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Analytical study of *Vanga Bhasma*

Research article

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Abstract

Bhasma is a special dosage form as mentioned in *Ayurveda* texts. It is an incinerated metal or mineral prepared after several rounds of processing. Properly prepared *Bhasmas* have been proved to work wonders in clinical practice. Quality of a drug depends upon its formulation, processing and applications. It is essential to fix some standards for manufacture of drugs so that the genuineness of the drug is not compromised. There have been concerns regarding the safety and efficacy of *Ayurvedic* drugs mainly the *Bhasma*. Keeping this fact in mind, the *Vanga Bhasma*, which has been acclaimed to be efficacious in *Sukra Dosa*, and also prevents *sukrakshaya* was prepared for the present study and analyzed for quality control checks, on the parameters described in *Ayurvedic* texts as well as modern technology. The ancient methods of organoleptics were conducted and the modern parameters like, EDX, XRD, FTIR were done to find out the nature and form of the drug prepared. After analyses, it was inferred that the drug was converted into its oxide form and had a highly reduced particle size. Study also confirmed the formation of organometallic compound at the end of the manufacturing process.

Key Words: *Vanga Bhasma*, X-Ray Diffraction, FT-IR, EDX,

Introduction

Analytical study is the application of a process or a series of processes in order to identify and/or quantify a substance, the components of a solution or mixture, or the determination of the structures of chemical compounds.

Quality of a drug depends upon its formulation, processing and applications. It is essential to fix some standards for manufacture of drugs so that the genuineness of the drug is not compromised. Ayurvedic texts have described several methods for quality control of finished products like *Varitaratva, Nishchandratva, Nirutha* etc. to achieve a specific acceptable standard *Bhasma*.(1) There have been concerns regarding the safety and efficacy of Ayurvedic drugs mainly the *Bhasma*.(2) But as far as the *Bhasma* are concerned, Rasacharyas have described various parameters for its qualitative evaluation.

Ayurvedic classical texts have taken serious note of the potential toxicity of certain herbs, minerals, and metals. Traditionally, Ayurvedic drugs are purified through *Shodhana* which is aimed at reducing the drug toxicities through different physical & chemical processes.(3) The actions of medicines as described in Ayurveda are through their various properties like *Rasa, Guna, Virya, Vipaka and Prabhava*, based inherently on

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their eternal composition.(4) It is the need of the hour to use modern technology to explore the relevance of these concepts so that, they may be interpreted in the light of contemporary scientific language to make it relevant with the modern health care.

With this aim, the *Vanga Bhasma* was prepared and analyzed for quality control, on the parameters described in Ayurvedic texts as well as modern technology.

Material and Methods

Procurement of Raw Material:-

The raw material, namely *Vanga* was procured from the Charak Govt. Ayurvedic Pharmacy, Paprola, Distt. Kangra, Himachal Pradesh.

Preparation of *Vanga Bhasma*:-

It included three stages namely, *Shodhan* (purification), *Jaran* and *Maran* (incineration).

I. *Shodhan- Samanya*(5) and *Vishesha*(6):- Raw Vanga was heated to red hot stage & then quenched in *Tila* oil, *Takra, Gomutra, Kanjika, Kulattha Kwath* respectively 7 times each. Then, *Vishesha shodhan* was performed and *samanya shodhit Vanga* was quenched in *Nirgundi Kwath* mixed with *Haridra* powder for 3 times.

II. *Jaran*:- *Shodhit Vanga* was put in an Iron vessel & heated over flame till it melted. Then equal quantity of *Asvatha* bark was added to it and rubbed till it turned into powder form.(7)

III. *Maran:-Maran* was done with *hingul* media.(8) *Jarit Vanga* was put in a pestle – mortar & *Hingul* ($1/8^{th}$) was added to it and levigated with Aloe vera pulp. Then contents were dried, cut into pellets & subjected to heating at a temperature of 900C in an electric furnace. This process was repeated 10 times till *Bhasma* was obtained.

Observations and Results

The observations were made on the basis of the features mentioned in the classics, Macroscopic and Microscopic description, Physico-chemical tests, Qualitative/Quantitative tests like SEM (Scanning Electron Microscope), XRD (Phase Identification of Diffractogram using X-ray Diffraction), FT- IR (Fourier Transform Infrared Spectrometry), PSA (Particle size Distribution) and EDX (Energy-Dispersive X-ray spectroscopy).

The organoleptic analysis as mentioned in the texts of Ayurveda revealed that it was very soft *sparsh* (touch). The *varna* (colour) of the *Vanga Bhasma* was greyish light pink. The *Bhasma* did not produce any taste when kept on tongue, nor did it emit any odour when it was smelt. These tests were also conducted at Government Drug Testing Lab which also revealed the same results. The appearance of the drug was fine powder and pH was 8.75. (Table-1)

Table-1, Organoleptic Characterization of *Vanga Bhasma*

Parameters	**Vanga Bhasma**
Varna (Colour)	Light pink
Rasa (Taste)	Tasteless
Gandha (Odour)	Odourless
Sparsh (Touch)	Very Soft
Varitaratva	Positive
Rekhapurnatva	Positive
Nishchandratva	Positive
Apunarbhava	Positive
Appearance	Fine powder
pH	8.75

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Among the Physico- Chemical Tests total ash value of the *Vanga Bhasma* was determined. This test was performed as per the protocol stated in Ayurvedic Pharmacopoeia of India.(9) A total of 3 g accurately weighed, Bhasma was incinerated in a tarred silica dish at a temperature not exceeding 450º until free from carbon, It was allowed to cool and weighed. The percentage of ash with reference to the air-dried drug was calculated and was found to be 99.75%.

Acid insoluble ash was calculated by boiling the ash obtained previously for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated and found to be 93.15%. Similarly Water Soluble Extractive (WSE) was calculated. The sample was weighed 5gm. To it 50ml of distilled water was added and kept covered overnight. It was stirred intermittently in the initial period. Next day, it was filtered. 20ml of the filtrate was accurately measured with a pipette and transferred to the already weighed evaporating dish. The evaporating dish was placed on a water bath for evaporation of the water. After evaporation of the water it was dried at 105^0C weighed immediately. From the weight of the residue obtained, the percentage of water soluble extractive was calculated and was found to be 0.37 %w/w. Alcohol soluble extractive (ASE) of the sample was determined in the similar way like water soluble extractive by using Alcohol instead of water and was found to be 0.86%. (Table- 2)

Table 2 Results of Physico- chemical tests

S. No.	Test	Vanga Bhasma
1	Total ash	99.75%
2	Acid insoluble ash	93.15%
3	Water Soluble Extractive	0.37%
4	Alcohol soluble Extractive	0.86%

Fourier Transform Infrared Spectrometry (FT-IR) of *Vanga Bhasma* was performed using Perkin Elmer, USA, Spectrum GX, Range 30-15600/cm instrument to detect functional groups and to characterize the covalent bonding information. FT-IR is based on the fact that bonds of particular groups in a molecule vibrate at specific frequencies when exposed to infrared (IR) rays. During FTIR analysis, a spot on the specimen is subjected to a modulated IR beam. The specimen's transmittance and reflectance of the infrared rays at different frequencies is translated into an IR absorption plot consisting of reverse peaks and a resultant graph is produced which is then correlated to the reference table. The resultant FT-IR graph of *Vanga Bhasma* revealed that various functional groups like $-NH_2$ (amide), -OH (hydroxyl), CH_3, O-CH_3, C=O (ester, aldehyde, ketone), C=C, C-H are present. (10),(11),(12) Moreover an interesting fact was observed that organometallic bonds were formed in the *Bhasma.* Sn-O bonding and Sn-C bonding was present in the *Vanga Bhasma*. (Table - 3), (Figure- 1)

Figure – 1, Graph of FT-IR for *Vanga Bhasma*

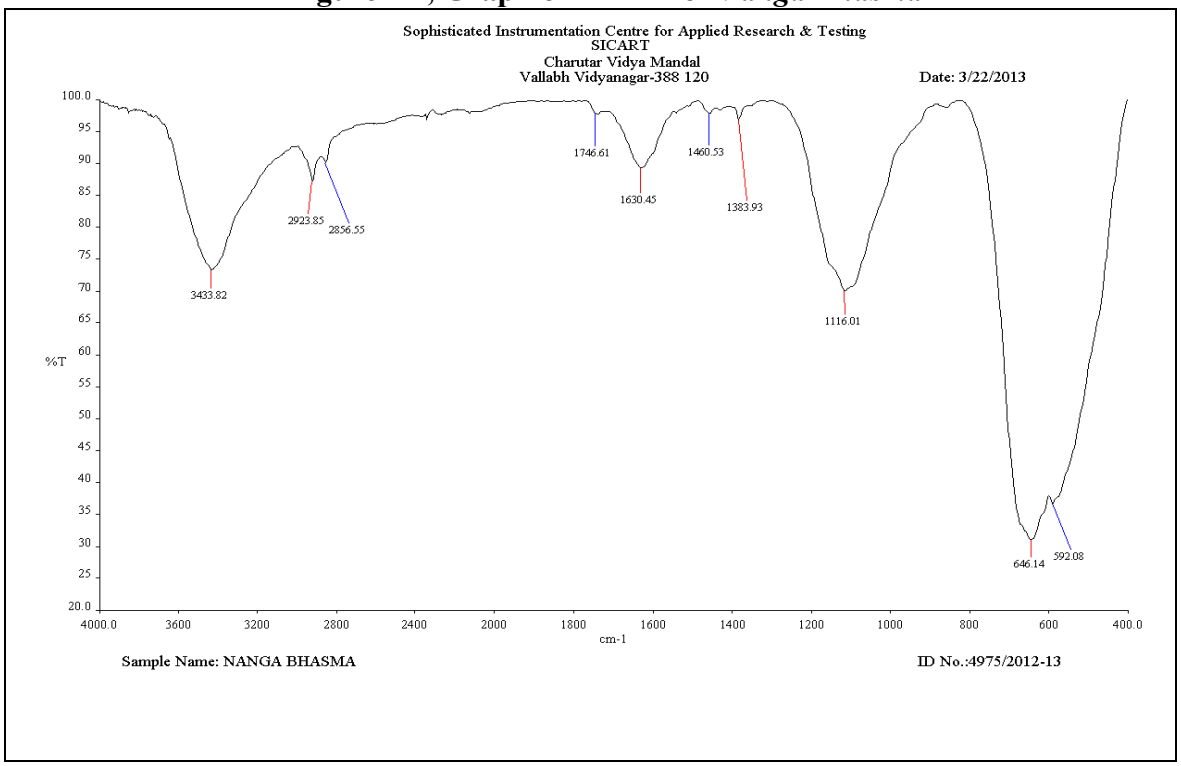

Table- 3, Bonding present in *Vanga Bhasma*

FTIR wavenumber	Bond Present
3433.82	-NH_2 , -OH str.
2923.85	CH_3 str.
2856.55	O-CH_3 str.
1744.57	C=O vibration (ester, aldehyde, ketone)
1630.45	C=C str. C=O, C-H,
1383.93	C-H vibration, fingerprint region
1116.01	C-O vibration, fingerprint region
646.14	Sn-O bonding, fingerprint region
592.08	Sn-C bonding, fingerprint region

Particle size distribution analysis of the sample showed the Volumetric Mean diameter of the *Bhasma* as 7.63µm. About 50% particles were of the size range 5460 nm falling in the category of coarse nanoparticles, while 16% particles were below 2020 nm falling under the category of fine nanoparticles. (Figure- 2)

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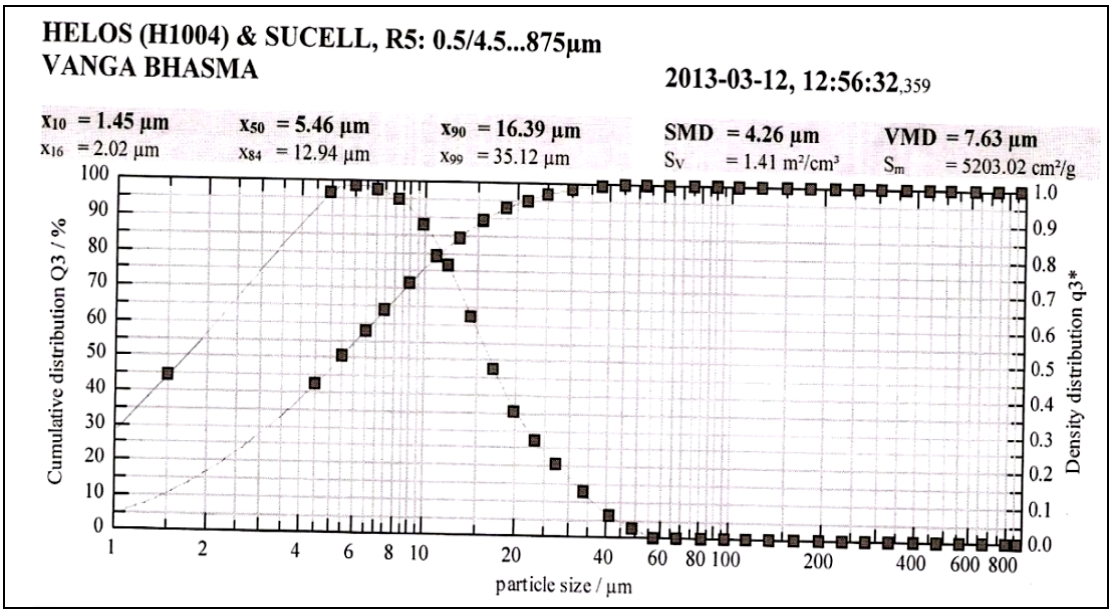
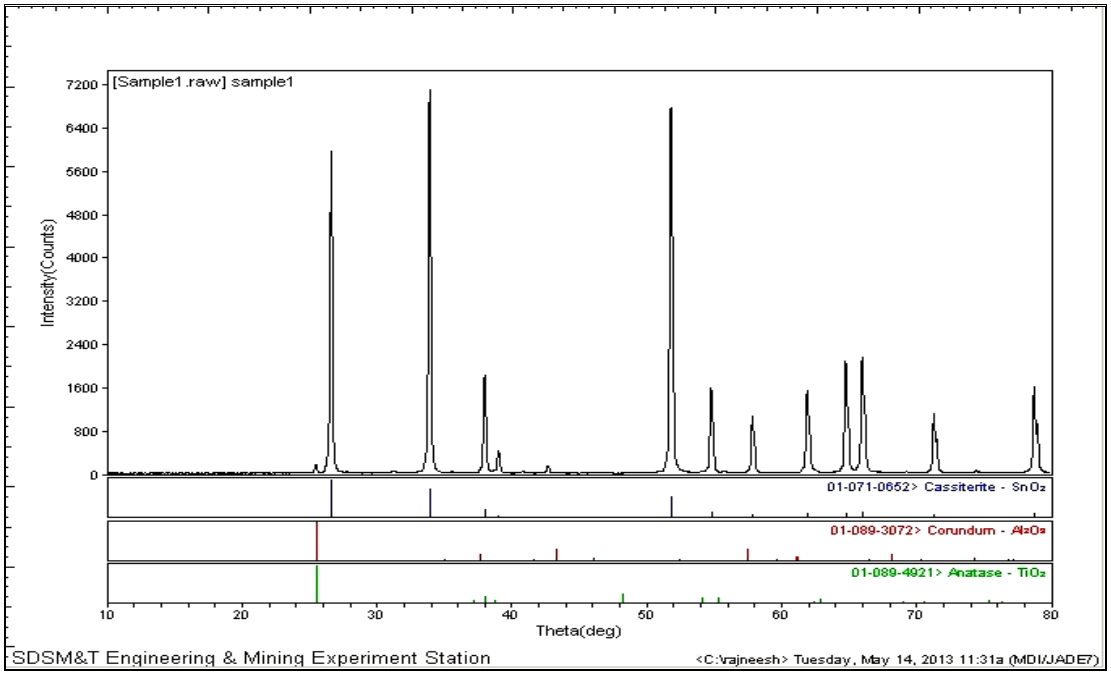
Figure- 2, Particle Size Distribution of *Vanga Bhasma*



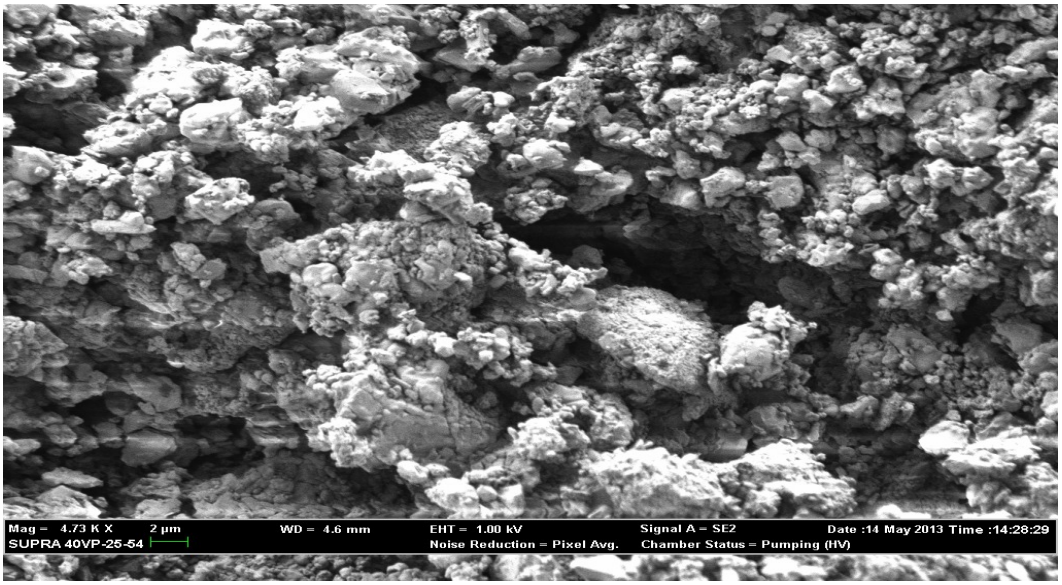
X-ray powder diffraction (XRD) analysis was conducted on the sample. The XRD showed that *Vanga Bhasma* has a crystalline structure. The major component (over 95%) is Tin Oxide, possibly Cassiterite and Aluminium oxide. The predominant peaks in Sample (Vanga Bhasma) correspond to major phase comprising SnO_2 (Figure -3).

Figure -3, XRD graph of *Vanga Bhasma*



Scanning electron microscopy (SEM) yielded high resolution Figures of the sample surface. SEM Figures showed a characteristic three dimensional appearance and were useful for determining the surface structure of the sample. (Figure-4)

Figure -4, SEM Figure of Vanga Bhasma



It is observed from SEM Figures that particles of Vanga Bhasma show granular appearance and porous morphology. There is no particular pattern in structure.

Energy-Dispersive X-ray spectroscopy (EDX) analytical technique was used for elemental analysis or chemical characterization of the sample. It showed the presence of Na, Mg, Al, Si, S, Fe & O in the *Bhasma.* (Figure-5, Table-4)

Figure-5 EDX of Vanga Bhasma

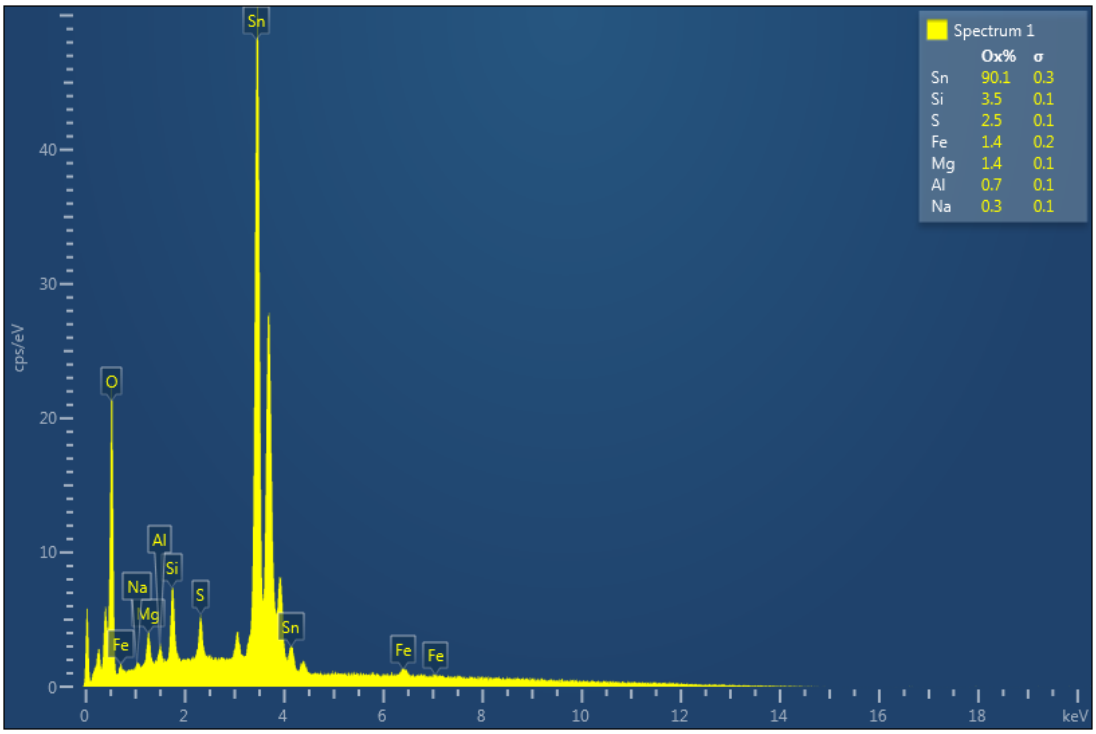


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Table-4, EDX Analysis Report

Element	Wt%	Atomic %	Oxide	Oxide %
O	23.82	66.02		
Na	0.21	0.41	Na_2O	0.29
Mg	0.84	1.54	MgO	1.40
Al	0.39	0.63	Al_2O_3	0.73
Si	1.64	2.59	SiO_2	3.51
S	1.02	1.41	SO_3	2.54
Fe	1.11	0.88	FeO	1.42
Sn	70.97	26.52	SnO_2	90.11
Total:	100.00	100.00		100.00

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Discussion

The macroscopic tests were in compliance to the analytical standards as mentioned in Ayurveda texts. The colour of the *Bhasma* was light pink, as it was prepared by *Hingul* media. This colour of the *Bhasma* was in concurrence to the works done earlier.(13) The *Bhasma* complied with the *varitaratva* test. Floating of the *Bhasma* particles over water is due to surface tension over water and low specific gravity of the *Bhasma*. The particles of *Bhasma* were very soft to touch and had a fine powder appearance.

FT-IR is most frequently used for characterization of organic molecules in a particular sample. It offers quantitative and qualitative analysis for organic and inorganic samples and identifies chemical bonds in a molecule. The major finding in the FT-IR was that the *Bhasma* is an organometallic compound. Formation of a bond between tin (Sn) and carbon (C) (Sn-C) was formed and appeared at wavelength of 592.08. Bonding at wavenumber 646.14 represented the bond formed between Sn and Oxygen (O).

Particle size analysis is an objective parameter for the assessment of subjective property of *Bhasma* called '*Rekhapurnatva*' which is mentioned in our Ayurveda classics. Smaller the particle size, larger is the surface area and greater are the chances of absorption. Moreover, the particle size of the sample corresponds to coarse nanoparticles.(14)

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions of the molecules present in the sample. The most widespread use of powder diffraction is in the identification and characterization of crystalline solids, each of which produces a distinctive diffraction pattern. The XRD of *Vanga Bhasma* showed its crystalline structure. The major component (over 95%) was Tin Oxide, possibly Cassiterite. The predominant peaks in the sample (*Vanga Bhasma*) corresponded to major phase comprising SnO_2. During the synthesis of nanomaterials, amorphous materials are subjected to calcination to transform the same to crystalline materials, with degree of crystallinity increasing with increasing calcination temperature.(15) Vanga Bhasma is also prepared by calcination at a high temperature of 800-1000 °C. This also facilitates the formation of nano sized *Vanga Bhasma* particles.

Energy-Dispersive X-ray spectroscopy (EDX) is an analytical technique used for elemental analysis or chemical characterization of a sample. It relies on the investigation of an interaction of some source of X-ray excitation and a sample. This analysis confirmed the

presence of various elements viz. Na, Mg, Al, Si, S & Fe in their oxide form. The major percentage was tin oxide. The source of the other elements can be attributed to the fact that various processes involving different herbal drugs were used in the pharmaceutical manufacturing of *Vanga Bhasma*. The processing containers and *sarava* (earthen vessel) used during *puta* (calcination) may also have contributed to the addition of these elements.

Scanning electron microscopy (SEM) is an analytical technique that uses electron beam rather than light to form an Figure. It is capable of producing high resolution Figures of a sample surface, which means that closely spaced features can be examined at a high magnification. Due to the manner in which the Figure is created, SEM Figures have a characteristic three dimensional appearance and are useful for determining the surface structure of the sample. It was observed from SEM Figures that particles of Vanga Bhasma showed granular appearance and porous morphology. The particles were adhered together as agglomerates. There was no definite pattern in their structure.

Conclusion:

The present study reaffirms the fact that Bhasma are nano sized particles that have been used in Ayurveda since centuries. *Vanga Bhasma* is in fact organometallic compound comprising of Sn-C bond and tin oxide (SnO_2) as major phase. The processing for the preparation of Bhasma adds into it various micro elements essential for the body. These elements come from the herbal ingredients used in various sub processes of *Bhasma nirman.*

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