

Comparative study of Marketed samples of *Yeshada Bhasma*, An Ayurvedic Medicinal Preparation based on XRF, XRD and Particle Size Analysis

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Abstract

Yeshada Bhasma, marketed by various drug manufacturers is observed prepared by using different methods. The label *Yeshada Bhasma* prohibits differentiation between these products. Prepared, using different plant materials, these formulations differ in characteristics. The situation underlines the need to assess the uniformity and difference in their physical and chemical characteristics. Considering this need, seven marketed samples of *Yeshada Bhasma* were analysed in this study with an object to study the uniformity or its absence in their physico-chemical characteristics, using XRF, XRD and Particle size analysis. Hexagonal crystals of ZnO were detected in all the samples irrespective of their method of preparation in this study. In addition, ZnS, ZnSO₃ and ZnSO₄ were also detected in two samples. Zinc and light elements, the principal elements, with iron, calcium and other elements in traces to significant amounts, were detected in the analysed samples. Not much difference was observed in the particle size and shape distribution of these samples. The present study concludes that variation in physico-chemical characteristics observed in the marketed samples of *Yeshada Bhasma* is likely to affect its therapeutic efficacy.

Key words: *Yeshada Bhasma*, Zinc oxide, X-Ray Fluorescence (XRF), X-ray Diffraction (XRD), Particle Size Analysis

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Key message

- *Yeshada Bhasma* prepared by different methods is found marketed by Ayurvedic drug manufacturers with the same name on the label.
- Physico-chemical characteristics of *Yeshada Bhasma* are observed varying from product to product and batch to batch in the marketed products.
- Variation in physico-chemical characteristics is likely to affect the therapeutic efficacy of the drug. While prescribing the drugs, physicians should take this issue into consideration.
- Absence of product uniformity appears to be a burning issue in marketed Ayurvedic drugs. This study underlines a dire need to standardize the SOPs of preparation of

Bhasma and similar Ayurvedic drugs with an object to bring such uniformity.

- Strict quality control measures need to be adopted in preparation of drugs by Ayurvedic drug manufacturers.

Introduction

Bhasma is a class of Ayurvedic mineral-metallic medicinal preparation, always debated for its therapeutic efficacy by Ayurvedic practitioners on one side and receiving a harsh criticism by conventional medical professionals for being harmful to the human health due to its toxic metal ingredients on the other side. Research articles reporting presence of heavy metals in *Bhasma* preparations and scattered case reports holding them responsible for causing liver injury are found published every now and then.^{1,2,3} On the other side experimental studies producing

evidence of safety and efficacy of *Bhasma* preparations in therapeutic doses are also found published.^{4,5} In either case, use of *Bhasma* preparations by Ayurvedic practitioners is on the rise and many *Bhasma* preparations are manufactured and sold in the market. *Bhasma* preparations are prepared from naturally occurring minerals and metals, and are processed with multiple herbal, mineral and animal products. In addition, traditional process of preparation of *Bhasma* involves manual interventions, producing considerable variation in physical characteristics and chemical composition of the final product, the *Bhasma*. Currently, technology has reduced manual interventions to a considerable extent but variation in product characteristics affecting the quality appears to be still an issue of concern. Moreover, different methods of preparation of *Bhasma* of the same metal or mineral are described in Ayurvedic classics. Manufacturers are free to select any of these methods. Thus, a *Bhasma* of one single metal / mineral bearing the same name on the label, is prepared differently by different manufacturers using different methods of processing with widely varying processing materials. Therefore, *Bhasma* formulations bearing the same name marketed by different manufacturers are likely to differ widely in their physical characteristics and chemical composition as well, affecting their pharmaceutical characteristics and therapeutic activity. However, the end user of the *Bhasma*, a patient or an Ayurvedic practitioner prescribing the same, is very unlikely to be aware of this difference. On this background quality of these products ever remains questionable. Government of India has published drug monographs in Ayurvedic Pharmacopeia of India, prescribing pharmacopeial quality standards mandatory to be followed by Ayurvedic drug manufacturers. However, recent studies indicate that few marketed Ayurvedic drugs qualify these quality standards. In a recent XRF and XRD analytical study, only one marketed sample out of the seven samples of *Svarnamakshika Bhasma*, was found to qualify pharmacopeial quality standards prescribed by API.⁶ On the background of widely rising criticism of Ayurvedic mineral and metal medicinal preparations, particularly

with respect to concern on their safety, it is necessary to ensure their quality. In this regard manufacturers need to maintain uniformity in the physico-chemical characteristics of their marketed products as required by regulatory guidelines.

In furtherance of our studies in this direction, a comparative study of multiple samples of *Yeshada Bhasma* considering its wide use by Ayurvedic practitioners for various ailments like diabetes, and urinary and male sexual disorders, was undertaken. Few studies on physico-chemical characterization of *Yeshada Bhasma* are found published in the past but comparative studies on multiple marketed samples of *Yeshada Bhasma* are not much known.

On this background the present study compares seven marketed samples of *Yeshada Bhasma* using X-Ray Fluorescence (XRF), X-Ray Diffraction (XRD) and Particle size and shape analysis. XRF study is used to detect the elemental composition of a formulation. Whereas, XRD detects the compounds present in it along with the crystal shape of the particles of detected compounds. The element and compound composition affects the therapeutic efficacy of the drug. The particle size and shape along with crystal shape of the particles affect the absorption and bioavailability of the drug. Thus, all these characteristics have a determining effect on the overall pharmacodynamics of the drug, which is a subject of future study.

In this study seven samples of *Yeshada Bhasma* manufactured by six different manufacturers were purchased from the domestic market. In these seven samples, two samples from two different batches manufactured by the same manufacturer were also collected to assess the product uniformity in two batches. All the samples are marketed with one and the same name '*Jasada Bahsma*' referred by its synonym '*Yeshada Bhasma*' in this article, and as a classical Ayurvedic medicinal preparation requiring mandatory display of reference of the Ayurvedic classical text on the label. Labels on two samples refer classical text *Rasatantrasara*

Siddhaprayoga Sangraha, two refer Rasatarangini, one refers to Ayurveda Sara Sangraha, and another one refers to Rasarajsundar. However, preparation method of one sample could not be known as it is not mentioned on the label. *Yeshada Bhasma* referring Rasatarangini is prepared by using Mercury and Sulfur in its processing. However, other products are prepared by using plant material only. *Yeshada Bhasma* prepared by processing Zinc metal with various herbal, animal and mineral materials is reported to be converted into Zinc oxide as its principal component in the end product.⁷⁻¹¹ In addition to ZnO, traces of other elements are also reported detected in *Yeshada Bhasma*. Apart from its chemical composition, physical characteristics such as particle size and crystal shape and amorphous content of *Bhasma* also play a significant role in determining its pharmacological action. Most interesting part is that *Bhasma* preparations are referred as a form of nano-medicine by some experts. The present study is undertaken to study all these issues using XRF, XRD and Particle size distribution analysis for seven marketed samples of *Yeshada Bhasma*. The study has confirmed earlier findings regarding presence of Zinc Oxide and trace elements. However, it raises questions regarding referring the *Bhasma* formulations as nanoparticles. Bhowmik et al in their study concluded that *Yeshada Bhasma* contains oxygen deficient ZnO. The present study raises concern and suggests additional analysis by other techniques for validation of this conclusion as well.

Materials and Methods

Seven samples of *Yeshada Bhasma* manufactured by six different manufacturers using classical methods and Zinc Oxide (AR grade) 98% purity, collected from the local market were subjected to XRF, XRD and Particle size distribution analysis. Vanta handheld X Ray Fluorescence (XRF) Analyzer, Terra-II portable X-ray Diffraction analyser and BIOVIS PSA 2000 Particle size and shape analyser were used for the respective analysis.

X-ray fluorescence (XRF) analysis

Vanta handheld X Ray Fluorescence (XRF) Analyzer used

in this study, is an energy dispersive X-ray fluorescence spectrophotometer. It is used to perform the identification and analysis of elements contained in a test sample from Magnesium to Uranium (Mg to U). The identified elements in this method are quantified up to ppm level. An adequate amount of powdered sample under test is filled in a XRF sleeved sample cup, spreading it evenly to form a flat surface at the opening of the cup. Thereafter, the powder filled open end of the cup is closed by attaching a thin mylar film. Care is taken to keep the surface of the powder flat under the film. The X-ray emitting opening of the Vanta XRF analyser is then positioned directly over the flattened surface of the film covered powder filled sample cup. The test is initiated after setting the time of exposure. Elemental analysis is started by tapping the start button of the analyser. The status bar on the screen indicates the test progress. The results of analysis are displayed on the screen immediately after the completion of the test.

X Ray Diffraction

A monochromatic X-ray beam is used to irradiate a sample in XRD analysis. X-ray diffractogram obtained in the analysis is used to identify the crystallographic structure and chemical composition of the sample. X-ray powder diffraction (XRD) analysis is primarily used for phase identification of a crystalline or amorphous material. It can provide information on unit cell dimensions as well. Terra-II portable X-ray Diffraction analyser was used for XRD analysis of the samples in the present study. The analyser is accompanied with a sample carrier, which is fitted with sample carrier cells. The instrument is useful only for analysis of dry powder samples. The particle size of the sample powder needs to be about 50 micrometers. Approximately 2 g powder is required for the analysis. However, the test can be performed with as little as 20 mg powder. To initiate the procedure the sample powder is loaded in the sample cell fitted to the cell carrier. The sample filled carrier is then inserted in the sample chamber, which is then locked. The diffraction instrument is then connected to the computer. The SwiftMin software

is started and the information of the sample is filled in the dialogue box appearing on the screen. Analysis is then started selecting the appropriate mode and necessary exposures. Results are displayed on the screen as the test progresses. A diffractogram is obtained at the completion of the analysis. The obtained result is then downloaded. The downloaded result displaying diffractogram is then subjected to analysis using X- powder software with PDF2 database. Peaks in the diffractogram related to specific chemical compounds, are then identified at the end of analysis with the help of the PDF2 database.

Particle size and shape analysis

BIOVIS PSA 2000 was used for particle size analysis in this study. The equipment comprises of a microscope and high-resolution digital camera with powerful particle analysis with compliant software. It analyses the images of the sample and distinguishes discrete particles. It obtains complex measurements of these particles based on the computer programming software. The instrument can identify particles ranging from 0.5 microns to 5000 microns. Morphological measurements such as length, breadth, area etc. are evaluated in this analysis.

The particle size of all the seven samples of *Yeshada Bhasma* and one sample of Zinc oxide was analysed by spreading each sample evenly on a slide to obtain a thin film. The slide so prepared was then placed on the platform and the microscope was set appropriately. The image of the sample was then captured. It was then saved on the computer screen. Moving the slide a little on the platform of the microscope, another field was exposed. Image of the particles was again captured and saved on computer screen as earlier. The procedure was then repeated to get images of minimum five fields for all the samples. The results of the particle size analysis of each sample were obtained and saved by computer software at the end of each test.

Results

Chemical analysis of all the samples was carried out using XRF and XRD analysis as described above.

Legends

Zinc Oxide – ZnO, Light Elements - LE

Samples of *Yeshada Bhasma*: DPJB-1, DPJB-2, BDJB, UNJB, KNJB, ABJB, ATJB

Table 1: Elemental composition of the samples – XRF analysis

Sample	Method of Preparation Classical reference	Compounds				
		Zn %	S %	LE %	Fe %	Ca %
ZnO	-----	88.94 +/- 0.38	610 ppm	9.55 +/- 0.38 %	-	1856 ppm
<i>Yeshada Bhasma</i>						
DPJB-1	Rasatarangini 19/104-107	79.36 +/- 0.34	8.118 +/-0.079	10.14 +/- 0.36	7140 ppm	2650 ppm
DPJB-2	Rasatarangini 19/104-107	79.43 +/- 0.41	5.650 +/-0.069	12.36 +/- 0.42	1.494 +/- 0.022	4810 ppm
BDJB	Rasaraja Sundar	82.28 +/- 0.43	720 ppm	11.80 +/- 0.42	1.399 +/- 0.021	1.937 +/- 0.026
UNJB	Rasatantra Sara	84.32 +/- 0.40	3080 ppm	10.15 +/- 0.38	1.599 +/- 0.022	8420 ppm

KNJB	Rasatantra Sara	80.8 +/-1.4	1470 ppm	9.97 +/-0.45	3.42 +/-0.10	8420 ppm
ABJB	Ayurveda Sara Sangraha	84.32 +/-0.4	3080 ppm	10.15 +/-0.38	1.599 +/-0.022	8420 ppm
ATJB	Not known	89.93 +/-0.30	690 ppm	7.24 +/-0.30	3336 ppm	4416 ppm
Average	-----	82.92%+/-3.71		10.26%+/-1.63		

All the seven samples of *Yeshada Bhasma* and one sample of Zinc oxide were subjected to XRF analysis. Zinc content in *Yeshada Bhasma* ranged from 79.36% +/- 0.41 to 89.93% +/- 0.3. with average of 82.92%+/-3.71. Whereas, Zinc oxide was found to contain 89.94%+/- 0.38 Zinc. Another major component in *Yeshada Bhasma* was light elements ranging from 7.24%+/-0.30 to 12.36%+/-0.42 with average of 10.26%+/-1.63. Zinc oxide was also found to contain 9.55% +/-0.38 light elements. Two of the *Yeshada Bhasma* samples DPJB-01 and DPJB-02 contained Sulfur 8.118%+/- 0.079 and 5.650%+/-0.069 respectively. Iron was another element found in significant amount in *Yeshada Bhasma* samples DPJB02-1.494% +/-0.022, BDJB- 1.399% +/-0.021, UNJB and

ABJB- 1.599 %+/-0.022 and KNJB- 3.42% +/-0.10. However, iron content in DPJB-01 and ATJB was insignificant 7140 ppm and 3336 ppm respectively. Iron was found absent in Zinc oxide. Another element Calcium was found in the amount of 1.937% +/-0.026 in only one sample of *Yeshada Bhasma* BDJB. Calcium content in other samples of *Yeshada Bhasma* was in ppm quantities and was found 1856 ppm in Zinc oxide. In addition to the elements listed in the table more than 30 elements were detected in traces amounting to ppm concentrations in all the samples taken together. These elements were Al, Si, P, Cl, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, Sn, Sb, Ba, La, Ce, Pr, Nd, Hg, Pb and Bi.

Table 2: Compounds found in the samples in XRD analysis

Sample	Compounds			
	Zinc Oxide	Zinc Sulfide	Zinc Sulfite Hydrate	Zinc Sulfate Hydroxide Hydrate
Std. Zinc Oxide	ZnO			
<i>Yeshada Bhasma</i>				
DPJB-1	ZnO	ZnS		
DPJB-2	ZnO		ZnSO ₃ !0.5 H ₂ O	Zn ₄ SO ₄ (O H) ₆ !3 H ₂ O
BDJB	ZnO			
UNJB	ZnO			
KNJB	ZnO			
ABJB	ZnO			
ATJB	ZnO			
Crystal shape	Hexagonal	Hexagonal	Unknown	Triclinic

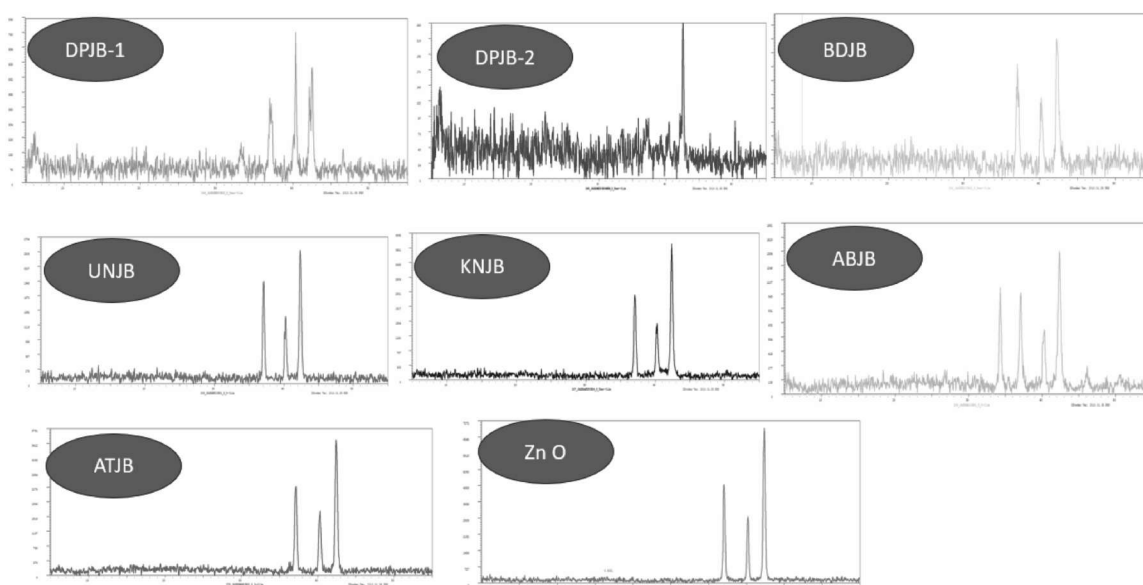
Table 3 - Peak positions of ZnO in X-ray diffractogram

	2 theta	D spacing	Crystal Shape
Std. ZnO	37.022 40.175 42.305	2.8174 2.6044 2.4788	Hexagonal
ZnO in <i>Yeshada Bhasma</i>			
DPJB-1	37.077 40.148 42.361	2.8133 2.6009 2.4757	Hexagonal
DPJB-2	37.050 40.203 42.084	2.8154 2.6026 2.4665	Hexagonal
BDJB	37.022 40.148 42.306	2.8174 2.6061 2.4788	Hexagonal
UNJB	37.022 40.175 42.305	2.8174 2.6044 2.4788	Hexagonal
KNJB	37.022 40.175 42.305	2.8174 2.6044 2.4788	Hexagonal
ABJB	37.022 40.148 42.306	2.8174 2.6061 2.4788	Hexagonal
ATJB	37.022 40.258 42.444	2.8174 2.5992 2.4711	Hexagonal

Table 4: Peak positions of Zinc Sulfide, Zinc Sulfite and Zinc Sulfate in *Yeshada Bhasma* DPJB-1 and DPJB-2 detected in XRD analysis

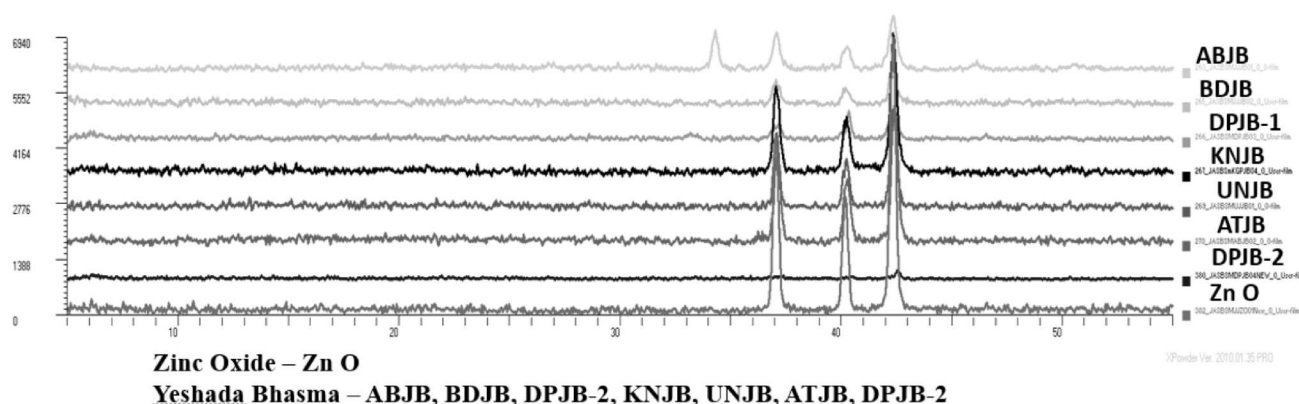
	Compound	2 theta	D spacing	Crystal Shape
DPJB-1	Zinc Sulfide	31.379 33.205 35.666	3.3077 3.1306 2.9208	Hexagonal
DPJB-2	Zinc Sulfite Hydrate · ·	16.580 33.426 42.637	6.2039 3.1104 2.4604	Unknown
	Zinc Sulfate Hydroxide Hydrate ·	22.195 31.434 42.084	4.6471 3.3020 2.4912	Triclinic

Figure-1 X-ray Diffractograms of samples of *Yeshada Bhasma* and Zinc Oxide



Yeshada Bhasma- ABJB, BDJB, DPJB-1, KNJB, UNJB, ATJB, DPJB-2 Zinc Oxide- Zn O

Figure-2: Stacked X-ray Diffractograms of samples of *Yeshada Bhasma* and Zinc Oxide



Zinc Oxide – Zn O

Yeshada Bhasma – ABJB, BDJB, DPJB-2, KNJB, UNJB, ATJB, DPJB-2

XRD patterns of all the samples were obtained using Terra II portable X ray diffraction analyser. The samples were scanned from 5 - 55° (2 θ). Crystalline phases were identified by matching phases using PDF2 database. The major crystalline phases of ZnO detected in samples of *Yeshada Bhasma* were similar to the phase of diffraction pattern of standard Zinc oxide. Three peaks corresponding to ZnO with max. intensity were detected at 2 theta 37.022° -37.077°, 40.148°-40.258° and 42.305° to 42.444° in all samples. Crystals of ZnO were hexagonal in shape

in all the samples. In addition, peaks of Zinc Sulphide with hexagonal crystals in DPJB-1 and that of Zinc Sulphide, Zinc Sulphite Hydrate and Zinc Sulphate Hydroxide Hydrate in DPJB-2 were detected in addition to phases of ZnO. Zinc Sulfate Hydroxide Hydrate crystals were trigonal in shape. However, crystal shape of Zinc Sulfite Hydrate could not be identified.

Table 5: Crystalline and Amorphous content of compounds detected in samples

Sample	Crystalline content %	Amorphous content %
Zinc Oxide	93	7
<i>Yeshada Bhasma</i>		
DPJB-1	77.3	22.7
DPJB-2	68.8	31.2
BDJB	73.6	26.4
UNJB	87.1	12.9
KNJB	89.2	10.8
ABJB	82	18
ATJB	88.8	11.2
Average	80.97+/- 7.96	19.03 +/- 7.96

ZnO was found in 93% Crystalline form in standard Zinc oxide and only 7% in amorphous state. Whereas highest 31.2% amorphous content of compounds was found in

Yeshada Bhasma sample DPJB-2 and lowest 12.9% in UNJB. Average amorphous content of *Yeshada Bhasma* was 19.3% +/- 7.96.

Table 6: Particle Size Distribution of samples of *Yeshada Bhasma*

Sample	0 to 2.5 μ	2.5 to 5 μ	5 to 10 μ
DPJB-1	90.28%	9.56%	0.15%
DPJB-2	92.72%	7.23%	-
BDJB	95.19 %	4.78%	-
UNJB	89.57%	10.31%	0.10%
KNJB	92.26%	7.63%	0.10%
ABJB	86.59%	12.84%	0.57 %
ATJB	95.19%	4.78%	-
Average	91.68% +/-3.12	8.16% +/- 2.95	0.20% +/-0.24

Table 7: Particle size of 10%, 50% and 90% particles of *Yeshada Bhasma*

Sample	d10	d50	d90
DPJB-1	< 0.868 μ	< 1.418 μ	< 2.456 μ
DPJB-2	< 0.709 μ	< 1.228 μ	< 2.12 μ
BDJB	< 0.709 μ	< 1.326 μ	< 2.127 μ
UNJB	< 0.709 μ	< 1.418 μ	< 2.74 μ
KNJB	< 0.709 μ	< 1.418 μ	< 2.352 μ
ABJB	< 0.709 μ	< 1.418 μ	< 2.746 μ
ATJB	< 0.709 μ	< 1.326 μ	< 2.127 μ
Average	< 0.731 μ +/-0.06	< 1.364 μ +/-0.07	< 2.381 μ +/-0.28

Table 8: Minimum area, Major and Minor axis length of particles of *Yeshada Bhasma*

Sample	Area min	Axis – major	Axis minor
DPJB-1	0.197 Sq μ	0.444 Sq μ	0.314 Sq μ
DPJB-2	0.197 Sq μ	0.444 Sq μ	0.314 Sq μ
BDJB	0.197 Sq μ	0.444 Sq μ	0.314 Sq μ
UNJB	0.197 Sq μ	0.444 Sq μ	0.314 Sq μ
KNJB	0.197 Sq μ	0.444 Sq μ	0.314 Sq μ
ABJB	0.197 Sq μ	0.444 Sq μ	0.314 Sq μ
ATJB	0.197 Sq μ	0.444 Sq μ	0.314 Sq μ

Particle size distribution analysis was conducted using BIOVIS PSA 2000 particle size and shape analyser. Percentage of particles falling between the particle size 0 – 2.5 μ , 2.5 – 5 μ and 5 – 10 μ was also recorded. 91.68% $\pm$ 3.12 particles were between the particle size 0 – 2.5 μ in all the samples of *Yeshada Bhasma* taken together (Table 6). 90% particles in all the samples of *Yeshada Bhasma* taken together were observed to be falling below 2.38 μ $\pm$ 0.28. Only 10% particles were observed below 0.731 μ $\pm$ 0.06. in all samples of *Yeshada Bhasma* taken together (Table 7). Smallest i.e. Minimum and largest i.e. maximum area covered by any particle in all the samples was also measured. It was found to be the least 0.197 Sq μ and highest 0.444 Sq. μ measurable by the instrument. Same was the case with minor axis of particle measured to be 0.314 Sq μ for all the samples (Table 8).

Stacking of X ray diffractograms of all the samples of *Yeshada Bhasma* with the diffractogram of standard Zinc oxide showed peaks of Zinc oxide (Zn O) from all the tested samples matching with each other (Fig 2).

Discussion

Seven samples of an Ayurvedic drug marketed by the name '*Yashada Bhasma*' or '*Jasada Bhasma*' manufactured by six manufacturers, analysed in this study, were found prepared by using methods described in the classical Ayurvedic texts Rasatarangini, Rasarajasundar, Rasatantrasara Siddhayoga Sangraha and Ayurveda Sara Sangraha. Samples DPJB-1 and DPJB-2 are the two batches of *Yeshada Bhasma* manufactured by the same

manufacturer. These two samples are prepared by using Mercury and Sulfur as processing ingredients for its *Marana* i.e. *Bhasmikiranana* (incineration) as described in the classical Ayurvedic text Rasatarangini as mentioned on the label of the marketed drug. Other five samples were prepared by using different herbal materials in their processing. The present study was undertaken to study the uniformity between different samples, marketed in the name of *Yeshada Bhasma*, using XRF, XRD and Particle size analysis. Zinc oxide (AR grade) with 98% purity was also included in this study as a standard based on the reports of presence of ZnO in *Yeshada Bhasma* in earlier studies. Earlier studies have reported presence of hexagonal Zinc oxide as the major phase in *Yeshada Bhasma*.⁷⁻¹² Zinc was found present in all the samples in varying quantities in the present study during XRF analysis. Standard Zinc oxide (AR grade) was found to contain 88.94% $\pm$ 0.38 Zinc. Whereas, highest 89.93% $\pm$ 0.30 and lowest 79.36% $\pm$ 0.34 Zinc was detected respectively in the *Yeshada Bhasma* samples ATJB and DPJB-1 with average of 82.92 $\pm$ 3.71 all the samples taken together (Table 1). Interestingly, not much difference in Zinc content was observed in the two batches of *Yeshada Bhasma* DPJB-1 (79.36 $\pm$ 0.34) and DPJB-2 (79.43 $\pm$ 0.41) manufactured by the same manufacturer. This uniformity in Zinc content in these two batches appears to be a result of the same raw material used in preparation for both the batches. Whereas, the variation in quantities of Zinc in other samples of *Yeshada Bhasma* appears to be the result of raw material Zinc of varying quality used in the preparation by different manufacturers. Zinc content of the *Yeshada Bhasma* analyzed in the

present study is higher than found in *Yeshada Bhasma* studied by earlier researchers. Nitu Bhatnagar, Avani Parik in their studies reported presence of 49.20%, 38.58% and 35.50% Zinc in the three marketed samples of *Yeshada*

Bhasma collected by them for research study.¹³ Interestingly in this study the *Yeshada Bhasma* prepared by researchers was reported to contain 65.18% Zinc (Table 9).

Table 9 : Zinc and Oxygen content in *Yeshada Bhasma* reported by earlier researchers.

	<i>Yeshada Bhasma</i>	Zinc	O
Parik & Bhatnagar ¹⁰		65.18	30.45
Bhatnagar & Parik ¹³	BYB1	49.28	20.92
	BYB2	38.58	20.72
	CYB	35.50	18.50
	OMM	65.18	30.45
Bhowmik et al ⁷		78.86	11.7
	Na, Mg, Al, Si, S, K, Sn, Ca, Mn, Fe, Cu. – Bhoumik et al		
Geeta G Gadad ¹¹	<i>Yeshada Bhasma</i>	77.13	-
	Ca, Al, Fe, P, K, P, SI		

Oxygen is the other important ingredient detected in *Yeshada Bhasma* in almost all the past studies. Bhowmik et al reported 11.7% oxygen in *Yeshada Bhasma*.⁷ Whereas, oxygen was found in the range of 18.5% to 30.45% in four samples of *Yeshada Bhasma* analysed using EDAX in earlier studies by Bhatnagar, Parik.¹³ Independent Oxygen content was not detectable using XRF analysis used in the present study. However, light elements comprising of Oxygen, Hydrogen, Sodium, Potassium and others were observed to be the other principal ingredients of all the tested samples of *Yeshada Bhasma*. Considering ZnO as the principal compound detected in *Yeshada Bhasma* in the past studies supported by the observations of XRD analysis in the present study, Oxygen appears to be the principal light element present in *Yeshada Bhasma*. 9.55 % $\pm$ 0.38 light elements were found present in standard Zinc oxide (AR grade). Whereas, the quantity of light elements ranged between ATJB- 7.24% $\pm$ 0.30 and DPJB-2 - 12.36% $\pm$ 0.42. averaging 10.26% $\pm$ 1.63 in *Yeshada Bhasma* (Table 1).

Sulfur was another significant detection in *Yeshada Bhasma* samples DPJB-1 and DPJB-2. Presence of Sulfur in DPJB-1 (8.118 % $\pm$ 0.079) and DPJB-2 (5.650 % $\pm$ 0.069) (Table 1) is attributed to use of Sulfur in the

Marana i.e. *Bhasmikarana* (incineration) of Zinc. Other manufacturers have not used Sulfur in processing. Therefore, Sulfur is almost absent in those samples. Presence of Sulfur in DPJB-1 and DPJB-2 is validated by detection of Zinc sulphide, Zinc sulphite hydrate and Zinc sulphate hydroxide hydrate in these sample in XRD analysis (Table 2). The presence of Sulfur is attributed to use of Sulfur in the processing. Interestingly, mercury was not found in these samples although it was used in processing of *Yeshada Bhasma* DPJB-1 and DPJB-2.

Iron (Fe) was another element found in significant quantity in KNJB (3.42% $\pm$ 0.10). Whereas, although not in very significant amount it was found between 1.399% $\pm$ 0.021 to 1.599% $\pm$ 0.022) in DPJB-2, BDJB, UNJB and ABJB. Iron is absent in standard Zinc oxide (Table 1). Presence of iron in *Yeshada Bhasma* is attributed to the adulteration resulting from the processing of the material. Calcium is another important element. It is found in significant quantity in one sample of *Yeshada Bhasma* BDJB (1.937 % $\pm$ 0.026 %). In other samples it was present in ppm concentrations (Table 1). Presence of calcium in BDJB is possibly due to use of slaked lime in *Shodhana* (purification) of Zinc, which was desired to be removed in washings.

In addition to the major components Zinc, Oxygen, Sulfur, elements Al, Si, P, Cl, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, Sn, Sb, Ba, La, Ce, Pr, Nd, Hg, Pb and Bi were also detected in the samples of *Yeshada Bhasma*. However, the quantity was in ppm measures and hence prima facie not much significant. Presence of all these elements in trace amounts is a result of different plant materials used to process the *Yeshada Bhasma*. Presence of these trace elements in standard Zinc oxide appears to be a result of adulteration. Iron, calcium found in significant quantities and elements like Al, Mg, etc. and light elements sodium, potassium etc. found in traces in *Yeshada Bhasma*, are likely to form their silicates, oxides, sulfides etc. These compounds were not reported, not being the target compounds in this study. However, due to their presence the end product *Yeshada Bhasma* is likely to be containing multiple herbo-metallic complexes. Role of these complexes in determining the pharmacological action of these formulations needs to be assessed. Detection of these trace elements in *Yeshada Bhasma* in the present study confirms the reports of earlier studies conducted by various researchers. Some researchers have quantified oxygen content in *Yeshada Bhasma* using EDAX analysis. Bhowmik et al have reported Oxygen content of *Yeshada Bhasma* as 11.7%.⁷ Whereas, Nitu Bhatnagar, Avani Parik have reported Oxygen content of four samples as 20.92%, 20.72%, 18.50% and 30.45%.¹³ (Table 1) In this context Bhowmik et al have reported presence of particles in oxygen deficient state on the basis of Zinc: Oxygen atomic ratio close to 2:1 in *Yeshada Bhasma* analysed by them. Further the authors suggest that out of 54% atoms of Zinc only half is bound with Oxygen (20%). This study is based on SEM-EDAX and XPS analysis of *Yeshada Bhasma*.⁷ Presence of oxygen deficient particles has many therapeutic implications like increase in bioavailability, enzyme activity like insulin regulation, antioxidant action and immune response modulation. However, detection of oxygen deficient particles of ZnO in *Yeshada Bhasma* reported by Bhowmik et al needs further validation by complimentary methods like Electron Paramagnetic Resonance (EPR), Fourier Transform Infrared

Spectroscopy (FTIR), Raman and Thermogravimetric Analysis (TGA)-Differential Scanning Calorimetry (DSC).

In the present study XRF analysis was followed by XRD analysis of *Yeshada Bhasma*. X-ray Diffraction analysis has revealed presence of hexagonal crystals of ZnO in all the samples of *Yeshada Bhasma* and Zinc oxide as well. The major three reflection peaks of ZnO in all the samples of *Yeshada Bhasma* and standard Zinc oxide appear identical in comparative XRD pattern analysis. The peak 2 theta positions of ZnO with highest intensity were around 37.022, 40.175 and 42.305 (Table 3). However, the peaks of ZnS were also detected in the sample *Yeshada Bhasma* DPJB-1 and DPJB-2. In addition, peaks of Zinc sulphite and sulphate were detected in DPJB-2. Peak positions of Sulfur compounds in these two samples of *Yeshada Bhasma* were ZnS (31.339, 33.205, 35.666), ZnSO₃ (16.580, 33.426, 42.637) and ZnSO₄ (22.195, 31.434, 42.084) (Table 4). The detection of Sulfur compound in DPJB-1 and DPJB-2 is a result of use of Sulfur in the *Marana* (incineration) processing of *Yeshada Bhasma* as mentioned on the label of the marketed samples in the current reference. The crystal shape of ZnS in this study was identified as hexagonal. ZnSO₄ (OH). 3 H₂O crystals were Clinic in shape. However, the crystal shape of ZnSO₃. 5 H₂O) could not be identified by the software used in this test. The crystal shape of drug molecule impacts significantly on its pharmacokinetic behaviour ultimately affecting its therapeutic efficacy. The solubility, dissolution and hence bioavailability of the drug varies with variation in crystal shape of the drug particle. Greater the surface area of the crystal greater will be its absorption. In the present study ZnO crystals were found hexagonal in shape exposing larger surface area facilitating its faster absorption. Whereas, ZnSO₄ (OH). 3H₂O particles trigonal in shape are likely to minimize their absorption.

Amorphous content of the seven samples of *Yeshada Bhasma* (average 19.3% +/- 7.96,) was found significantly increased as compared to standard ZnO (7%). (Table 5)

This increase in amorphous content of *Yesahda Bhasma* appears to be a result of *Marana* (incineration) process prescribed in Ayurvedic classical texts, carried out on Zinc metal. In addition, a wide inter sample variation ranging between 12.9% (UNJB) to 31.2% (DPJB-2) in amorphous content was also found. Such variation was also observed in *Yeshada Bhasma* prepared by different manufacturers using similar and different methods. This variation appears to be a result of method variation as well as variation in quality of raw materials used by them. However, variation between two batches of *Yeshada Bahsma* DPJB-1 (22.7%) and DPJB-2 (31.2%) prepared by the same manufacturer using the same method appears to be a result of inappropriate quality control during manufacturing process. (Table 5).

Particle size and shape analysis

Particle size and shape analysis of samples of *Yeshada Bhasma* was carried out through digital image analysis by optical microscopy and high-resolution digital camera using Biovis PSA 2000 analyser. All the particles screened from all the samples analysed in this study were below 10 μ in size (Table 6). Further 90%, 50% and 10% particles in all the samples together were found to be below 2.746 μ ., 1.418 μ and 0.868 μ respectively (Table 7). Whereas maximum percent distribution of particles in all the samples together was found as follows: 95.14% between 0 to 2.5 μ , 12.84% between 2.5 to 5 μ and 0.57% between 5 to 10 μ (Table 6). The shape of the particles of *Yeshada Bahsma* being irregular, the minimum area covered by the particle, major and minor axis of the particle were measured. Minium area covered by the smallest particle was found to be less than 0.197 μ . Whereas Major axis and minor axis of the particles were found to be respectively less than 0.444 μ and 0.314 μ (Table 8). The measures of area and major and minor axis mentioned are the lowest possible measurements with the method used in this analysis. A particle having dimensions between 1 to 100 nano-meters is defined as a nano-particle. Few researchers have reported presence of such nano-particles in Ayurvedic *Bhasma* preparations

and hence classify Ayurvedic *Bhasma* formulations as a type of nano-medicine. Avani Parik, Bhatnagar have reported detection of crystals with average size of 32.79 nm in *Yeshada Bhasma* analysed with XRD using Debye-Scherrer equation.¹³ Bhowmik et al have detected particles with crystal size in 25 to 50 nm range in nano-fractionated part of *Yeshada Bhasma*.⁷ However, the particle size of particles in unfractionated part of *Yeshada Bhasma* was between the range of 0.85 to 1.35 μ . In our earlier study we have reported less than 10% particles below the size of 130 nm in analysis of a study involving particle size analysis of different types of Ayurvedic *Bhasma*.⁶ In the present study although the nano-particles were not specifically looked for. Only 10% particles were found to be having size below 2.5 μ (Table 6). This finding confirms the conclusion in our earlier studies that Ayurvedic *Bhasma* formulations can't be referred as a form of nano-medicine.

Conclusion

XRF, XRD and Particle size and shape analysis of marketed samples of *Yeshada Bhasma* conducted in this study, indicates that a wide variation in elemental and chemical composition of compounds found in these samples, is observed. The variation is not only limited to the samples of *Yeshada Bhasma* prepared by using different methods but it is also observed in the samples of *Yeshada Bhasma* prepared by using uniform methods by different manufacturers. Moreover, variation was also observed in chemical composition of two batches of *Yeshada Bhasma* prepared by using one single method by the same manufacturer. However, hexagonal crystals of Zinc oxide were found present in all the marketed samples of *Yeshada Bhasma* irrespective of their method of preparation. Two samples DPJB-1 and DPJB-2 from two batches of *Yeshada Bhasma* prepared by one manufacturer using the same method, were also not uniform in their chemical composition. DPJB-1 contained Zinc oxide and Zinc Sulfide. Whereas, DPJB-2 contained Zinc Sulfite Hydrate and Zinc Sulfate Hydroxide Hydrate in addition to Zinc oxide. The proportion of amorphous

and crystalline content of compounds detected in *Yeshada Bahsma* as compared to standard ZnO was found markedly altered in this study. The amorphous content was found significantly increased as a result of *Marana* (incineration) of Zinc metal. Wide variation in amorphous content of different samples of *Yesahda Bahsma* manufactured by different manufacturers along with variation in amorphous content of two batches of *Yedhada Bahsma* marketed by the same manufacturer is a matter of concern. Not much difference in Particle size and shape of particles from all the samples of *Yeshada Bhasma* was observed irrespective of their method of preparation. The observations in this study underline the significance of observation of strict quality control measures on Ayurvedic products for ensuring uniformity in products marketed as one and the same drug, under the same name.

Credit Statement

Function	Responsibility
Conceptualization	SSS
Methodology	SSS
Software	US
Validation	SSS
Formal analysis	US
Investigation	SSS & US
Resources	MS
Data Curation	MS
Writing - Original Draft	US
Writing - Review & Editing	SSS
Visualization	US
Supervision	SSS
Project administration	MS
Funding acquisition	MS

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Glossary:

Yeshada – A Sanskrit term used for the metal Zinc

Bhasma: An Ayurvedic medicinal preparation prepared by incinerating the base metal or mineral pre-processed with product specific plant and other materials.

Shodhana: Product specific processing of a raw mineral, metal or other base material to make the material suitable for further processing like incineration to produce Bhasma or similar medicinal products.

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