abhraka* is more than water so they settle at the bottom; but that of bhasma is less, hence bhasma floats. It strengthens the *rasashastra* theory of changing the *guru* substance to *laghu* by trituration (*mardanam*) and calcination (*puta*). In KVB, any particles settling down in water were not seen. The thin film of bhasma particles did not allow the grain put on top to drown, which indicated uniformity of the low density particle size (Fig. 5). It corresponds with the DLS results showing that 98 percent of the particles bear mean size of 92.3 nm.

4.2.1.4. Niruttha test. The thin silver sheet of 600 nm containing a small amount of KVB, was heated to red hot using heating gun, for 5 min [27]. After annealing non-adherence of bhasma particles to the sheet and also no weight gain of silver foil indicated no alloy formation.

4.2.1.5. Apunarbhava test. The bhasma was triturated with the mixture of *mitrapanchaka* (see note) in equal amount for 5 min. It was heated in muffle furnace till 900°C [12,44]. After annealing the mixture was tested for appearance of luster (*chandrika*), which indicates metal's physical character. The sample was tested using magnet because *Abhraka bhasma* might yield metallic iron particles. These two tests indicate irreversible transformation of original material.

4.2.2. Chemical tests

4.2.2.1. The pH. The pH gives an idea of absorption of bhasma. The near neutral acidic pH 6.5 is suggestive of stomach as the site of absorption, which needs to be confirmed by further study.

Comparison of volumetric tests of raw *abhraka* with bhasma have shown that the Si and Mg percentage increases (34.22–36.66%, 1.83 to 3.00% respectively), while Al and Fe percentage decreases (21.5–13.57% and 12.4 to 7.81% respectively). The chemical methods show presence of marker compounds like Si, Fe, Mg qualitatively and quantitatively both. The combination of ayurvedic and simple physico-chemical tests is very cost effective and serve useful for a pharmacy which adheres to specific manufacture method, SOP and quality control measures; as against the preconceived notion of subjectivity and non-reliability towards the tests.

Having said this, it must be noted that though the tests together with physical methods like appearance, colour, odour (*sartha gunatah pariksha*) confirmed the endpoint of bhasma preparation, they cannot differentiate between different samples of *Abhraka bhasma* or give any clue about ingredients used during process because all the *bhasmas* of *Abhraka* (including those available in the market) show similar appearance and comply with the above mentioned tests. Therefore it is necessary to use instrumental methods.

4.3. Tests for characterization

The tests used to map the structural change during conversion of Biotite to Biotite Bhasma in our study are discussed below and are compared with the findings of similar tests from other studies.

4.3.1. SEM

From the raw to bhasma stages the samples revealed change from micron size particles having multilayered structure to agglomerates of nanoparticles. Unlike other studies, our

dhanyabhraka sample made using distilled water does not show fibrous strands which seem to appear owing to the treatment with rice vinegar. The KVB particles are square in shape concurrent with the findings of Kantak et al. where cow milk, paddy husk and leaves *R. communis* are common ingredients [3]. In the same study the other sample of *Abhraka* bhasma made using cow urine, paddy husk, *cyperus rotundus* and *calotropis procera* produce mixed shaped viz. spherical and rod-shaped particles. Similarly, the *sahasra-puti* (1000) *abhraka bhasma* of Shree Dhootpapeshwara Ltd studied by Bhatia et al. shows heterogenous agglomerates of particle size 28–88 nm, irregular grains with spherical to oblong shape [27]. The differences can be attributed to 960 times more puta cycles with about 60 plants used for trituration. Use of a different make of testing instrument which uses different method of sample fixation could be another reason.

4.3.2. EDAX

The elemental concentration of our samples at the raw, *shuddha*, *dhanyabhraka* and bhasma stages as shown in Table 2 when compared with the findings from other studies reveals that amounts of Si, Mg and O remain more or less similar; tendency of increase in amounts of Fe, Ca, Na and decrease in amounts of C, K and Al is also comparable. Presence of carbon and oxygen highlights the organic nature of KVB and thereby possibility of its claimed non toxicity in therapeutic dose. We may like to agree with Kantak et al. that reduction in amount of Al is a marker of detoxification. The difference in the quality of raw material biotite may be responsible for the differences in the amounts of major and minor elements in final product when compared with other reported studies, where biotite schists (unacceptable variety of mica) are used instead of sheets.

4.3.3. XRD

Our results of XRD have clearly shown the phase change in six successive samples from *shuddha krishna vajra abhraka* to KVB, which is a result of specific processing ingredients and the process. The sample of same biotite heated between 500°C to 900°C reveals that the heat treatment alone is not sufficient to form the oxide phases as seen in the bhasma samples (Fig. 7). The intensity of peaks sharpens in successive stages and the size of a crystallite decreases with each processing step of *shodhan*, *dhanyabhraka* and *maran* which ultimately forms nanosized bhasma particles of spinel shape (two squares attached to each other in a particular fashion), which is concurrent with the findings of Kantak et al. Comparison of the results of our bhasma made in 40 puta, by Kantak et al. (35 puta), by Bhatia et al. (1000 puta SDLpharma), Gupta V. et al. (40 puta) show similarity in nano size of particles and all of them comply with the *Rasashastra* testing criteria; with some differences of the level of phase change [3,27,44]. The differences can be attributed to quality of raw material and personal errors in *Gajaputa* process which can be rectified by using sophisticated controlled heating technology. It also poses the question regarding the accurate number of puta required to achieve desired size, shape and functional groups of the bhasma particle.

4.3.4. DLS

In our study unlike others, 98 percent of the particles show unimodal distribution. The possible hindrance to use it in cell lines to study its mechanism due to agglomerates seen in bimodal distribution may be reduced. Also, it may be able to give accurate results in experimental toxicity studies.

4.3.5. FTIR

Our study shows presence of hydroxyl group, carbonate group and presence of carbon, similar to other studies. However, presence of free alcohol, intramolecular bonded alcohol and benzene derivatives should be attributed to the plants used in our processing of KVB. It has been reported earlier that larger and complex molecules of alcohol are often isolated from volatile oils of plants by steam distillation [45]. In the manufacturing process of KVB in the presence moisture in pellets and intense heat during puta, the alcohol groups from plants may have been attached to bhasma molecule. In our selected method we did not use *kanji* (rice vinegar) which is acidic with pH 2.4 and comprises of acetic acid with small proportion of malic, oxalic, tartaric acids and phenolic groups [46]. This could be the reason for the variation seen in the functional groups in our KVB and other bhasmas.

4.3.6. NMR

It confirms the findings of FTIR and also elaborates the presence of organic groups and protons in tune with the processing ingredients viz. *milk*, *Vata*, *Kantakari*, *Vasa*, *Bibhitaka* and *Eranda* known to have actions in respiratory system. This might explain the targeted effect of the KVB in *shwasa* (~asthma) *kasa* (~cough) and *kshaya* (~pulmonary tuberculosis).

Based on the test results, plotting of total chemical change was attempted. The raw biotite of the formula $[K(Mg,Fe)_3(AlSi_3O_{10})(OH_2)]$ was transformed into a mixture of individual oxides of possible formulae (a) $Mg(AlFe)_2O_4$ (b) $KAlSiO_4/Si_2O_6$ with spinel structure. However, it was not possible in this study to map the complete change because KVB is a compound mineral consisting of many major, minor and trace elements. It can be a separate study.

The selected traditional method seems to be a combination of physical, chemical, thermal and green synthesis method of making nanoparticle of biotite mica, using top down approach, targeted towards respiratory system. Regarding synthesis, we strongly feel that it is possible to replace the tedious manual process of making KVB which is a source of errors causing batch to batch variation. Mechanization of the process using modern technologies by confirming nature of resultant product is possible. The study of KVB characterization is a proof of advanced process of formulation development. The basis of transformation of natural substances into potent medicine is explained as *gunantaradhan* which means alteration in biochemical properties of the substance by specific *sanskar* (process) on the material in the presence of water/liquid and heat, because *gunas* (characteristics) do not have independent existence (they are always with the substance). In case of conversion of KVB, the repetition of heating, quenching into cow milk, followed by trituration with herbal decoctions and subsequent heating in *gajaputa* converts the *parthiva* (inorganic) heavy, lustrous, substance into bio-organic, light, completely lusterless *bhasma* having altered set of *gunas*. *Gunas* are responsible for *karmas* (pharmacological actions) which depend on the nature of the substance. The peculiar process of *bhasma* making of biotite mica changes its nature and subsequently properties and activities.

Actions of *bhasmas* i.e. metals impregnated with organic molecules have dual set of properties, their own and acquired through process. Each combination has a specific structure hence a specific activity may be justified. The particular formula of KVB prepared for pulmonary disorders is a unique combination of Si, Fe, Ca, Mg and other elements attached to specific alkaloids like Eicosane, Solasone, Vasine and many others. Further structural and molecular understanding of the KVB and subsequent experimental

studies may lead to use it in various respiratory diseases like bronchial Asthma, COPD, and others.

5. Conclusion

The procured sample of KVB befitted *Rasashastra* and mineralogical selection criteria. The KVB prepared using the specific processing ingredients and SOP complied with the confirmatory tests of *Rasashastra*. The physical and physicochemical tests correlate with the results obtained by instrumental analytical methods. SEM revealed square shaped particles of mean size of 92.3 nm. EDAX showed presence of Si, Mg, O, Fe, Ca, Na, C, K and Al. XRD revealed the crystalline nature of bhasma with mixture of various individual oxides. DLS showed that the particles are unimodal in nature. FTIR and NMR show the organic functional groups corresponding with milk and selected herbs, indicating bio-inorganic nature of the bhasma targeted towards particular activity.

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Conflict of interest

None.

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