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Research Article

PHARMACEUTICAL AND PHYSICO-CHEMICAL STUDY OF SHILAJIT BHASMA

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ABSTRACT

Although there were mention of Shilajit Bhasma (Calx) in various classical texts, but no specific study or usage of the same found mention in Google Scholar, ARD, Pub-Med, NCBI. AYUSH Research Portal and other open source database. This study is intended for preparation of Shilajit Bhasma (Calx) and assessments of physico-chemical properties of the same. **Methods:** The *Bhasma* (Calx) of *Shilajit* was being prepared using classical references, from Shilajit purified with two different methods, namely, Ushna Jal Shodhita Shilajit (Agnitapi) and Triphala Kwatha (decoction) Shodhit Shilajit (Survatapi). Main constituents used in Marana (incineration) were purified Gandhak (sulphur), Haritala (orpiment), Manasila (realgar) in equal proportion to Shilajit. IEC No, for the study is IEC-RAC-18/R.S.B.K.-18-M.D.(Avu.). Results: Result of the FE-SEM shows that both samples contain crystalline of nano size, with size ranged from 100nm – 1μ m, with irregular shape and sizes. The findings of FE-SEM are in concordance to results of classical examinations of Bhasma (Calx). XRD intensity plots of both samples show various sharp and small peaks, when diffraction patterns were observed at different 20 angles ranges from 10° to 70°, various peaks identified to correspond with known peaks for S & AsS and have been marked on the plots. ICP-MS and flame photometry of both samples reveals the presence of Na, Ca, K, Mg, Mn and As in different ratio. Conclusions: It has been concluded that the elemental composition of Shilajit Bhasma have a strong similarity with Purified Shilajit.

INTRODUCTION

It has been mentioned about *Shilajit* in classical Ayurvedic texts that "There is no curable disease on earth, which cannot be cured with the help of *Shilajit*. When administered at right time, well prepared, and in the right manner, it will secure for the healthy subject the optimum measure of vitality".^[1] A number of research works are available regarding physicochemical properties of *Shilajit* in literature, but there is no specific work found among available research database about usage and physico-chemical properties of *Shilajit Bhasma* (Calx), while database of Ayurvedic Research (ARD-VII), Google Scholar, Ayush Portal, Pub-Med, NCBI and other online platforms are being



searched for. In a general presumption some of the scholars believed that the Shilajit was being formed with a similar processes and mechanism like petroleum, and advocates to not placing the Shilajit in direct contact of fire. In classical text usage of Shilajit Bhasma (Calx) are being advocated in a strong way, as like, when equal proportions of Kantloha Bhasma (Calx of magnetic iron), Vaikrant Bhasma (Calx of Tourmaline), and Shilajit Bhasma (Calx of Asphaltum Punjabinum) in a total amount of 375mg (125mg each) administered for a duration of six months in accordance to Rasayanavidhi, one should fight with adverse effect of ageing, and could attain hundred years of age.^[2] The commentator on the above verse have noted that 375mg dose of Shilajit Rasayana could be a high amount of dose for a Madhyama Prakariti human being, and a dose amount of 187mg is sufficient. The Shilajit Rasayana may be administered as cure of anemia, tuberculosis, digestive impairment, increased frequency and turbidity of urine, piles, abdominal lump, splenic disease, diseases of abdomen,

diseases of female genital tract and Nanavidh Shula in a amount of 375mg along with Anupana of Triphala *Churna, Trikatu Churna* and *Ghrita*".^[2] Apart from the usage as described in classical texts any specific assessment of the Shilajit Bhasma (Calx) for its chemical and physical properties on modern parameter did not being carried out in any study as mentioned above. Pradhan Nilakshi et al. have described scope of the physico-chemical study of *Shilajit Bhasma* (Calx) in a review study.^[3] During the search of literature and available databases it has been found that Bhasma (Calx) of Karpura Shilajit is widely being used in some parts of southern India and it has been studied for its physico-chemical properties.^[4] On the basis of the above mentioned facts the aim and objective of present study have been formulized. The aim and objective of present study to prepare Shilajit Bhasma (Calx) as described in the various classic texts.^[5,6,7,8,9,10], and to evaluate the physico-chemical properties of *Shilajit Bhasma* (Calx), and to analyse the Shilajit Bhasma (Calx) for its elemental composition through XRD, ICP-MS and other modern techniques, and apart from all to compare the elemental composition of *Bhasma* (Calx) with the elemental composition of purified *Shilajit* as available in literature to determine whether it have any correlation or not.

MATERIAL AND METHODS

In present study we have selected two samples of purified Shilajit for preparation of the Bhasma (Calx). The samples selected so have been obtained from the two purification methods adopted for purification of raw Shilajit. One of the samples was purified by soaking the *Shilajit* in lukewarm water and then drying the same on hot plate, the sample obtained so is known as Ushna Jala Shodhit Shilajit (Agnitapi).^[11] Second sample was purified by soaking the raw *Shilajit* in decoction of Triphala, and drying the same in sun light, and known as Triphala Kwatha Shodhit Shilajit (Survatapi).^[12] The reference for Shodhana (purification) process was taken from *Rasa Tantrasar* for Ushna Jala Shodhit Shilajit (Agnitapi),^[11] and from Rasa Tarangini for Triphala Kwatha Shodhit Shilajit (Suryatapi).^[12] The study was approved by Internal Ethical Committee vide IEC No. IEC-RAC-18/R.S.B.K.-18-M.D. (Avu.) dated 30.12.2019. The rationale behind selection of two different types of purified *Shilajit* is to make a differentiative inference from the final analytical findings. As it is not clear initially that what the yield percentage of incineration of *Shilajit* will be found, what its chemical characters will be, and so we were a little bit confused on the selection of Shodhita Shilajit samples. As winter approaching at the time of experiment starts and drying Shilajit soaked in lukewarm water in sunlight will definitely take a longer time, while there might be a little chance of adverse chemical processes while drying *Triphala Kwatha* soaked *Shilajit* on a hot plates, as *Triphala Kwatha* is slightly acidic media, and *Triphala Kwatha* soaked *Shilajit* takes a short time span in drying in sunlight in comparison to lukewarm water soaked *Shilajit*, that was why we have selected two different process of drying the *Shilajit*.

Raw *Gandhaka* (sulphur) was purified by Dhalana Methods (melting and pouring) using cow's milk and *Goghrita* (clarified butter of cow milk).^[13] Crystalline dark yellow Gandhaka (sulphur) turned into granular and dull yellow form after purification. A weight loss of 10% was observed during purification process of *Gandhaka* (sulphur). Raw Haritala (orpiment) was purified following the reference of Rasa Raj Sundar.^[14] No considerable weight loss was being observed during process, while slight elevation in pH, from 10.23 to 10.32, of purification media Churnodaka (lime water) was noticed after purification. Manasila (realgar) was purified following reference of Rasa Ratna Sammuchchaya.^[15] Raw *Manasila* (realgar) was levigated with extract of *Ardrak* (Zingiber Officinale) in a cyclic manner and for seven cycles. Purified Manasila (realgar) obtained so was dried on room temperature.^[16]

The whole process of incineration of *Shilajit* in brief could be summarized as; "one part of purified Shilajit (Image-1), one part of purified Gandhak (sulphur) (Image-2), one part of purified Haritala (orpiment) (Image-3) and one part of purified Manasila (realgar) (Image-4) was being taken in equal proportion and for levigation Matulunga Swarasa (extract of pulp of Citrus medica) was used in required proportion. All the four materials were levigated in mortar with the use of extract of Matulunga (Citrus medica), till the mixture was dried out with little moisture content. Then pelletizations were done, and approximately equal size pellets were prepared (Image-5). Then pellets were put into Sharava (earthen saucer) and placed on room temperature to be dried. Dried pellets in the Sharava (saucer) was covered with another Sharava (saucer) and sealed with the help of double folded mud smeared cloth and again let to be dried. The mud sealed saucer, i.e., Sharava Samputa (Image-6) was placed in such a manner that it was completely covered between Puta of 8 Uppalas (cow dung cake) known as Laghu Puta or Kapota Put^[17] (Image 7) Weight of the Uppalas in both case recorded approximately as 4.6kg. The fire was lit and observation of temperature recorded with interval of 10 minutes with the help of HTC Infrared LASER guided pyrometer. The variation in temperature might be visualized with the help of following line graph (Graph-1). According to a study self cooling time for a Laghu Puta is 3.5 hours, while attains a maximum temperature of 800°C which sustains for about half hour approximately.^[18] Self cooling time in the present experiment was observed as 2.25 hours, while attaining a maximum temperature of 796°C. The materials remains after *Putapaka* was collected and grounded. The weight of finally prepared *Bhasma* (Calx) was taken, and colour was observed. (Image 8) The brief of observation are being tabulated in Table-1.



Analytical Study

Both the samples are subjected to classical examinations. For *Varitara* (lightness and fineness) small quantity of *Bhasma* (Calx) had been spread on cold and still water and there floating character was assessed. While for *Rekhapurnatvam* both the samples of *Bhasma* (Calx) have been placed between index finger and rubbed to check whether it get easily into the crevices and lines of the fingers or not. *Unnama* or *Uttama* characteristics of both samples were been assessed by placing a rice grain on *Varitara* surface of the *Bhasma* (Calx). *Slakshanatvam* of the samples were examined by placing them between the fingers and assessing their fineness. Taste and other organoleptic characteristics have also been assessed.

Both the samples were analysed for their pH of 10% aqueous solution, ash value, acid- insoluble ash, water soluble ash, loss on drying and solubility in water using methods given in API.^[19]

X-Ray Diffraction: Both the sample have been submitted for analysis and to measure diffraction pattern, through XRD, as XRD is a non-destructive, fast and easy analysis, giving highly accurate results for dspacing and result could be simply verified with the help of JCPDS database. Smart lab X-Ray Diffractometer (Rikagu, Japan) was used to perform powder XRD pattern using Cu Ka radiation, as X-Ray source (λ =0.15418nm), done at room temperature, with power source voltage as 45Kv, and current at 100mA, generating high source X-Ray beam. After placing sample in a glass sample holder, the diffraction pattern at 20=10° to 70° was observed.

Scanning Electron Microscopy: SEM in principle have advantage of producing high resolution images and dataset, easy to handle and user friendly operation, and produce data in a short period of times as short as in 5 minutes time frame, and could be used to analyse various types of surface, and sub-surface analysis. Both the samples were @20µl was drop casted on slides and then the slides were sputter-coated with 2nm gold thickness @ 20mA. Thereafter CarllZeis model supra 55 VP was used to image the samples.

ICP-MS & Flame Photometry: Inductively coupled plasma mass spectrometry (ICP-MS) is an elemental analysis technology capable of detecting most of the periodic table of elements at milligram to nanogram levels per liter. The samples were submitted to assess the actual quantity of elements which was found present in purified *Shilajit* in various previous references and different study in the field by various scholars. It has also been presumed, based upon the finding of XRD results and methods used in preparation of material for incineration that arsenic might present with a significant level. So, sample had been submitted to RATL, Bangalore, with a request to determine quanta of 6 elements in the both *Bhasma* (Calx). 4 elements out of 6 had been assessed through ICP-MS, while Na and K had been determined through flame photometer.

	Table:1 Details of observation during preparation of Bhasma, Sample 'A' & 'B'								
S. No	Sample Name	Material/Comp onent	Weight (gms)	Dimension of Saucer	Pellets Dimension and weight	Total Pellets weight	Maximum Temperature	Final Remains of the <i>Bhasma</i>	Colour of the <i>Bhasma</i>
1 Ushna Jala Shodhita Shilajit (Agnitapi) Bhasma, Sample 'A'	Shodhita Shilajit	Ushna Jala Shodhita Shilajit (Agnitapi)	60	Diameter =14.6cm & Depth	Diameter= 2.3cm (Approx)	240gm	796°C attains 30 Minutes	32 gm	Black
	Purified Gandhaka (S)	60	4.5cm	and weight 6gms (Approx)		after litting fire			
	Purified <i>Haratala</i> (As ₂ S ₃)	60							
	Purified <i>Manashila</i> (As ₂ S ₂)	60							
	Matulunga Swarasa (Extract of Citrus Medica)	37ml							
Triphala Kwatha Shodhita Shilajit 2 (Suryata pi) Bhasma, Sample- 'B'	Ushna Jala Shodhita Shilajit (Agnitapi)	60	Diameter =14.6cm & Depth 4.5cm	Diameter= 2.5cm (Approx) & Weight=	240 gm	796°C attains 30 minutes after litting	33gm	Black	
	Kwatha	Purified <i>Gandhaka</i> (S)	60	LECEPTION.	5.9gm (Approx)		fire		
	Shilajit (Suryata	Purified <i>Haratala</i> (As ₂ S ₃)	60						
	<i>Bhasma,</i> Sample-	Purified Manashila 60 [As ₂ S ₂]							
		<i>Matulunga Swarasa</i> (Extract of Citrus Medica)	37ml						

Table:1 Details of observation during preparation of Bhasma, Sample 'A' & 'B'

Table-2: Comparative Table: Elemental Composition of Sample 'A' & 'B' Through ICP-MS & Purified Shilajit

Sr. No.	Elements Assessed through ICP-MS	Elemental Composition of Sample-'A' (ICP-MS & Flame Photometry)	Elemental Composition of Sample-'B' (ICP-MS & Flame Photometry)	Elemental Composition of Processed Shilajit (Purified Shilajit) ^[21,22]
1.	Arsenic	5.754gm/kg*	5.528gm/kg*	NA
2.	Sodium	4 gm/kg [#]	3 gm/Kg [#]	0.04gm/kg
3.	Manganese	0.058gm/kg*	0.056 gm/kg*	0.08-0.1gm/kg
4.	Magnesium	13.117gm/kg*	11.726gm/kg*	14.00gm/kg
5.	Calcium	8.462gm/kg*	16.5gm/kg*	27.00gm/kg
6.	Potassium	42g/Kg#	36 g/kg#	60g/kg

*Through ICP-MS, #Through Flame Photometry





Image 1: Pellets of Mixed Contents





Image 3: Puta



Image 4: Finally Prepared Bhasma





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Image-6 (i): FE-SEM Image of Sample 'A' at Magnification ratio 1:25000



Image-7 (i): FE-SEM Image of Sample 'B' at Magnification ratio 1:5000

RESULT AND DISCUSSION

Heat flows in the Sharava Samputa by the mechanism of conduction due to temperature gradient formed by application of heat from all sides. Selection of Puta depends upon the quality of metal and minerals, other materials used for levigation and final composition to be formed. 'Hess law' explains selection of specific Puta for different material. Less heat is required for chemical changes of easily fusible material like lead, tin and more heat required for chemical changes of hard and tough material.^[20] As components used in preparation of *Shilajit Bhasma* (Calx) is mainly Arsenical Chemical and Sulphur, which have boiling points of 613°C and 444.6°C respectively, apart from the *Shilajit* itself, the selection of *Laghu puta* may be justified on following lines. It has been described that maximum temperature of 8 Upala is reach upto 800°C and sustained up to for $\frac{1}{2}$ hr. and self-cooling time is 3.5hrs. The temperature curve of present work also follows a similar pattern, however a less cooling time was observed. The same may be due to the sharp temperature gradient present between Samputa and environment, as work was done in winter season. The maximum temperature observed is definitely above the melting points of 'As' and 'S'.



Image-6(ii): FE-SEM Image of Sample 'A' at Magnification ratio 1:80000



Image-7 (ii): FE-SEM Image of Sample 'B' at Magnification ratio 1:50000

It has been found that the fine particles of both the Bhasma (Calx) samples were floated on water. without breaking the surface tension. The surface floating character of the *Bhasma* (Calx) remains when a small rice grain placed cautiously on the floating Bhasma (Calx). This implies the fineness and light weights of Bhasma (Calx) nano particles. Both the sample of *Bhasma* (Calx), namely sample 'A' and sample 'B', when rubbed with index finger and thumbs it enter into the furrow of fingers and does not cause any irritation. This implies that both the samples were complying with the standards of classical examination. Varna (colour) of both sample was black, and did not produce any nauseates sensation. Both the samples are found of smooth texture, of black colour, tasteless and odourless while examined for their organoleptic characters.

pH for sample 'A' and sample 'B' was recorded as 6.07 and 3.24 respectively, means sample 'B' was more acidic than sample 'A'. pH of sample 'A' was greater than the sample 'B', the difference might be associated with the purification media of *Shilajit* for both the samples. As *Triphala* is slightly acidic than the lukewarm water, *Bhasma* (Calx) prepared with *Shilajit* which was purified by soaking in *Triphala Kwatha* (decoction), was more acidic than *Bhasma* (Calx) prepared with *Shilajit* purified with lukewarm water. Ash value for samples is residue remaining after incineration and consists of inorganic salts of the carbonates, phosphate, silicates of sodium, potassium, calcium and magnesium etc. Ash value for the sample 'A' and 'B' was recorded as 52.84% and 62.86% respectively. Acid insoluble Ash, and Water soluble ash for sample 'A' and 'B' was recorded as 6.07 and 7.25, 33.24 and 36.42 % of weight respectively. While loss **Graph-2**

on drying was 3.31% and 4.99% of weight respectively. Sample 'A' have 63.79% solubility in water while sample 'B' have slightly less solubility of 60.86%. The results obtained so are being incorporated in the bar diagram on log scale as below (Graph-2). Sample 'A' has less ash value than Sample 'B'; this indicates that Sample 'B' has more inorganic salts content than sample 'A'. Sample 'A' has higher solubility in water than sample 'B', while less water soluble Ash contents.



The intensity plots of Sample 'A' (Image 9(i)) at different 2-theta degree angles show various strong and weak peaks. Some of the main peaks are identified as corresponding peaks of sulphur and arsenic sulphides used during the preparation of *Bhasma* (Calx), in equal proportion to purified *Shilajit*. These peaks correspond to effective incineration of Bhasma (Calx) using As₂S₃, As₂S₂ and S compounds. Sample 'B' have shown similar pattern of diffraction (Image 9(ii)) in comparison to sample 'A', but have more sharp peaks corresponding to Arsenic Sulphides (AsS) than sample 'A'. FESEM scan shows that both samples namely sample 'A' (Image 10(i) & 10(ii)) & sample 'B' (Image 11(i) & (ii)), have irregular shape and size of nano size crystallite. This may be attributed due to multiple stages of *Bhavna* (levigation) and *Shodhana* (purification), as well as at best parts heat treatment to the Chakrikas (pellets) used to prepare Bhasma (Calx). The average particle mean size for Sample 'A' ranged from 200nm - 1µm, while for Sample 'B' it ranges from 100nm - 1µm. It may easily be deduced from the above findings that results of examination of both the samples using modern tools and techniques are in concordance with findings of classical examinations. As Bhasma (Calx) Complies with the Rekhapurnatvam, Varitara and Unnama, the fineness of Bhasma (Calx) are also established when FE-SEM analysis revealed

that both the Bhasma (Calx) consist of nano-crystalline of size 100/200nm to 1µm. The findings of FE-SEM evaluation at different magnification stages reveals that the sizes of crystals of Bhasma (Calx), sample 'A' and sample 'B', are 200nm- 1µm and 100nm- 1µm respectively, while both samples consist nano crystalline of irregular shape and sizes. The findings of FE-SEM are seems in concordance to the observation on Ayurvedic parameters, as like Rekhapurnatvam. Presence of sulphur crystal could easily be identified while analysing FE-SEM images. Results and analysis of XRD scan at various 20 angles shows different sharp peaks, in which peaks corresponding to sulphur and arsenic sulphide are shown on the graphs of scan in analytical parts of this work. While various unidentified peaks might be due to the presence of elements and compounds corresponds to the various elements founds in Shilajit. According to the findings of a study it has been established that processed Shilajit have Potassium-60g/kg, Calcium-27g/kg, Magnesium-14g/kg, Sulphur-6g/kg, Sodium-40mg/kg respectively.^[21] The percentage of elemental constituents in processed Shilajit is approximately 10-12% of total weights, and may vary in accordance to the source and site of Shilajit exudation and process used in processing of raw Shilajit. Both the samples have been submitted for ICP-MS analysis and the

results obtained so, are summarized as below in Table-2 along with elemental composition of processed *Shilajit*.^[21,22]

Elemental composition of the *Shilajit Bhasma* (Calx) clearly implies the presence of purified *Shilajit* elemental constituents in the finally prepared *Bhasma* (Calx). As like. presence of magnesium approximately in equivalence to the purified *Shilaiit* sample analysed by another scholars. However, lukewarm water processed Shilajit Bhasma (Calx) have a slightly higher quantity of Magnesium. That might be due to the different processes used during the purification of *Shilaiit*. Presence of considerable amounts of calcium, and potassium also signifies the observations. While a slightly elevated same concentration of sodium in the *Shilajit Bhasma* (Calx) might be due to the spatial variation and others aspects of the *Shilaiit* sample selected for study, as for present study sample was obtained from Himalavan region.

However, a considerably high limit of arsenic founds in both the sample of *Shilajit Bhasma* (Calx).

The quantity of the arsenic determined by ICP-MS study in both the samples are seems to be considerably very high, when we compare the same with the reference ranges given in API. Part-I. Vol-VIII. 2008. which is mere 3mg/Kg. This seems alarmingly high when we consider the same without taking the Bhasma (Calx) preparation methods in consideration. Peaks corresponding to arsenic sulphides also seen in XRD scan graphs. But before deducing any inference from the above tabulated value we have to consider the facts that equal amounts of As₂S₂ & As₂S₃ with S and *Shilajit* being used while preparing the Bhasma (Calx) of Shilajit. As per a study done by P. Satadru et al, on Haritala (orpiment) Bhasma (Calx) & Rasamanikya, it has been found that wt% of arsenic in Haritala (orpiment) Bhasma (Calx) 58.69%, and sulphur is 11.69%, while in Rasamanikya wt% of arsenic is 41.77%, and sulphur is 15.81%.^[23] While comparing the wt% of arsenic founds in *Shilaiit Bhasma* (Calx) in present study, a range of 0.51-0.58% of weight of arsenic found presents (for comparison please see graph below, Graph- 3).





The presence of arsenic however could be explained on the basis of composition of *Haritala* (orpiment) *Bhasma* (Calx) and *Rasamanikya* as established by the above cited study. However, it could be kept in mind that the *Puta* used for incineration of *Chakrika* (pellets) in present study was *Laghu Puta*, and no cyclic incineration was carried out, but the final composition of the *Bhasma* (Calx) while compared with respect of arsenic presence are very different from the *Haritala* (orpiment) *Bhasma* (Calx) and *Rasamanikya*. This might be due to the use of *Shilajit* as main and primary constituents during the incineration. **CONCLUSION**

Main elemental constituents identified as Ca, Mg, Mn, K, Na and S in the both sample of *Shilajit Bhasma* (Calx). While a significant amount, approximately 0.55% of weight, of arsenic also found present. It has been found during search of literature that a more significant amount of arsenic, >40% of weight, found present in Haritala (orpiment) Bhasma (Calx) as well as in Rasamanikya prepared from incineration of Haritala (orpiment) as a primary constituent. So, presence of arsenic on such a level might be due to the usage in equal proportion of Haritala (orpiment), Manshshila (realgar) and *Gandhaka* (sulphur) in preparation of *Shilajit Bhasma* (Calx). So, it has been concluded that while approx. 0.55% of arsenic found present in the final Bhasma (Calx), primary constituents of *Shilajit* as like Ca, Mg, Mn, K, Na formed approx. 6.5% of the total weight. Presence of other elements and constituents also might not be denied, as Bhasma (Calx) was analysed only for few elemental traces. The elements found so are

definitely converted to a nano form during incineration as reveals by FE-SEM results as well as findings of classical examinations. While presence of sulphur may be identified from XRD scan results. sulphur might be present in a higher ratio than purified *Shilajit* as it was being used for incineration processes in an equal ratio of *Shilajit*. It may be concluded that *Shilajit Bhasma* (Calx) contains elemental trace of purified *Shilajit* as main elemental constituents of *Shilajit* as like Ca, S, Mg, Mn, K and Na are present in a comparable ratio. It may be suggested from the findings of the above study that further evaluation of *Shilajit Bhasma* (Calx) should be carried out to know its exact chemical compositions as well as toxicity, to evaluate its clinical efficacy in the various disease given in classical texts.

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