Original Research

A Novel Approach for Quality Control of the *Bhasmas* of *Mukta*, *Muktashukti*, and *Shankha*

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Abstract

Objective

To evaluate and characterize the mukta, muktashukti, and shankhabhasmasin order to help preventing adulteration.

Methods

All the three bhasmas were subjected to pharmaceutical procedures such as *shodhan*, *bhavana*, and *maran*, Weight gain and weight loss were measured. Analytical procedures such as pH, ash value, namburi phased spot test, X-ray diffraction (XRD), scanning electron microscopy, (SEM) and diffuse reflectance spectroscopy (DRS) were carried out. Market samples of mukta were procured and similar procedures were followed and compared with prepared samples of all three bhasma.

Results

SEM revealed the crystalline nature of all bhasmas. In DRS, the reflectance of light decreased in the order *shankha*>*muktashukti*>*mukta* and showed good absorbance in ultraviolet light rangingfrom 200 to 350nm. Market samples 1 and 2 had many differences when compared to our prepared samples. XRD graph showed similar lattice and high crystalline structure for *mukta* and *muktashuktibhasma*.

Conclusion

Market samples 1 and 2 showed many differences when compared to the prepared sample. DRS can be applied to differentiate the *sudhavargiyadravyas* (calcium containing compounds).

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Introduction

Ayurvedic drugs and their formulations are prepared by using plant and marine origins such as *mukta* (pearl), *muktashukti* (pearl oyster), and *shankha* (conch). Ayurveda physicians use marine drugs for treating bone structure disorder, muscular movements, and regulation of gastrointestinal secretions. Materials of marine origins are abundantly rich in minerals such as calcium (Ca) and iron (Fe), and the drugs formulated from marine origins contributes their bio active products for pharmaceuticals and cosmetics applications.[1]

Bhasma (ash) is an incinerated metallic or mineral formulationused in combination with herbal extracts for the treatment of various diseases such as diabetes and cancer.[2] *Bhasma* prepared using materials of marine origins such as *mukta* and *muktashukti* are found to be effective even in small doses. These are mainly used in treating netraroga (eye deformities) and asthisosa (bone disorders).[3] *Muktabhasma* is prepared using the pearl, which is rich in calcium and cures diseases caused by *pitta* and *kapha* vitiation and enhances antibiotic efficacy, stimulates strength, intellect, and semen production.[3, 4] *Bhasma* preparation techniques such as *shodhana* (purification) and *marana* (calcination) processes shrink

the particle size and facilitates for assimilation and absorption into the body.[2, 5] *Mukta* is regarded as one of the *navaratnas* that can eradicate disorders, especially calcium deficiency disorders.[6] However, improper collection, inappropriate manufacturing practices, and low-grade raw material usage may lead to variation in its efficacy and reduces safety of *muktabhasma*.[3]

In current clinical practice, to overcome high cost of genuine natural pearls, cultured pearls are used in most of the formulations by many pharmacies. Escalation in costand increased demand of cultured pearls preparation (*mukta*) as medicine led to adulteration. Precious *mukta* is adulterated with cheaper *muktashukti* or *shankha* commercially. This adulteration alters the therapeutic properties and may contribute to adverse complications. Thus, a proper identification and standardization of market samples of *muktabhasma* for adulteration becomes mandatory for ensuring its safety and efficacy.

Hence, current study intended to evaluate the quality parameters including the structure and particle size for characterization of the three *bhasmas* including *mukta*, *shankha*, *muktashukti*, and using Scanning electron microscope (SEM), X-ray diffraction (XRD), and Diffraction reflection spectroscopy (DRS) analysis, which assist in preventing the adulteration.

Methods

Raw materials

Mukta, *muktashukti*, and *shankha* are the raw material used in this study.These were procured from professional suppliers and collected according to the *grahya* and *agrahyalakshanas* mentioned in Rasa classics. Samples of *Mukta* were procured from 2 different spaces namely Hyderabad (S1) and Bangalore (S2).

Shodhana of mukta

Jayanti swarasa nirmana

The Jayanti was collected from the forest and was authenticated by the subject experts as *Sesbaniaegypticapers* plant. The plant was then pounded in *khalwayantra* to prepare *kalka* without adding water and was then squeezed through a clean cotton cloth to obtain *swarasa* out.

Using *Jayanthiswarasa*, *shodhana* was performed using dolayantra according to the scheme proposed by Sharangdhar.[7] *Pottali* was prepared using *ashodita mukta* and was suspended in *dolayantra* containing Jayanti *swarasa* as a liquid media. *Dolayantra* was subjected to *madhyamagni* for 3 hours. Fresh *swarasa* was added throughout the procedure as the *swarasa* was getting evaporated. After *swedana* process, *pottali* was taken out and *mukta* was washed using lukewarm water. Then it was spread on clean cloth and dried.

After *shodhana*, *churnikarana* of *shodhitamukta* was carried out to bring the material in a fine powder state.

Marana of mukta

Marana is performed as per Rasa Tarangini[7, 8] to obtain *muktabhasma* using *laghu puta* (mode of heating). *Sharavasamputa* was subjected to *lagu puta*. The pit was filled up to 2/3 part with 30 cow dung cakes and *sharavasamputa* were placed over it. Thermocouple was placed vertically and the remaining upper 1/3 part of the pit wasfilled with cow dungcakes. Temperature and time were recorded before ignition and throughout the process of *marana* until complete burning of cow dung cakes in *puta*, using thermocoupleat every 10 min interval. After complete burning, it is allowed for self-cooling.

Then the *sharavasamputa* was taken out, layer of cloth smeared with multanimitti were scrapped off carefully with knife and two sharavas were separated. The *marita mukta* was collected from sharava and powdered as well as weighed.

After first *laghu puta*, second *laghu puta* was performed

using bhasma after first *laghu puta* and subjected to *bhavana* with *godugdha* followed by *chakrikanirmana*. Similar procedure was followed for third *laghu puta*.

However, the bhasma obtained after three *laghu putas* did not pass all *bhasma parikshas*. Hence, fourth *puta* was given with following same procedure to clear all *bhasma parikshas*. For *muktashukti*, it needed five *laghu putas*. After respective *puta*, the *bhasma* passed through all the classical *bhasma parikshas*.

Shankha shodhana* and *marana

Similar *shodhana* and *marana* procedure was followed for *shankhabhasma* preparation with jayanthi swarasa and *gajaputas*. It took four *gajaputas* in *marana* to clear all *bhasma parikshas*.

Mukta shukti shodhana* and *marana

Nimbuswarasa nirmana

Fresh nimbu was collected, washed with water, and squeezed using squeezer. The obtained *swarasa* was used for *shodhana* of *muktashukti* and *bhasma*.

Similar *shodhana* and *marana* procedure were followed for *muktashuktibhasma* preparation using nimbuswaras and *gajaputas*.

Analytical study

All the samples were analyzed for organoleptic properties. Physicochemical parameters such as weight loss on drying (LOD), total ash value, specific gravity, pH were measured. Samples were subjected to SEM, XRD, and DRS analysis for more detailed analysis to get particle size, structure, and reflectance.

Results

Light gray *muktabhasma* passed all the *bhasmaparikshas* after four *laghu puta* to form bright white. For, *shankhabhasma*, four *gajaputas* were required and for *muktashukti* needed five *gajaputas* to give pale white *bhasma*. Texture was smooth and soft and were odorless, tasteless, and lusterless.

Comparable results of pH, ash value, calcium content (Ca%), weight loss on drying, and acid insoluble ash content are shown in Table. 1.

Namburi phased spot test (NPST)showed that market sample 1 (*muktabhasma*) had similar trend as that of prepared *muktabhasma*; however,market sample 2 (*muktabhasma*) showed different pattern resembling the prepared *muktashuktibhasma*.

The XRD study revealed that *muktabhasma* (Fig. 1) and *muktashuktibhasma* (Fig 2) could not be differentiated because of its similar lattice parameter and high crystalline structure. SEM observation showed that the particles were clearly differentiated. Morphology of *mukta* was crystalline but can be differentiated from other, as it is slightly smooth structured. Whereas, *muktashukti* and *shankha* showed crystalline in nature and *muktashukti* showed more compactness then *mukta*, and *muktashuktibhasma*.

DRS analysis of *shankha* showed high reflectance in visible region and that of *muktashukti* and *mukta* showed good absorbance in ultraviolet range.

XRD analysis showed sharp peak which indicates crystalline nature where *mukta* and *muktashukti* is aragonite form and *shankha* forms vaterite. Pattern shows that crystallinity of *mukta* and *muktashukti* samples were higher compared to *shankha* where peak corresponds to 2 theta values of 29.40. XRD peak height of *shankha* was less compared to *mukta* and *muktashukti*.

SEM image of *mukta* showed crystalline morphology but can be differentiated due to its spherical nature. *Muktashukti* also shows crystalline nature of *muktashuktibhasma* at 2 kV it shows particle size (50 - 1μm) at magnification of 500 X to 20,000 X. *Shankha* also showed crystalline nature at 20 kV particle size of 50–1μm.

DRS of *mukta* and *muktashukti* showed better reflectance in 200–350 nm wavelength whereas, *shankha* showed significant reflectance in visible region (350–800).

On XRD and DRS, both the market samples showed similar profile to that of prepared *muktabhasma*. XRD showed that both market samples had crystalline structure and the obtained peak corresponded to 2 theta value of 29.40. Prepared *muktabhasma* also showed 2 theta value of 29.40. In DRS, both the market samples as well as mukta showed good absorbance at 200–350 nm. Also, pH and NPST results of market sample 2 was similar to that of prepared *muktashuktibhasma*.

Discussion

The traditional belief that the ayurvedic entities are valid and do not require any research to prove its authenticity, this misconception is harming the significance of ayurvedic science.[9] Research is required to prove the quality control parameters of dynamic ayurvedic entities using modern equipment to authorize it as flawless formulations.

In the present study, an attempt has been made to draw the quality control parameters of the *bhasmas* of *mukta*, *muktashukti*, and *shankha* analytically. *Mukta* possess qualities such as *sheetavirya, madhuravipaka, kapha-pitta shamaka, vrishya, balakara*, and *brihmana*. Cultured pearls are of two varieties—freshwater and seawater cultured pearls. Freshwater-cultured pearl was investigated in the present study. It possesses effective therapeutic prominence such as immunomodulatory effect.[10] *Shankha* is of two varities—*dakshinavarta* and *vamavarta*. *Vamavarta* was used in the present study, as it is easily available and possess medicinal properties on dyspepsia and gastroesophageal reflux diseases [11, 12] Due to higher expenditure, mukta has been adulterated with cheaper *muktashukti* or *shankh* commercially. This does not serve as the effective treatment instead leads to unknown complications.

Mukta is an aquatic gem having less chances of getting contaminated implying the less compulsion in the view of purification. However, to enhance the therapeutic properties of *muktashodhana* (purification) becomes necessary. Current shodhana procedure involves *jayanthiswarasa*, which comprises various phytochemicals such as flavonoids, tannins, and sugar. These phytochemicals are believed to possess antibiotic, antitumor properties, which in turn improve the therapeutic efficacy of *mukta*.[13]

Heat plays a major role in *shodhana* procedure. It assists in altering the structure of matter. By intense heating and mode of cooling makes liquid media to enter into expanded intermolecular space of the drug contributing to brittleness by reducing the hardness.

Organoleptic characters displayed that all the three drugs became clean and their lusterreduced due to removal of external impurities by acidic media that is, *nimbuswaras* for *shankha* and *muktashukti* and *Jayanti swaras* for *mukta*. The color of *muktabhasma*, *muktashuktibhsama*, and *shankhabhasma* was whitishgrey, pale white, bright white, respectively which is comparable to the studies.[14-16] *Sparsha* was smooth and soft, odorless, tasteless, and lusterless.

Jayanti swarasa showed a pH of 6.28 before *shodhana*. After *shodhana*, it became 6.42. This alteration in pH might be due to continuous heating for 3 h or due to interactionof *swarasa* with *mukta*. Similarly, pH of *nimbuswaras* increased after *shodhan* (2.51 to 2.80) in *muktashukti* and 2.51 to 3.21 in *shankh*, which may be due to the reaction of $CaCO_3$ with acidic medium.

The pH of market sample 1 was 8.98 and the pH of the market sample 2 was 9.8. In sample 1, pH matches with that of prepared sample (8.92) and the pH of the sample 2 matches with that of *muktashukti* (9.5). By analyzing these pH results,it was inferred that the *mukta* of market sample 2 might have been adulterated with *muktashukti* as the pH matches with it. Also, *kshariyata* (waste) goes on decreasing in the order *shankhabhasma> muktashuktibhasma> muktabhasma.*

Ash values of all the prepared samples were as per the limit. Ash value inboth the market sample was high. This may be due to procedure variation, such as change of *bhavana* drug in *shodhana*, *marana* and, even selection of drug.

NPST is a direct method by which we can differentiate easily by the patternformed over the whatman filter paper no.40.[17]Market sample1 showed similar patterns that of prepared *muktabhasma* and market sample 2 showed different pattern quite similar to prepared *muktashuktibhasma*. This suggests there may be anadulteration in market sample 2.

XRD was carried out for *muktabhasma*, *shankhabhasma*, *muktashuktibhasma*, market sample 1 (*muktabhasma*), and market sample 2 (*muktabhasma*).The sharp peak indicates that the substance is well crystalline in nature. It was found that *mukta* and *muktashukti* were ofaragonite form according to the lattice matter. In suman lata et.al. [18] study, the XRD pattern of *Muktabhasma* showed crystalline nature with 2 theta value of 29.36and peaks were corresponding to calcite form of calcium carbonate. Sawanth et al[19]study the XRD peaks were corresponding to aragonite form of calcium carbonate. In another study by Jayanth et al[16] the XRD revealed rhombohedral crystal and peaks were identified corresponding calcite form of calcium carbonate.

From the XRD pattern, the crystallinity of the *mukta* and *muktashukti* samples were found to be higher than *shankha*. The peaks correspond to two theta values of 29.40, which can be seen in all the three samples; however the XRD peak height of *shankha* was less when compared to *mukta* and *muktashukti*. Both market sample 1 and 2 had crystalline structure, but we can find the peak height difference in both the samples. Because of the same lattice parameter and high crystallinity, differentiation between *muktabhasma* and *muktashuktibhasma* becomes impossible. (Figs. 1–5)

SEM showed the crystalline morphology of *mukta* but can be differentiated from others, as it is slightly spherical.*Muktashukti* and *shankha* showed crystalline morphologywith particle size of 50 –1µm at 20kV. *Mukta* market samples 1 and 2 also showed crystalline nature with particle size 50–1 µm at 20kV. (Fig. 7)

In DRS, *shankha* showed good reflectance in the visible region (350–800nm) of solar light spectrum, but the reflectance decreased below 350 nm. Absorbance in the range of 200–350nm showed that *muktashukti* and *muktabhasma* can absorb ultraviolet light The reflectance of light decreases in this order *shankha*> *muktashukti*> *mukta*. Market sample 1 and 2 showed good absorbance in the range of 200–350nm.This reflectance is depended on the color of the substance. As *shankha* is bright white, it reflects the maximum amount of light, where other *bhasma* do not reflect with that intensity. Hence, this test can be specifically applied to *sudhavargiyadravyas* (Figs. 7–8).

Conclusion

Our findings suggests that the study market sample 1 and 2 has many differences when compared to prepared samples which conclude that the proper standard operational procedures were not followed in the preparation. Hence the samples were not genuine.

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Table 1. Physicochemical evaluation of *bhasmas*

Tests	*Mukta*	*Muktashukti*	*Shankha*	Market sample 1	Market sample 2
pH	8.92	9.5	11.81	8.98	9.84
Ca%	41%	40%	40%	41%	41%
Ash value	2.5%	1.52%	17.25%	38%	35%
Loss on drying	0.17%	0.25%	0.25%	0.39%	0.38%
Acid-insoluble ash	0.25	0.28	0.25	0.32	0.28

Figures

Fig. 1. X-ray diffraction of *muktabhasma*

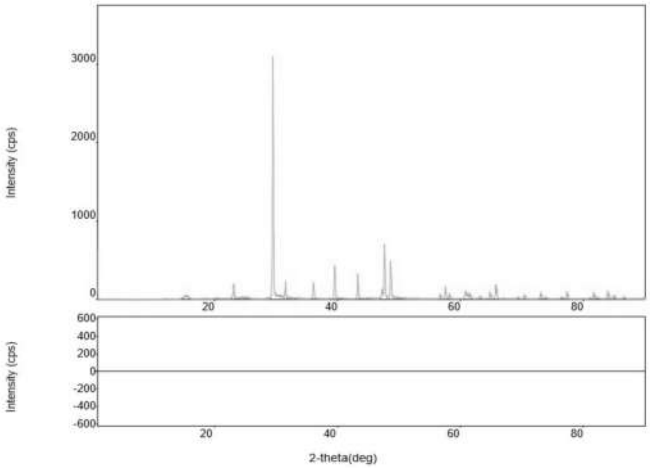

Fig. 2. X-ray diffraction of *muktashuktibhasma*

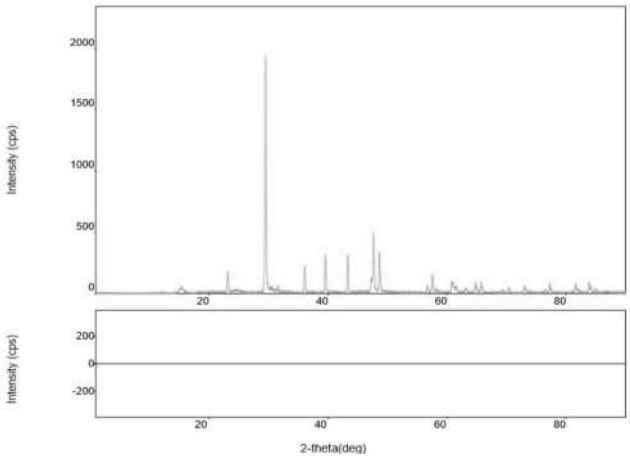

Fig. 3. X-ray diffraction of *Shankhabhasma*

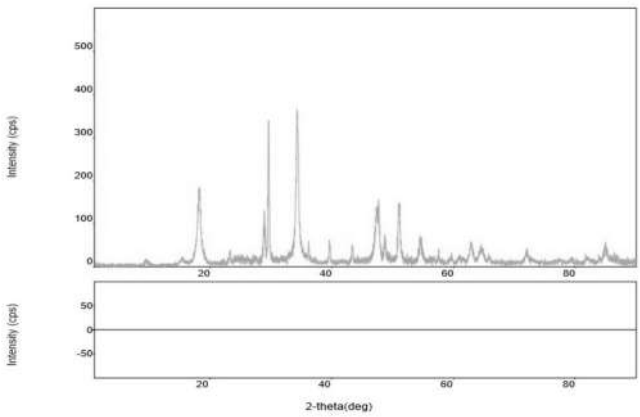

Fig. 4. X-ray diffraction of Market sample no 1

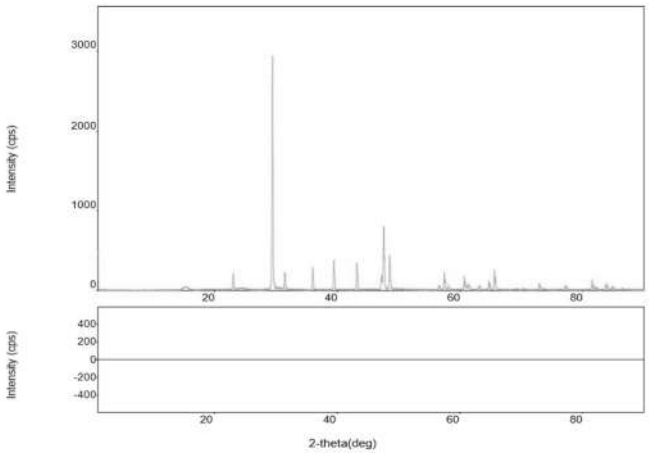

Fig. 5 X-ray diffraction of Market sample no 2

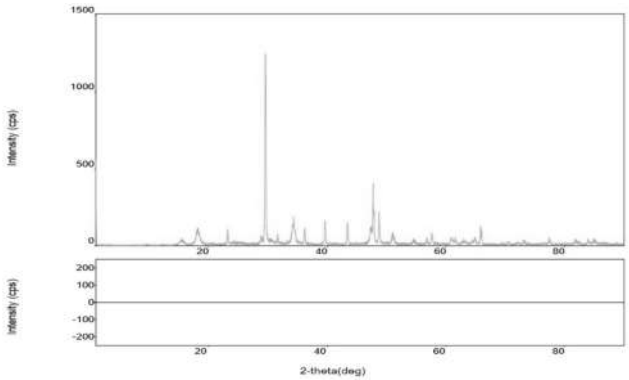

Fig. 6. Scanning electron micrograph of a. *mukta*, b. *muktashukti*, c.*shankha*, d. sample 1, e. sample 2

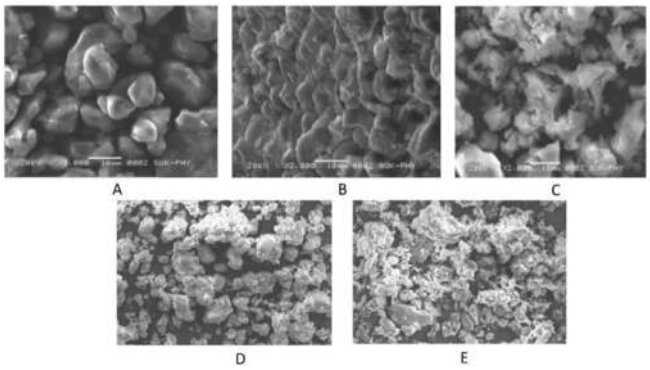

Fig. 7. Diffusion reflectance spectroscopy of *mukta*, *muktashukti*, and *shankha*

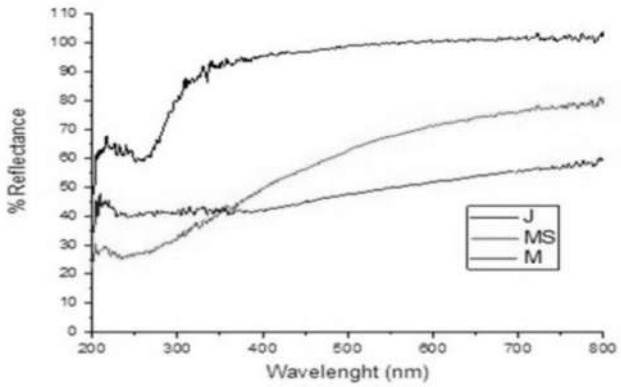

Fig. 8. Diffusion reflectance spectroscopy of sample 1 and 2