Research Article

PHARMACEUTICAL AND ANALYTICAL STUDY OF KASISA

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ABSTRACT

Bhasmas are unique Ayurvedic preparations of metals/minerals formulated with herbal extracts or juices and used for the treatment of a variety of ailments. Owing to their micro/ nano fineness, ease of administration and comparatively small dose, they have been known for their vast area of application and therapeutic value. Kasisa ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) is an iron containing used in the treatment of eye diseases, pruritis, leucoderma, dysurea, helminthiasis. The present study was conducted to perform physicochemical characterization for the Kasisa Bhasma as per the procedure mentioned in the literature by using several analytical tools. Kasisa was taken for preparation of Bhasma with the help of Shodhana and Marana procedures as per traditional references. The Kasisa Bhasma and its intermediary in-process were distinguished for their physicochemical characters using Atomic Absorption Spectroscopy (AAS), Energy Dispersive X-ray Spectroscopy (EDAX), X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM) analysis. EDAX analysis revealed that iron, sulfur, and oxygen was found to be 29.52%, 20.03%, 50.45% respectively in raw Kasisa, 46.48%, 21.38%, 31.30% respectively in Shodhita Kasisa and 68.48%, 0.86% and 29.26% respectively in Kasisa Bhasma. The XRD pattern of Kasisa Bhasma shows that all the strong medium and weak peaks correspond only to Fe_2O_3 . The SEM analysis confirmed that the particle size of raw and Shodhita and Bhasma of Kasisa were found to be 90 μ , 2 μ and 350 nm respectively. Therefore, by using advanced analytical technique this work grants an applicable correlation between the traditional information on characterization and processing of Kasisa Bhasma.

Keywords: Bhasma, EDAX, Kasisa, SEM, XRD

INTRODUCTION

In Ayurvedic classical literature has documented several texts which make sure the suitable transformation of essential metal into bio-absorbable calcined (Bhasma) form¹. Bhasma is the calcined preparation of minerals and metals having various advantages over herbal formulations². Bhasmas are generally used in the healing of diseases because they have vast therapeutic value due to its easily assimilated, micro fineness, little dose and also great therapeutic effect. The preparation of Bhasma was a sophisticated process relating purification of the drug, levigation with different herbal juices, and formation of pellet along with incineration (Putra)³. But in the current scenario, certain people raised questions regarding the quality, efficacy and safety issues of Bhasma. It is recommended to use the advanced modern technology to make sure the suitable formation of Bhasmas⁴. Kasisa (green vitriol) is an iron containing Ayurvedic formulation, composed of ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) used in the treatment of eye diseases, pruritis, leucoderma, dysurea, helminthiasis, fever, splenomegaly, primary and secondary amenorrhea⁵. The crude form of Kasisa contains iron along with various other toxic heavy metals and minerals which if taken without purification may produce various poorly effects within the body like palpitation, insomnia, burning sensation⁶. So Kasisa Bhasma was used as a medicine for treatment of various diseases after proper purification and incineration process. In addition to the main metal (Iron), several other metals are also expected during the preparation of Bhasma⁷. This may be present in its elemental form during

pharmaceutical processing. In addition, different pharmaceutical procedures used for the different manufacturing companies produce similar Bhasma with the diverse characteristic. This may lead to the difference in the metal content of samples which have to contain these types of Bhasma. For this motive, it is necessary to standardize such type of Bhasma on the basis of their classical tests as well as using the advanced analytical parameters like AAS, XRD, SEM, TGA etc. The present study was conducted to perform physicochemical characterization for the Kasisa Bhasma as per the procedure mentioned in the literature by using several analytical tools.

MATERIAL AND METHODS

The analytical study was conducted at Department of Metallurgy, Indian Institute of Technology, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

Procurement and authentication of raw materials

Raw Kasisa was procured from a genuine supplier of Varanasi and authenticated as per their accepted characters (Grahya Lakshan) specified in the authoritative texts of Rasa-Shastra.

Shodhana of Kasisa

Shodhana of Kasisa was done by giving Bhavana (dry through trituration) with lemon juice for one day⁷.

Marana of Kasisa Bhasma

The Kasisa Bhasma was prepared by incineration process in this process Shodhita Kasisa was kept in a mortar and triturate with Kanji (sour gruel). After trituration uniform size, thick and small pellets of Kasisawas prepared. The sun dried pellets of Kasisa were kept in an earthen pot. The earthen pots were smeared seven times with mud and cloth and subjected to the heating system (Laghuputa). This process was repeated for seven times after that bright red colour of Kasisa Bhasma was formed⁷.

Figure 1: Photograph Showing A. Raw Kasisa, B. Triturate With Mortar Pestle, C. Soaked With Lemon Juice, D. Raw Kasisa Triturate With Lemon Juice, E. Shodhita Kasisa F. Shodhita Kasisa in powder form, G. Triturate with Kanji for pellet preparation, H. Pellet of Kasisa, I. Pellet in Sarav Samputa, J. Incineration process, K. Temperature detector, L. Sarav Samputa after Incineration Process, M. After 1 Puta color changed, N. Again given Bhavana with Kanji, O. Pellet of Shodhita Kasisa, P. Pellet of Kasisa Bhasma

Physicochemical evaluation

Raw, Shodhita Kasisa and Bhasma were analyzed by using organoleptic parameters, Bhasma Pariksha (Test of accurately prepared Bhasma), Scanning electron microscope, Energy dispersive X-ray analysis, and X-ray diffraction analysis.

Bhasma Pariksha

Bhasma Pariksha is a very important part in the field of Ayurveda to ascertain the fact that the prepared Bhasma are best in quality so that maximum therapeutic results can be obtained from it. Therefore, to conclude the fact that Bhasma prepared are standard and genuine, Ayurvedic literature prescribes the following tests⁵.

- Rekhapuranatva (enterability in the furrows of the finger tips)
- Varitaratva (Floatability on still surface of water)
- Anjanabhatva (Fineness like collyrium)
- Nischandratva (lustrelessness)
- Mridutva (softness on touching)
- Slakshanatva (smoothness to touch)
- Nirdhumatwa (smokelessness)
- Apunarbhavatva/Niruthatva (Irreversibility)
- Gatarasatwa (Tastelessness)
- VisistaVarnotpatti (development of specific colour)

Atomic absorption spectroscopy Study⁸

Preparation of the samples

250 mg of the raw, Shodhita and Bhasma of Kasisa were taken in a beaker and mixed with 25 mL of aqua-regia. It was heated slowly till a complete solution was formed. The digested sample is filtered through a filter paper into a volumetric flask and diluted to 250 mL by adding distilled water drop by drop. This solution was used for the determination of metallic elements present in the samples.

Detection of the metals in the sample

All three sample of Kasisa were aspirated and mixed with combustible gasses and was ignited in a flame having temperature ranges from 2100 to 2800°C. The desired element in the sample was reduced to the ground state, which absorbs light at a characteristic wavelength. A light ray from a lamp whose cathode is the makeup of the diverse elements being determined was passed throughout the flame. A device such as a photon multiplier was used to detect the amount of the reduction of the light intensity due to absorption by the analyte. The amount of reduced light intensity is directly proportional to the amount of element present in the sample.

X-ray diffraction study⁹

A small quantity of powder of all three sample of Kasisa was crushed to very fine size in an agate mortar. These powders were then mounted on the sample holder of a commercial high-resolution X-Ray power diffractometer fitted with a curved monochromator. This diffractometer operates on "Bragg-Bretano geometry". An 18 kw rotating anode generator was used as a source of X-Ray. This machine was manufactured by Rigaku (Model No. Rint 2000/PC Series). The XRD scans were made on randomly oriented samples from 2θ value- 10-60° with a step size of 0.02° and 1 sec time per step. The data were collected in fully automatic mode and stored in a computer.

Scanning electron microscopy Study¹⁰

The mounted sample was placed inside the microscope's vacuum column through the air tight door, and then the air was pumped out. After the air was pumped out of the column, a high electron beam was emitted by an electron gun from the top. The ray run downward through a chain of magnetic lenses designed to focus the electrons to a very fine spot. The scanning loop which prepared the fixed beam to move backward and forward

transversely on all the samples of Kasisa mounted on close to bottom line by line.

RESULTS

Table 1: Physical Characteristic

Characters	Raw Kasisa	Shodhita Kasisa	Kasisa Bhasma
Colour	Pale Green	Greenish yellow	Brick Red
Odour	Metallic	Metallic	Metallic
Taste	Sour	Sour	Tasteless
Touch	Rough	Smooth	Smooth
Appearance	Crystalline	Crystalline	Amorphous

Table 2: Organoleptic Characters

Sample Properties	Raw Kasisa	Shodhita Kasisa	Kasisa Bhasma
Colour	Green	Greenish White	Brownish Violet Red
Odour	Metallic	Metallic	Metallic
Taste	Astringent	Metallic	Tasteless
Consistency	Soft	Soft	Soft

Table 3: Observation of Bhasma Pariksha

Parameters	Kasisa Bhasma
Varna	Brownish Violet Red
Sparsha	Soft
Gandha	Metallic
AmlaPariksha	Nirmalatva
Rekhapurinatva	+
Varitaratva	+
Anjanabhatva	+
Nischandratva	+
Mridutva	+
Slakshanatva	+
Nirdhumatva	+
Apunarbhatva	+
Gatarasatva	+
Visista Varnotpotti	+

Table 4: Results of Pharmaceutical Study

Initial Wt. of Crude Kasisa (gm)	Wt. of Shodhita Kasisa (gm)	Loss (gm)	% Loss	Wt. of Kasisa Bhasma(gm)	Loss (gm)	% Loss
500gm	360 gm	140 gm	28%	210 gm	150 gm	41.66%

Table 5: Temperature Pattern observed in puta system of heating for 20 cow dung cakes at 15 minutes interval

Time (min)	Temperature (°C)
0	26
15	80
30	225
45	390
60	550
75	690
90	670
105	650
120	620
135	580
150	535
165	480
180	415
195	350
210	270
225	200
240	140
255	80
270	30

Figure 2: TGA analysis of Kasisa Bhasma

Table 6: TGA analysis of Kasisa

Time (min)	Sample weight (mg)	Temperature (°C)
0	15.175259	30.2700
5	13.316482	108.2300
10	10.229187	213.1300
15	9.248300	316.1100
20	8.971083	419.6400
25	8.735284	520.6400
30	7.550594	620.6100
35	4.713122	719.4100
38	4.647379	789.1400

Table 7: Presence of different elements in the sample

Sample	Weight of elements in percentage			
	Fe	Zn	Pb	Cd
Raw Kasisa	27.6	0.022	0.001	0.001
ShodhitaKasisa	43.1	0.014	0.001	0.000
KasisaBhasma	63.3	0.009	0.000	0.003

Figure 2: Energy Dispersive X-Ray Analysis of raw Kasisa

Table 8: Microanalysis Report of Raw Kasisa

Element	Wt%	At%
OK	50.45	73.22
SK	20.03	14.51
FeK	29.52	12.27
Matrix	Correction	ZAF

Figure 3: Energy Dispersive X-Ray Analysis of Shodhita Kasisa

Table 9: Microanalysis Report of Shodhita Kasisa

Element	Wt%	At%
OK	31.30	58.15
SK	21.38	11.56
CaK	00.84	00.76
FeK	46.48	29.54
Matrix	Correction	ZAF

Figure 4: Energy Dispersive X-Ray Analysis of Kasisa Bhasma

Table 10: Microanalysis Report for Kasisa Bhasma

Element	Wt%	At%
OK	30.66	29.26
SK	00.86	01.18
FeK	68.48	69.56
Matrix	Correction	ZAF

Figure 5: Scanning electron microscopy of Kasisa [A] Raw Kasisa, [B] Shodhita Kasisa, [C] Kasisa Bhasma

Figure 6: XRD of Raw Kasisa

Figure 7: XRD of Shodhita Kasisa

Figure 8: XRD of Kasisa Bhasma

JOINT COMMITTEE ON POWDER DIFFRACTION STANDARDS (JCPDS)

JCPDS is a committee setting up XRD standards of various substances in powder form. Thus we generally compare the XRD curves of all three samples of Kasisa with those of the standards to conclude the elements present in the sample responsible for giving sharp and small peaks. The XRD curve of raw and Shodhita Kasisa corresponds to JCPDS File (No. 22-633) of XRD which identified the peaks as FeO, Fe(OH)₂, FeSO₄, FeSO₄ (H₂O), Fe(OH)₃, Fe₂O₃ and FeS₂. The XRD curve of Kasisa Bhasma corresponds to JCPDS File No. 85-1436 which identifies Fe₂O₃ as the compound solely responsible for all the peaks.

DISCUSSION

Efforts were made to prepare Kasisa Bhasma from raw Kasisa by adopting a classical reference of 'Rasa Tarangini' for its Shodhana and Marana purpose. The first step of pharmaceutical processing of Kasisa Bhasma is Shodhana of raw material. The objective of Kasisa Shodhana is mainly to remove unwanted materials from Kasisa and to enhance its potency¹¹. In the present study, Bhavana method was adopted in Shodhana process with lemon juice as media. Lemon juice is a rich supplier of vitamin C and vitamin B complex, which acts as an intrinsic-factor in the absorption of iron in the body providing a synergistic effect¹². The color of Shodhita Kasisa was pale green, fine powder, and soft, smooth in touch (Table 1). On exposing the sample to a gentle heat, the color of Shodhita Kasisa became changed to white due to loss of water. For the preparation of Kasisa Bhasma, Shodhita Kasisa was subjected to Bhavana with Kanji (Sour gruel)¹¹. After completion of one Bhavana, pellets of

the Shodhita Kasisa was prepared. The dried pellets of Kasisa was kept in a Sharava Samputa (closed earthen vessel) and heated to almost 650°C by applying traditional Puta (incineration cycles) method (Laghu Puta) using 20 cow dung cakes. This process was repeated seven times after that bright red colored Bhasma was obtained. The Bhasma were subjected to Samanya and Vishesh Pariksha to ascertain the fact that they were properly prepared or not. Rekha-purnatva, Dantagrenakachakachaiti and Anjana Sadrishya Sukshmatva indicate the fineness of the Bhasma (Table 3). Varitaratva indicates the lightness (density) of the Bhasma. Nischandratva indicates the absence of metallic luster in the Bhasma. The Kasisa Bhasma prepared to pass the entire Bhasma Pariksha (Tests)⁵. The physical analysis of raw, Shodhita and Bhasma of Kasisa was mainly done to know the physical characteristics, nature and various properties of the Kasisa (Table 1). The TGA analysis of Kasisa has confirmed the changes in physical and chemical properties of Bhasma that it undergoes transformation. The peak temperature obtained for 20 cow dung cake given in Laghu Puta was 670°C which remained for almost 10 minute in that intensity. The decomposition of Kasisa starts at 300°C and gets calcined with an increase in temperature. The Shodhita Kasisa was heated from 30°C to 800°C at an increasing temperature of 20°C per minute in a thermo balance to observe the effect of temperature on the way to the formation of Kasisa Bhasma. As the temperature was increasing various components of the Shodhita Kasisa was started to decompose. The weight percentage of Shodhita Kasisa was calculated after changing its mass. The gradual decreased in weight of Shodhita Kasisa with respect to increasing temperature or time, which confirms the fact about the rate and extent of decomposition of the Kasisa. The analysis gives us an idea the nature of thermal decomposition that takes place in unprocessed Kasisa when

subjected to the formation of Bhasma. The Atomic absorption spectrophotometer (AAS) was help in the quantitative determination of metal which is present in Kasisa Bhasma. The analysis was done by using the different lamp for different heavy metals to detect their presence in the sample and to determine the iron percentage of each sample (Table 6). The percentage of iron determined by this method was found to be 27.6% in raw, 43.1% in Shodhita and 63.3% in Bhasma of Kasisa whereas zinc, cadmium, and lead was found to be very less amount in all samples (Table 7-9). For elemental and particle size analysis of Kasisa, various sophisticated instruments were used viz. XRD, SEM, and EDAX. The XRD studies of Kasisa were performed by subjecting it to grinding into a very fine powder form manually. Then powder diffraction study was performed, and the XRD spectra was comprise strong medium and weak peaks were matched with the standard peaks recorded in JCPDS suggest the presence of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, FeSO_4 , $\text{FeO}(\text{OH})$, $\text{Fe}(\text{OH})_3$, Fe_2O_3 , FeS_2 .

The appearance of FeS_2 cannot be explained by any redox reactions. It may be present as impurity if the sample is exposed to H_2O . The XRD curve of Shodhita Kasisa was more or less same as raw Kasisa. The XRD pattern of Kasisa Bhasma shows that all the strong medium and weak peaks correspond only to Fe_2O_3 . Thus the XRD study clearly reveals the structural changes that it has undergone from its raw state to final product. The XRD finding justifies the fact that structural deformity occurred due to thermal agitation, during the Bhasmikarana process of Ayurvedic minerals (Figure 6-8)^{13,14}. In addition, some of the peaks also match up to organometallic compounds, due to purification and incineration process phyto-constituents of plant material may be reaction with a metallic compound. A scanning electron microscope study was done to expose very minute details about the surface morphology of the different samples of Kasisa (Figure 5). The data obtained from SEM confirmed the reduced particle size by purification and incineration process. The particle sizes of the raw, Shodhita and Bhasma of Kasisa were found to be 90 μm , 2 μm and 350nm. The EDAX analysis of Kasisa revealed that Kasisa Bhasma contained the highest percentage of iron in comparison with unprocessed and purified Kasisa (Figure 2-4). This may be concluding the fact that highest percentage of iron in Kasisa Bhasma was confirming the formation of Fe_2O_3 ¹⁵.

CONCLUSION

Chemically Kasisa Bhasma is the ferrous sulfate (FeSO_4) containing more amount of iron than sulfur along with other elements in traces. The SEM study reveals that fine particle of Kasisa Bhasma in the range of 10-100nm and such particles are found in the range of 350nm. Due to the calcination processes agglomerates, particles are formed. AAS, EDAX, XRD, and elemental analysis result confirmed that Kasisa Bhasma contained iron and sulfur as major element, therefore, the result of this research study will help in the founding well-

documented provision for the quality of Kasisa Bhasma along with well-documented manners for substantiating that quality.

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