Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [4] March 2024: 24-32

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Comparative Qualitative Analysis of Yasad Bhasma with Classical and Modern Parameters

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ABSTRACT

Yashada bhasma has proven significant efficacy in the treatment of diseases such as diabetes, eye disorder, urine disorder, and others. As bhasma is a metallic preparation, its quality must be proven, various classical as well as modern techniques are available for that among them are XRD, DLS, SEM and EDAX etc. Yasad bhasma containing zinc, Zinc has a distinct biological function, and its deficiency leads to many kinds of diseases. Hence, the present work deals the analysis of Yasad bhasma of three different brands, to compare and know the quality. Yasad bhasma were purchased from local Ayurvedic market Amravati, India. of three manufacturers, namely Shree Baidyanath (BYB), Shree Dhutpapeshwar (DYB) and Unjjha pharmaceuticals (UPYB). Based on a study, the classical method (BYB and DYB) produces better results; therefore, both samples should be proceeded for analytical analysis. The XRD analysis of both bhasma samples reveals a hexagonal ZnO crystalline phase, stability is confirmed by the zeta potential value, and the element zinc is the main constituent in addition to Fe, Pb, Cu, Sn, Mg, and Mn. by atomic absorption spectroscopy. ZnO, C=C, OH functional groups in both samples confirm by FTIR. DYB shows less particle size than BYB. So, bioavailability of Dhootpapeshwar Yashada bhasma was greater. By analytical method of examination along with classical method this is reveal that DYB is better for therapeutic efficacy. This step towards establishing significant move in revealing the scientific explanations for the safety and effectiveness of this traditional medical practice.

Keywords: Bhasma parikshan, Zeta potential, Namburi phased spot test, XRD, Yashada bhasm

Received 26.01.2024 Revised 21.02.2024 Accepted 19.03.2024

INTRODUCTION

"Alpamatropayogitvat arucheraprasangata
Kshipramarogyadayitvat aushdhebhyadhiko rasa"

The above phrase from Rasendra sara sangraha highlights the exceptional qualities of rasoushdhis, stating that they are administered in very minimal quantities, easily digestible, and have rapid effects [1]. Bhasmas are complex compounds consisting of metals or minerals, or specific proportions of herbal-minerals. They are developed through repeated incineration with liquid extracts [2]. Yashada Bhasma undergoes a specialized treatment involving zinc. Zinc is the second most prevalent transition metal in living organism, following iron. It is the only metal present in all known enzymes. Zinc serves a distinctive and important biological role in humans, and a lack of zinc can result in numerous disorders [3]. The name Yashada was initially documented in the Madanapalanighantu during the 15th century. Ayurvedaprakasha has extensively examined Yashada for the first time and categorized it as puthiloha, which is a group of metals [4]. In order to be suitable for medicinal purposes, Yashada must undergo a series of pharmaceutical procedures referred to as Shodhana (purification), Jarana (frying), and Marana (incineration) [5]. The conditions in which it is recommended to use this treatment include Prameha (diabetes mellitus), Pandu (anemia), Kasa Swasa (respiratory diseases), Nisha sweda (night sweating), Rajasrava (excessive menstrual bleeding), vrana (wounds), and Kampavata (Parkinsonism) [6]. Simultaneously, nanomaterials including zinc, specifically zinc oxide nanoparticles (ZnO NPs), are gaining popularity as cutting-edge substances for medical purposes. Zinc oxide exhibits excellent biocompatibility, enabling its utilization for its antibacterial, antifungal, antiviral, and antitumoral properties. Yashad bhasma has been demonstrated to be efficacious in treating diabetes [7]. To prove its quality, various classical as well as modern techniques are available for that among them are (XRD, FTIR, SEM, AAS and EDAX) etc.

MATERIAL AND METHODS

- Yasad bhasma were purchased from local Ayurvedic market Amravati, India. of three manufacturers, namely Shree Baidyanath, Shree Dhutpapeshwar and Unjjha pharmaceuticals consider to compare and know the quality of commercial bhasma in market. Their details are given in Table 1.
- Subjecting all samples to classical bhasma parikshas.
- Then comparison between two bhasma sample by analytical study.

Standardization of Bhasma:

Classical characterization of Bhasma:[8,9]

The classical methods of evaluation called as *Bhasma Parikshan*, for three commercial brands of Yasad bhasma and classical methods are *Varna* (colour), *Niswadu* (taste), *Rekhapurnata* (fineness) and *Varitara* (lightness) etc. are as:

***Varna* (colour):** A particular color is cited for every bhasma. Colour depending on the materialused in the process of incineration. If it complies with classical references, then it should be considered as authentic bhasma. Given in Figure 1[10].

***Niswadu* (Taste):** Naturally occurring metals has distinct tastes. In this test, a small amount of bhasma is applied to the tongue. If it lacks any taste, particularly a metallic taste, it indicates that the bhasma has been correctly prepared [11].

***Nishchandrata* (lusterless)**: For therapeutic action Bhasma must be Bhasma should possess a Nischandra appearance, free of any gloss. Luster, also known as chandratva, is a natural quality of metals. In properly incinerated metal, luster is not in existence. For Nishchandrata observation, bhasma is taken out in intense sunlight to verify the sustainability of its radiance; if it exhibits a lustrous appearance, it indicates requirement of more ignition [11].

***Varitara* (lightness)**: Varitara check is functional to determine weightlessness and the fineness of bhasma can be determined by observing its floating behavior on the surface of standing water. Essentially, this test works based on the principle of surface tension. The Bhasma sample is placed between the index finger and thumb, and then gently dispersed onto a still water surface from a narrow gap. Appropriately incinerated bhasma will drift on water superficially [11].

***Unama* test**: This check is the additional assessment of the Varitara test. The grain of rice should be delicately placed on the layer of suspended Bhasma. Observe whether the grain floats or sinks. If the grain remains unchanged in the layer, then the bhasma can be deemed as excellent (properly prepared) [11].

***Rekhapurnata* (fineness):** This test indicated the fineness of the particles. A small amount of bhasma was gently spread between the thumb and index finger. If bhasma particles are found within the creases of these fingers, it signifies that the metal has been thoroughly burned. The principle of pharmacology states that, finer the particle, fastest the absorption and quickest the action.

Nirdhuma : A small amount of yashada bhasma was sprinkled on the burning charcoal and observed for the fumes.

Physical characterization [12, 13]

1) Determination of Total Ash: One gram of Yasad bhasma (W1) was accurately measured and pulverized in a silicon crucible that had been weighed previously (W2). To obtain ash without any carbon, the crucible was placed inside a muffle furnace and maintained at a temperature between 450ºC and 600ºC for a duration of 4 hours. Afterward, it is cooled within a desiccator and measured using a scale (W3). Subsequently, the remaining substance was collected onto an ashless filter paper.

Total ash is determined by the following formula:

$$\text{Percentage of Total Ash} = \frac{W3-w2}{w1} \times 100$$

2) Acid Insoluble Ash: The ash was carefully put into a 250 mL beaker without any loss, and then 100 mL of diluted hydrochloric acid was added. The crucible was rinsed with 10 mL of acid and the resulting liquid was transferred to a beaker. The beaker was subjected to heat until the liquid reached its boiling stage. The solution proceeded filtration, resulting in the collection of the insoluble materials on ashless filter paper. Subsequently, the sample was dried using a heated plate and then subjected to ignition at a temperature of 600ºC in a muffle furnace. (Until it turned into white ash). The residue was cooled in appropriate desiccators for a duration of 30 minutes and immediately weighed.

$$\text{Percentage of Acid Insoluble Ash} = \frac{W3-w2}{w1} \times 100$$

W1= Weight of sample taken (yashada bhasma), W2= Weight of empty crucible, W3=(Weight of crucible + Acid insoluble Ash)

3) Water Soluble Ash: The entire ash was subjected to a 5-minute boiling process with 25 mL of water. The insoluble debris was accumulated on an ashless filter paper. The object was cleansed using heated water and then subjected to a controlled ignition process for a period of 15 minutes, ensuring that the temperature was below 450ºC- 600ºC. Take out the weight (W3) of the insoluble material from the weight of the ash; the resulting difference in weight indicates the amount of ash that is soluble in water.

Water Soluble Ash = Total Ash - Water Insoluble Ash (W3)

4) Determination of Loss on Drying: The weight of 10 grams of Yashada bhasma (W1) was measured accurately to the third decimal place (without prior drying) in a tarred evaporating dish, giving the weight of the bhasma (W2). The sample was subjected to drying at a temperature of 105ºC for a duration of 5 hours, after which its weight (W3) was measured. The drying process was continued and weighing was conducted at one-hour intervals until the difference between two consecutive weighing corresponded to no more than 0.25 percent. (The weight remained constant after two successive weighing following a 30-minute drying period.) Subsequently, the sample was allowed to cool for a duration of 30 minutes within a desiccator, showing a minimal weight variation of no more than 0.001 g.

$$\text{Percentage of Loss on Drying} = \frac{W3-w2}{w1} \times 100$$

5) Determination of pH: An aqueous solution is prepared and measurements are carried out at a temperature of 25ºC using a pH meter.

Standardization by Modern methods [14,15]

On classical, physical method of evaluation BYB & DYB is giving complimentary result so remaining modern methods of standardization for both commercial brands are as:

X-ray powder spreading (XRD): X-ray diffraction (XRD) is a rapid and systematic technique primarily employed for determining the phase composition of a crystalline solid and can provide valuable information on the dimensions of its unit cell. The substance under investigation is finely ground, calibrated and regular large structure is determined. It is based on helpful intrusion of unicolor X-ray and a crystalline sample. The X-ray diffractometer (Rigaku MiniFlex 600) was used to assess the crystallite size, structural identity, phase, and purity of Yashada bhasma samples. The analysis was based on a physiochemical investigation and utilized Cu Kα radiation with a wavelength of λ=0.1541 nm. The range of the two theta value extended from 30° to 90° [16,17] .

Zeta potential: The particle size, surface charge, polydisperse index (PDI), and stability of the yashada bhasma sample were determined using a particle size and zeta potential analyzer, namely the Nano-ZS series 633 nm laser (Malvern Instruments Limited, UK), at a temperature of 25 °C. Zeta potential analysis is a crucial method for assessing the surface charge of nanocrystals and can be used to evaluate the physical stability of bhasma. The presence of a high positive or negative zeta potential in nanocrystals indicates strong physical stability of nanosuspensions, as it signifies the electrostatic repulsion between individual particles. A zeta potential value outside the range of -30 mV to +30 mV is typically regarded as having enough repulsive force to achieve enhanced physical colloidal stability. See Table 5 [18].

Fourier transform Infrared spectroscopy: To investigate the organic and inorganic contaminations in the both commercial brands of Yashad Bhasma, IR spectroscopy was carried out. FTIR is a technique which is used to obtain an infrared spectrum of a compound. It is the superposition of absorption bands of specific functional groups. Bruker OPUS spectroscopic software was used. FTIR is a technique which is used to obtain an infrared spectrum of a compound. It is the superposition of absorption bands of specific functional groups.

FTIR analysis of Yashad Bhasma was carried out to identify the possible organic compounds in the bhasma. Spectra were recorded in the region 450-4000cm-1. The characteristic absorption frequencies and related functional groups observed during FTIR analysis of Yashad Bhasma given Table 6 and figure 4. [18]

Namburi phase spot test: The Namburi Phased Spot Test (NPST) is highly beneficial for evaluating the quality of Bhasma according to the Rasashastra guidelines. Dr. Namburi Hanumantha Rao developed and standardized the procedure in 1970. Its monograph has been accepted by CCRAS (Central Council for Research in Ayurvedic Science), New Delhi. The procedure is conducted using little equipment and utilizes chromatography. A clear drops of prepared bhasma is placed onto a specifically prepared reactive paper, resulting in the development of a series of colors at different time intervals. The sample undergoes a chemical reaction with the paper.

All three samples undergo NPST. At first, a centrifuge tube was filled with 0.2 gms of bhasma. Subsequently, 0.5 ml of 5N nitric acid (HNO3) were slowly introduced to the solution, followed by heating for a duration of one minute. The object was placed in an upright position for a duration of 50 hours, and was occasionally agitated during this period. Subsequently, it was permitted to settle, resulting in the formation of a distinct layer. A single droplet was extracted from the transparent layer and applied onto potassium iodide paper with a concentration of 10% (made using Whatman's filter paper no.1). Alterations in the color of the paper were noticed throughout three different time intervals [19,20].

1st phase: 0 to 5 min.

2nd Phase: 5 min to 20 min.

3rd Phase: 20 min to 1 day.

Heavy metal analysis by atomic absorption spectrometry: Atomic absorption spectrometry is a method used to quantify the quantity of an element in a sample. It works by using the fact that atoms in their lowest energy state absorb light of specific wavelengths when passed through a layer of atomic vapor containing the element. Atomic absorption spectrometry is employed for the quantification of heavy metal and certain non-metal elements in their atomic form [21].

RESULT AND DISCUSSION

Classical characterization:

BYB and DYB complied with all the Methods of classical analysis given in Table 2. Upon thorough characterisation, the BYB exhibited a yellowish-brown color, while the DYB displayed a black coloration. BYB and DYB were determined to be superficial, meaning they lacked any metallic taste. Both bhasma did not contain any fluorescent particles. Both BYB and DYB were observed to exhibit floating on the water's surface. The presence of floating rice grains above the layer of bhasma signifies the successful completion of the incineration process. It was seen that both bhasma entered the crevices of the finger and no fumes were observed coming from it.

Physiochemical parameters: BYB and DYB complied with all the physicochemical analysis. The physicochemical examination, such as the determination of total ash content, was conducted to verify the complete combustion of the metal samples. The significance of this parameter comes from the fact that improperly burned zinc has been documented to cause harmful consequences. The samples being studied exhibited effective zinc incineration, as shown by the total ash value. DYB (99.17%), and BYB (96 %), negligible moisture content DYB (0.01% loss on drying) and BYB (0.48%), acid insoluble ash DYB (46 %) and BYB (63.13%), lower solubility in water DYB (1.66%) and BYB (9.88%). The results exhibit a level of similarity to the values reported in the recent literature.

Modern characterization:

Modern characterization demonstrates that the process of transforming the metal into bhasma has undergone significant changes.

XRD analysis:

The XRD analysis of BYB and DYB indicates the existence of hexagonal ZnO (zinc oxide) which corresponds to the JCPDS card 36-1451[17]. This confirms that the main component of BYB and DYB is Zinc oxide given in Table 3 and table 4. The BYB peaks are situated at 2θ values of 31.770, 34.430, 36.260, 47.550, and 56.600, which correspond to the crystallographic planes (100), (002), (101), (102), and (110), respectively. The DYB peaks are found at 2θ values of 31.80, 34.45, 36.29, 47.58, and 5.66. The absence of distinctive peaks from other phases of ZnO and contaminants suggests that the acquired ZnO in both yashada bhasma samples is highly pure. Given in Figure 2 [22].

Zeta potential: The Zeta potential of both Yashada bhasma at neutral pH was found to be in standard range (+30 to -30 mV) as shown in Figure 3a & b . BYB & DYB shows -23.5mV & -29.5mV respectively found in neutral range that means both bhasma are stable. Partical size of DYB is found to be 1069 as compare with BYB.

Fourier transform Infrared spectroscopy:

The FTIR spectra of both Yashad Bhasma formulations exhibit a significant number of distinct peaks within the range of 450 – 4000 cm-1. The major peaks detected in the FTIR spectra of Yashad Bhasma indicate the existence of C=C, C=O, C-H, C≡C, and O-H bonds. The absorption peak observed at a wavenumber below 800 cm-1 can be attributed to the presence of a metal-oxygen (M-O) bond, namely the zinc-oxygen (Zn-O) bond.

Namburi phase spot test:

All three samples in NPST exhibited the desired results, however, sample 2 demonstrated superior accuracy in comparison to the remaining samples. [Figure 5].

Heavy metal analysis by atomic absorption spectrometry

Both two brands of Yasad bhasma shows different conc. of elements as shown in table 7 all are within limit as per recommended daily intake only Fe is higher in both brands of bhasma 18.4 mg/l (BYB) and 17.51 mg/l (DYB). However, as per RDI, only 15 mg/kg body weight is good for health, variation in quantity it can affect the function of other important trace minerals in body.

Table 1: Details of Bhasmas

Samples	Manufacturing license number	Batch number	Manufacture date	Method used in preparation	Price per gm
BYB	ND/AYU/4	191346002	11/2020	Sidddh Yog sangrah	145/10
DYB	AYU-10	P181000028	10/2018	Rasatrangini19/104-107	132/05
UYB	GA/418	130163	08/2020	NM	83/10

NM: Not mention, BYB: Baidyanath yasad bhasma, DYB: Dhootapapeshwar yasad bhama, UYB: Unjjha pharmaceutical yasad bhasma.

Table 2: Physical test of Yasad bhasma

Properties	BYB	DYB	UYB
Colour (Varna)	Yellowish brown	Black	Creamish
Taste (Niswadu)	Tasteless	Tasteless	Slight metallic taste
Lusturness (Nishchandrata)	No lusture	No lusture	lusture
Fineness (Rekhapurnata)	Fine powder	Fine powder	Slight roughness
Lightness (Varitara)	Positive	Positive	Negative
Unama	Positive	Positive	Negative
Total Ash Value	96%	99.17%	85%
Acid Insoluble Ash	63.13%	46%	67%
Water soluble ash	9.88%	1.66%	15%
Loss on drying	0.48%	0.01%	2.5%
pH value	9.88	8.53	11

Table 3 – Comparison of BYB observed XRD data with JCPDS data (CARD NO. 361451)

(*hkl*) planes	2θ (degree)		Intensity (arb. unit)		^{0}hkl		^{d}hkl (Å)	
	JCPDS	**Observed**	**JCPDS**	**Observed**	**JCPDS**	**Observed**	**JCPDS**	**Observed**
(100)	31.770	31.770	57	55	0.17	0.155	2.8143	2.8164
(002)	34.422	34.430	44	41	0.13	0.167	2.6033	2.6038
(101)	36.253	36.260	100	100	0.30	0.166	2.4759	2.4774
(102)	47.539	47.550	23	23	0.07	0.165	1.9111	1.9114
(110)	56.603	56.600	32	34	0.09	0.170	1.6247	1.6252

Table 4 – comparison of DYB observed XRD data with JCPDS data of (CARD NO. 361451)

(*hkl*) planes	2θ (degree)		Intensity (arb. unit)		^{0}hkl		^{d}hkl (Å)	
	JCPDS	**Observed**	**JCPDS**	**Observed**	**JCPDS**	**Observed**	**JCPDS**	**Observed**
(100)	31.770	31.80	57	55	0.17	0.23	2.8143	2.812
(002)	34.422	34.45	44	41	0.13	0.213	2.6033	2.602
(101)	36.253	36.29	100	100	0.30	0.233	2.4759	2.474
(102)	47.539	47.58	23	23	0.07	0.28	1.9111	1.909
(110)	56.603	56.66	32	34	0.09	0.19	1.6247	1.623

Table 5: Comparative zeta potential and particle size study between BYB & DYB

Sr. No.	Parameters	BYB	DYB
1	Z Average (r.nm)	1276	1069
2	Zeta potential (d.nm)	-23.5	-29.5
3	Zeta Deviation (mV)	5.36	7.41
4	Conductivity (mS/cm)	0.0140	0.0684

Table 6 : FTIR analysis of both Yashad bhasma

Functional group	Absorption Wavenumber /cm^{-1}	
	BYB	**DYB**
Zn-O	507	507
-N-H, C=C	1599	1597
C=O	1909	1985
CN	----	2224
C≡C	2345	2345
O-H	3151	3256

Sr.no.	Formulation	Phase	Observation
1.	BYB	1st - (0-5 min)	Faint pink color at periphery was seen
		2nd - (5-20 min)	Whitish purple spot along with light pink periphery
		3rd - (20 min onward)	Solid spot fades away and light pinkish brown periphery was observed
2.	DYB	1st - (0-5 min)	Light brown solid spot along with bluish green fluorescence at periphery was observed.
		2nd - (5-20 min)	Solid spot gets lighter in colour and dark brown fluorescence periphery was observed
		3rd - (20 mins onwards)	Light brown spot fade away at large extent and periphery gets darker
3.	UPYB	1st - (0-5 min)	Brick red color solid spot was observed along with bluish green fluorescence at periphery
		2nd - (5-20 min)	Outer periphery line gets darker and brown solid spot fades away
		3rd - (20 min onward)	Brick red spot gets lighter and peripheral outline gets more darker

Table 7: Observation of Namburi phase spot test

Table 8: Showing the AAS results of BYB and DYB

Sr. No	Elements	RDI	BYB mg/ L	DYB mg/ L
1.	Zn	15 mg	0.282 mg/l	0.192 mg/l
2.	Ca	1000 mg	9.27 mg/l	6.44 mg/l
3.	K	3500 mg	2.72 mg/l	1.44 mg/l
4.	Mg	350 mg	3.28 mg/l	0.84 mg/l
5.	Cr	120 µg	0.072 mg/l	0.097 mg/l
6.	Mn	5 mg	2.156 mg/l	0.94 mg/l
7.	Fe	15 mg	18.4 mg/l	17.51 mg/l
8.	Cu	2 mg	2.08 mg/l	0.99 mg/l
9.	Cd	25 µg/kg body weight	NA	NA

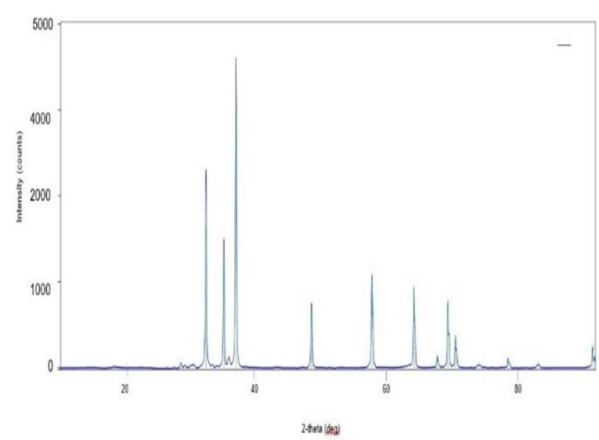
Values are expressed as mean. *RDI: Recommended daily intake


a) BYB

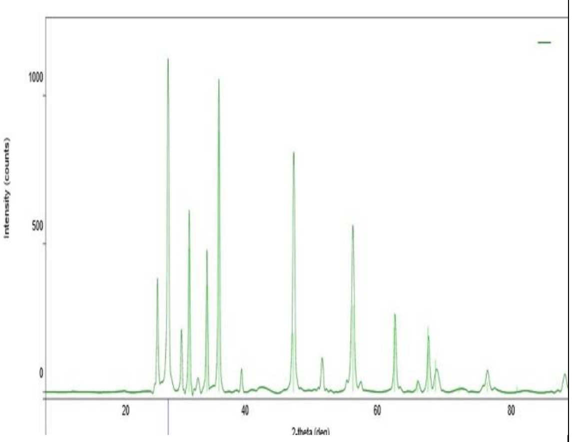

b) DYB


c) UYB

Fig. 1: Yasad Bhasma commercial formulation



a) XRD Spectra of Baidyanath Yasad bhasma



b) XRD Spectra of Dhootpapeshwar Yasad bhasma

Fig. 2: XRD spectra of both commercial sample

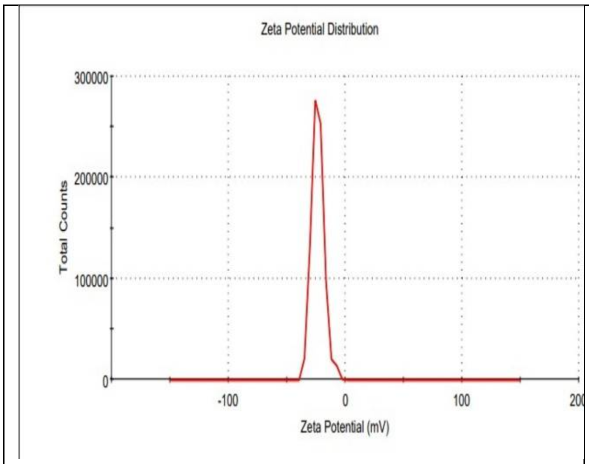
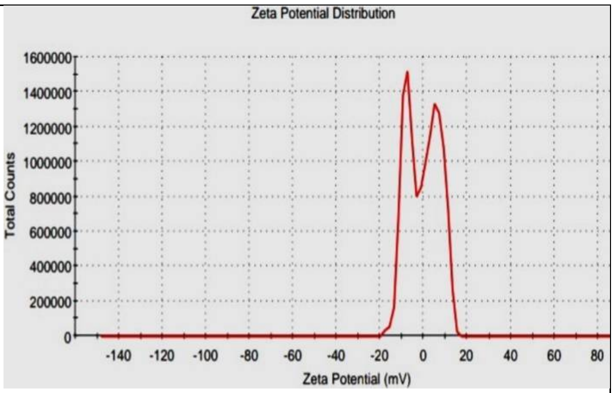


Fig. 3 a) Zeta potential of BYB



3b) Zeta potential of DYB

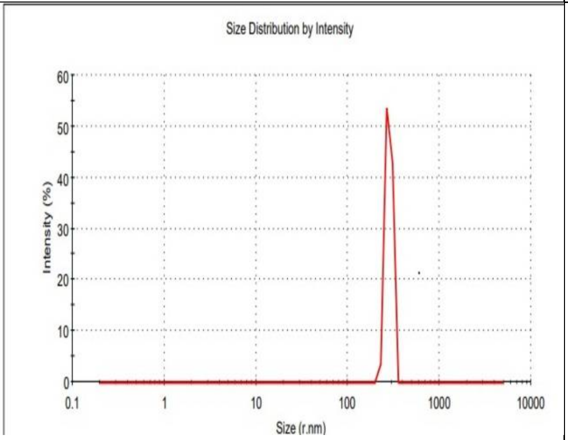


Fig. 3 C) Particle size analysis of BYB

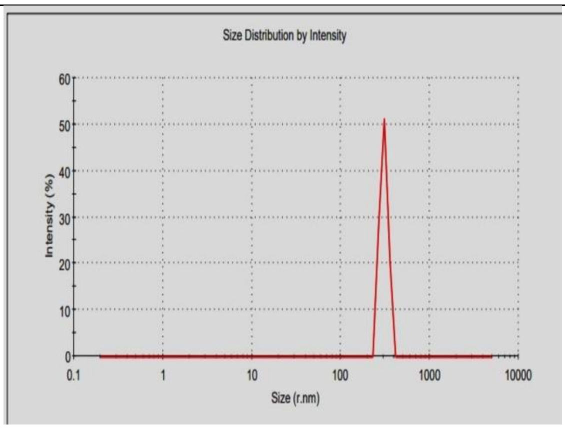


Fig. 3 d) Particle size analysis of DYB

Fig. 3: Zeta potential, particle size of both sample BYB and DYB

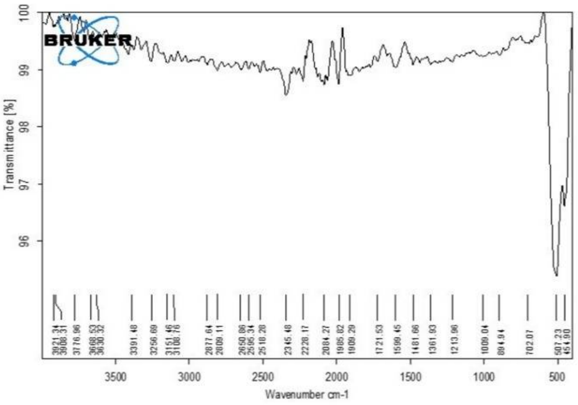


Fig.: 4a) IR spectra of Baidyanath YB

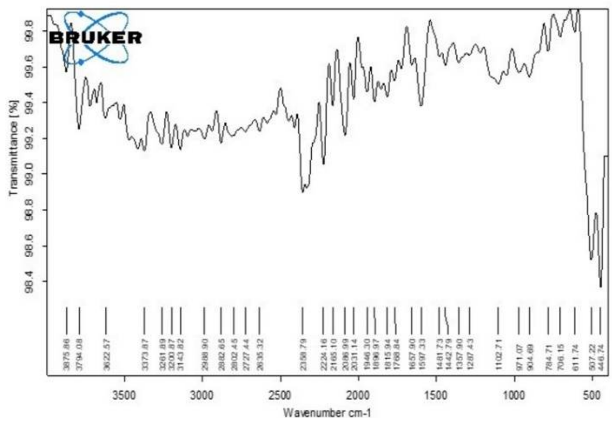


Fig.: 4b) IR spectra Dhootpapeshwar YB

Fig.4: IR spectra of both Baidyanath YB Dhootpapeshwar YB

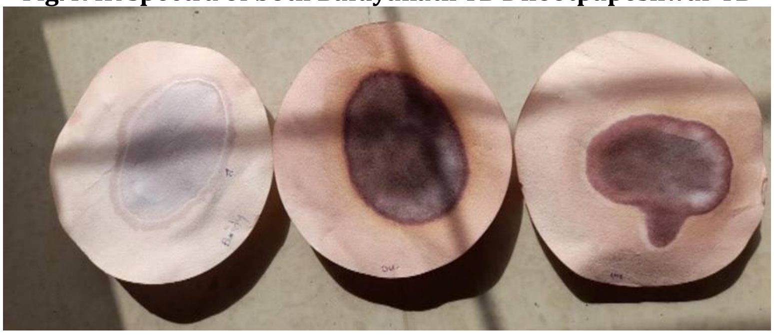


Fig. 5: Namburi phase spot test

CONCLUSION

This study is based on various bhasma studies that have been presented in Ayurvedic literature, both traditional and modern techniques. On the basis of these studies, we are able to confirm the quality of Yasad bhasma in comparison to other commercially available bhasma. In addition, the physicochemical study reveals that the DYB values are better. According to the results of the XRD analysis and the FTIR investigation, the primary component of both samples was zinc oxide. Both samples exhibited a number of different functional groups in their infrared spectra, but the Zn peak was the most prominent. It was established that the Zeta potential of Yashada bhasma at a pH of neutral was within the acceptable range of (+30 to -30 mV), which indicates that both bhasma are stable. It has been found that the only particle size of DYB is fine, which results in improved bioavailability and adsorption. It was therefore thought to be more suitable for internal administration. As a result, every single analytical investigation demonstrates that DYB is superior in terms of therapeutic efficacy. This is a huge step toward establishing the scientific explanations for the efficacy and safety of this traditional medical practice, which dates back thousands of years.

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Data is collected from various resources such as pubmed data base, google scholar and various research journals.

Competing interests: The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding: Not applicable

Authors' contributions
The Concept was discussed and writing original draft of the article by SC with validation, data analysis, checked the grammar and remove all the plagiarism by VA. Both the author read and approved the final manuscript."

ACKNOWLEDGEMENTS
We acknowledge Dr. S.N. Gawande, Department CIC, Mr. P. U. Ingle, Department Biotechnology, Sant Gadage Baba Amravati University Amravati. For their assistance in carrying out some of the analytical studies concerning Yasad bhasma.

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CITATION OF THIS ARTICLE
V. S. Adhao, S. A. Chavhan. Comparative Qualitative Analysis of Yasad Bhasma with Classical and Modern Parameters. Bull. Env.Pharmacol. Life Sci., Vol 13 [4] March 2024: 24-32