Research Article

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ANALYTICAL STANDARDIZATION OF MANDURA BHASMA

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ABSTRACT

Background: Rasa Shastra is an independent branch of Ayurvedic medicine, which deals with preparation of the drugs of metals and minerals with higher efficacy in lower dose and good palatability. Mandura Bhasma is an important formulation mentioned in Rasa Shastra texts obtained from the incineration of Raw Mandura. The pharmaceutical procedures involved in the preparation of Mandura Bhasma are Shodhana, Bhavana and Marana. Objective: To assess the safety and to understand about the identity, form, particle size and surface morphology of the Mandura Bhasma. Materials and Methods: Mandura bhasma was subjected to analysis through various techniques like X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDS), Particle size analysis (PSA), Zeta Potential (ZP), UV-Spectroscopy and Fourier transform Infra-Red spectroscopy (FTIR). Results and Conclusion: XRD of Mandura Bhasma showed major peaks of Fe_2O_3 (Iron oxide) and a minor peak K_2SO_4 (Potassium Sulphate). SEM micrographs showed irregular distribution of more or less Rhombohedral particles. EDS analysis confirmed the significant presence of elements viz. Fe-38.09%, O- 29.38%, Ca- 0.40%, and K-0.52%. Particle size was found to be 3.2 nm with Zeta Potential of -32.7 mV. UV- Spectrum of Mandura Bhasma showed maximum absorption at 300 nm. FTIR test showed 26 peaks between the wavelength 3696.60cm^{-1} to 632.67cm^{-1}. This shows that Raw Mandura has been completely transformed into compound form (Bhasma) without any traces of free elemental form.

Keywords: Mandura Bhasma, Analytical Tests, Safety, Toxicity.

INTRODUCTION

Analytical study is a process which helps in identification of quantitative and qualitative data of a substance, the components of a solution or mixture, or the determination of the structures of chemical compounds. It gives us the knowledge about identity, size, structure of chemical constituents and physical properties. It hints us about toxic properties of drugs, if any.

Ayurvedic drugs are proven for their efficacy and does not require any further validation for their administration to patients. Safety of the drug to be administered is at par with its efficacy. Analytical study is an important part in any research activity. It describes about the correlation between pre-determined hypothetical values and actual results obtained. It gives us clear information regarding safety, efficacy, stability, and contra-indications of any formulation. The presence of free metal in any formulation can cause damage to the vital organs of the body. Hence highly sensitive modern analytical parameters are used for gaining information about identity, form, particle size, and structure of contents of the formulation. Taking this into consideration, an effort has been made to analyze Mandura Bhasma through various modern parameters like X-ray diffraction, Scanning electron microscopy, Energy dispersive X-ray analysis, Particle Size Analysis, Zeta potential; UV-Spectroscopy and Fourier transform Infra-Red spectroscopy.

MATERIALS AND METHODS

Mandura and Triphala were obtained from local market of Chennai, Tamil Nadu. Gomutra was collected from the Goshala, TTD, Tirupati. Entire preparation of Mandura Bhasma was carried out in TTD's Sri Srinivasa Ayurveda Pharmacy and Department of Rasa Shastra and Bhaishajya Kalpana, S.V.Ayurvedic College, Tirupati. Requirement for XRD: Model-Powder X-Ray Diffractometer D8 advance, Manufacturer-Bruker Germany. SEM and EDS: Model- EVO MA 15, Manufacturer- Carl Zeiss – Germany; PSA and ZP: Model-Horiba scientific Partical Size and Zeta Potential Analyzer, Manufacturer- Horiba instruments, Irvine, CA 92618 USA; UV-Spectroscopy: Model- Nano drop 8000 Spectrophotometer, Manufacturer- Thermo Scientific India.

Pharmaceutical process

The main pharmaceutical procedures involved in the preparation of Mandura Bhasma are Nirvapa, Bhavana and Marana. Mandura was taken and subjected to Shodhana by Nirvapa in Gomutra Triphala Kashaya for 7 times[1]. Then the Shodhita Mandura was triturated with Kumari Swarasa and subjected to Marana by Gaja puta for 7 times, till the bhasma attains all the Bhasma lakshanas as mentioned in the classics[2]. Thus obtained Mandura Bhasma was collected and preserved in an air-tight container.

Analysis of Mandura Bhasma using ancient parameters (Bhasma Pariksha)

- Rekhapurnatva[3]: After proper trituration, small amount of bhasma was taken between thumb and index finger. It filled into the fine lines of fingers. Rekhapurnatva was obtained after 6th puta.
- Varitaratwa[4]: After proper trituration, small amount of bhasma was sprinkled on the surface of water. Bhasma being light floated on the surface of water. This was obtained after 7th puta.
- Niswadu Pareeksha: When a small amount of the bhasma was kept on tongue, there was not any feeling of taste / untoward sensation. This was obtained after 7th puta.

Analysis of Mandura Bhasma using modern parameters

X-Ray Diffraction (XRD)

Mandura Bhasma was subjected to XRD at Department of Physics, Yogi Vemana University, Kadapa, Andhra Pradesh.

Procedure: Sample was powdered in agate mortar to very fine powder and it was mounted in sample tray of machine. X-Ray beam bearing a wavelength of 1.5418740 A° from copper source is passed on the sample. Detector was set to identify diffracted beams between 10 -70 degrees of 2 range. Obtained soft files of XRD consisting values of 2θ and intensity are plotted on a graph (2θ on X-Axis and Intensity of Y-Axis) using "Origin Pro 8.5 SR2" Data Analysis Software. Various compounds consisting similar diffraction pattern were identified by matching their peaks with corresponding JCPDS Crystallographic cards. For even better accuracy and precision, XRD soft files were also analyzed for corresponding phase/entry matching with Crystallographic Open Data base (COD - 20120320) – USA, after plotting values in PANalytical X'pert high score plus software 3.0.0.123, UK.

Scanning Electron Microscopy and Energy dispersive X-Ray spectroscopy

The practical was performed at Department of Physics S.V University, Tirupati.

Procedure of SEM: Specimen of the sample to be analyzed was directly kept on the specimen holder for visualization. As the sample employed has nonconductive nature, the sample surface was coated by carbon by arc melting technique. Then the dried powder was observed under the microscope at 1,000X to 10,000KX and the micrographs were taken with the inbuilt camera.

Procedure of EDS: Electron beam excitation is used in scanning electron microscopes (SEM). A detector is used to convert X-ray energy into voltage signals; this information is sent to a pulse processor, which measures the signals and passes them on to an analyser for data display and analysis. The detector used in EDS is often the Lithium drifted Silicon detector which is operated at liquid nitrogen temperatures. Sample of Mandura Bhasma was placed on the specimen holder and subjected to Energy-Dispersive X-ray spectroscopy (EDS). When the sample was subjected to SEM's electron beam, bombardment occurs and the electrons are ejected from the atoms creating the sample's surface. Electrons are ejected from higher state to fill the resulting electron vacancies, and an X-ray is emitted to balance the energy difference between the two electrons' states. The X-ray spectrum thus obtained gives the elemental composition of the material.

Particle Size Analysis and Zeta Potential

The practical was conducted at Department of science and Technology, PURSE, S.V.University, Tirupati.

Procedure of PSA: The sample was mixed in water and centrifuged for 10 minutes. Then it is placed in the sample chamber, where the laser beam passes through the homogeneous particles. The scattering of light occurs over a wide range of angles upon interacting with the particles in the suspension which are moving by Brownian motion. Depending upon the scattering pattern of sample, distributions in particle size are calculated comparing with optical model.

Procedure of ZP: A 1% concentration of Mandura Bhasma was prepared using distilled water. The sample was taken in a 1ml syringe and injected slowly into the capillary cell (cuvette) through the sample port. When the sample comes out from the secondary port of the capillary cell, the injection process is stopped. Then the sample ports are covered with lids and capillary cell was placed into the sample holder of the zeta sizer instrument for analysis.

UV- Spectroscopy

Practical was performed at Department of science and Technology, PURSE, S.V.University, Tirupati.

Procedure: 5gm of Mandura Bhasma was diluted with 100 ml of solvent in a closed flask, and it was frequently shaken for six hours and allowed to stand for eighteen hours. Then it was filtered, and subjected for UV spectroscopic study. The Spectra was calculated at 200-800 nm, from the peak obtained the λmax value was calculated.

Fourier Transform Infrared Spectroscopy (FT-IR)

This practical was conducted at Padmavathi Mahila University, Tirupati.

Procedure: Sample was placed in the Potassium bromide plate of FTIR spectrometer and the interference pattern was detected by the infrared detector as variations in the infrared energy level, and the obtained spectral information was calculated.

RESULTS

X-Ray Diffraction Studies (XRD)

Table 1: Details of matching peaks of XRD data for Mandura Bhasma

S.No	Element/Molecule	JCPDS Ref.No	2θ	Intensity	FWHM	H	K	L
1.	Fe_2O_3 (Iron oxide)	00-013-0534	24.29	28.66	0.216	0	1	2
			33.28	100	0.216	1	0	4
			35.74	68.95	0.216	1	1	0
			49.55	29.21	0.12	0	2	4
			54.23	60.00	0.192	1	1	6
			57.71	16.00	0.288	0	1	8
2.	K_2SO_4(potassium sulfate)	01-071-1912	27.42	4.8	0.384	1	0	2

Table 2: Crystal details of JCPDS entries

Name	Fe_2O_3
Space group	R-3c
Crystal System	Rhombohedral
Cell Parameters	a = A˚ b= 5.0130 A˚ c = 13.7370 A˚
Z	6.0

Name	K_2SO_4
Space group	R-3c
Crystal System	Rhombohedral
Cell Parameters	a = 5.0310A˚ b= 5.0130 A˚ c = 13.7370 A˚
Z	6.0

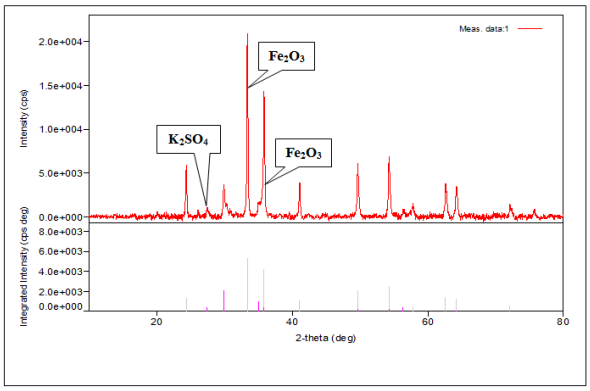

Figure 1: XRD peaks of Mandura Bhasma

XRD of Mandura Bhasma shows major peaks of Fe_2O_3 (Iron oxide) with Rhombohedral structure. A minor peak showed the existence of K_2SO_4 (Potassium sulphate) with Rhombohedral structure. The Fe_2O_3 peak was detected at diffraction angle of 24.29, 33.28, 35.74, 49.55, 54.23, 57.71, K_2SO_4 peak was detected at diffraction angle of 27.42.

Scanning Electron Microscopy

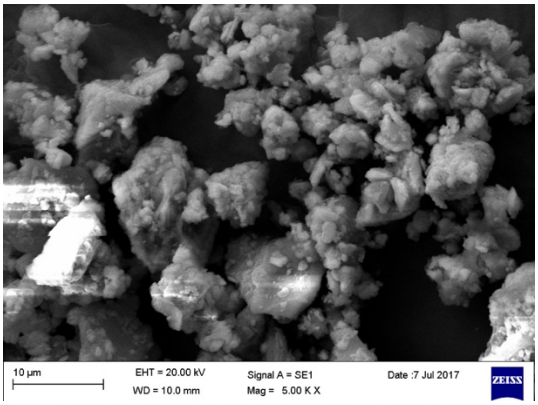

Figure 2: SEM result of Mandura Bhasma (Mag. 5KX)

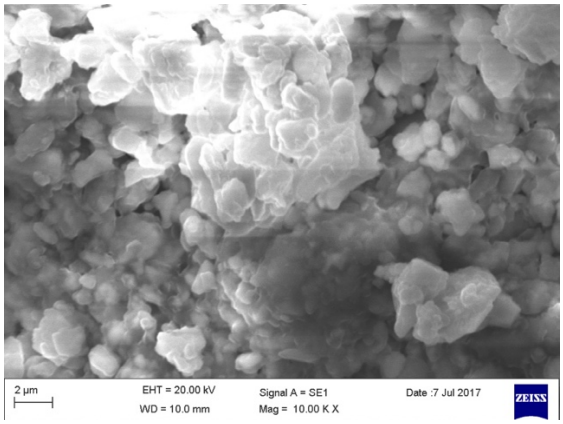

Figure 3: SEM result of Mandura Bhasma (Mag.10KX)

SEM micrographs of Mandura Bhasma showed irregular distribution of more or less Rhombohedral particles at 5KX and 10KX magnification

Energy Dispersive X-Ray Spectroscopy

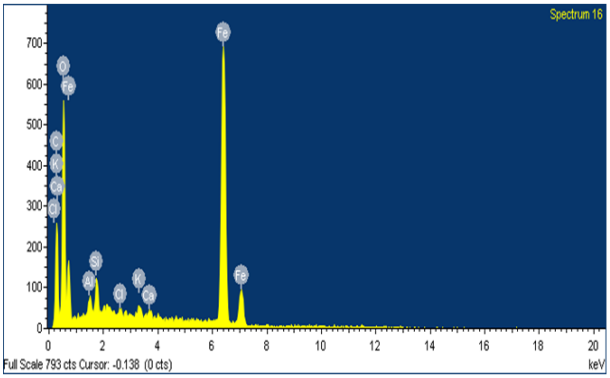

Figure 4: EDS graph of Mandura Bhasma

Table 3: Quantity of all the elements in Mandura Bhasma

Element	Weight%
C K	29.37
O K	29.38
Al K	0.71
Si K	1.21
Cl K	0.33
K K	0.52
Ca K	0.40
Fe K	38.09
Totals	100.00

EDS analysis of Mandura Bhasma confirmed the presence of following elements viz. C- 29.37%, O-29.38%, Al- 0.71%. Si-1.21%, Cl- 0.33%, K%- 0.52%, Ca- 0.40% and Fe-38.09%.

Particle Size Analysis

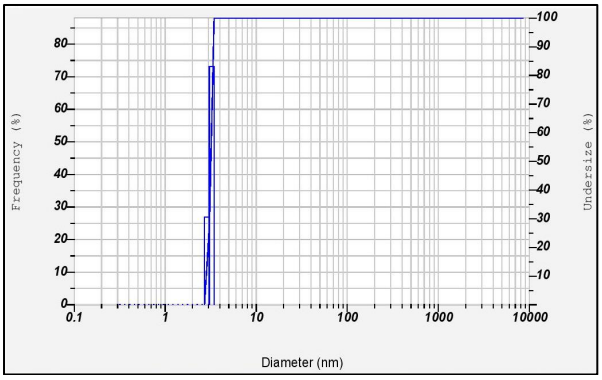

Figure 5: Result of Particle size analysis of Mandura Bhasma

The mean particle size of Mandura bhasma is 3.2 nm.

Zeta Potential

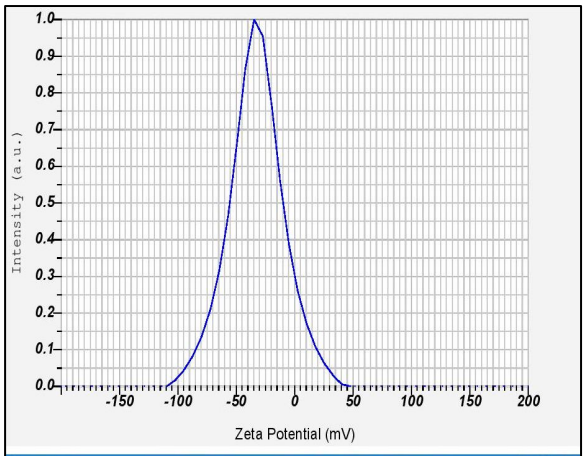

Figure 6: Zeta potential distribution of Mandura Bhasma

The Zeta Potential (mean) value of Mandura Bhasma was found to be -32.7mV which indicates high colloidal stability.

UV- Spectroscopy

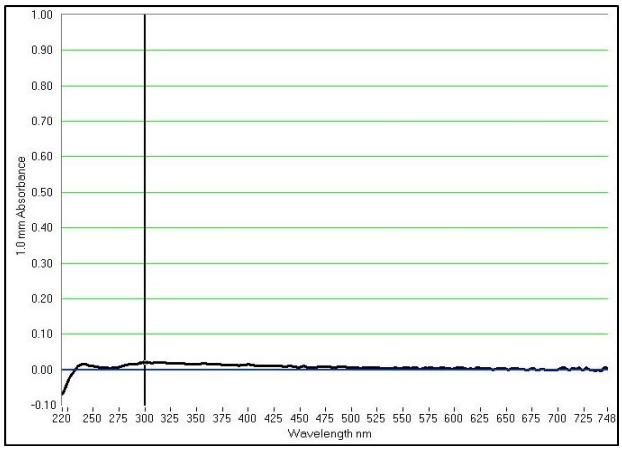

Figure 7: UV-Spectrum of Mandura Bhasma

UV- Spectrum of Mandura bhasma showed maximum absorption at 300 nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

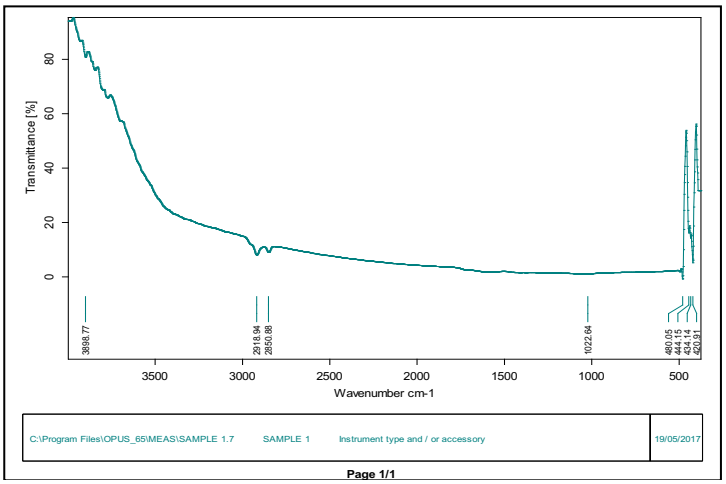

Figure 8: Various peaks obtained in FTIR analysis of Mandura Bhasma

Table 4: Details of Peaks obtained in FTIR analysis of Mandura Bhasma

Sample Name	No. of Peaks	Wave length
Mandura Bhasma	26	3948.38, 3910.29, 3877.61, 3835.65, 3777.79, 3696.20, 3661.14, 3530.76, 3482.79, 3440.28, 3408.47, 3350.91, 3173.91, 3101.49, 3034.32, 2934.72, 2683.49, 2380.68, 2343.95, 1754.98, 1659.29, 1578.47, 1479.61, 959.69, 632.67

Table 5: Various peaks obtained in FTIR analysis of Mandura Bhasma and their correlation with compounds

S.No.	Peak	Actual peak	Bond	Type of bond	Specific type of bond	Appearance
1.	3600-3700cm^{-1}	3696.20cm^{-1} 3661.14cm^{-1}	O -H	Alcohol Phenols	low concentration	Broad
2.	3500-3560cm^{-1}	3530.76cm^{-1}	O – H	Carboxylic acids	High concentration	Strong, Broad
3.	3400-3500cm^{-1}	3482.79cm^{-1} 3440.28cm^{-1} 3408.47cm^{-1}	N – H	Primary amines	Any	Strong
4.	3200-3400cm^{-1}	3350.91cm^{1}	O – H	Alcohols Phenols	High concentration	Broad
5.	2400-3200cm^{-1}	3173.91cm^{-1} 3101.49cm^{-1} 3034.32cm^{-1} 2934.72cm^{-1} 2683.49cm^{-1}	N – H	Ammonium ions	Any	Multiple broad peaks
6.	1640-1680cm^{-1}	1659.29cm^{-1}	C-C	C=C(both sp^2)	Any	Medium
7.	1450cm^{-1}	1479.61cm^{-1}	C-H	Alkyl	Methylene	Strong
8.	965cm^{-1}	959.69cm^{-1}	C-H	Vinyl	Trans-di substituted alkenes	Strong
9		632.67cm^{-1}	C-X	Bromo alkanes	any	Medium to strong

Mandura bhasma showed 26 peaks between the wavelength 3696.60cm^{-1} to 632.67cm^{-1} with different types of bonds like alcohols, phenols, ammonium ions, amines, alkyl, vinyl and bromo alkanes.

DISCUSSION

Analytical study is the series of process performed in order to identify or quantify a substance. Quality of a drug depends upon its formulation, processing and applications. Certain standards must be essentially followed while manufacturing a drug so that the genuineness of the drug is not compromised. Ayurvedic texts have described a number of methods for quality control of finished products like Rekhapurnatwa, Varitaratva, Nishchandratva, Nirutha[5] etc. to achieve a specific acceptable standard Bhasma. Usage of modern technology to understand the relevance of the concepts mentioned in Ayurveda have become quintessential, so that they may be interpreted in the light of present scientific language to mark it applicable with the current health care. Hence, Mandura Bhasma was prepared and analyzed for quality control, according to both modern and ancient parameters.

X-ray diffraction has been used in two main areas, for the finger print characterization of crystalline materials and the determination of their structure. Each crystalline solid has its unique characteristic X-ray powder pattern, used as a "fingerprint" for its identification. Once the material has been identified, X-ray crystallography may be used to determine its structure, i.e. how the atoms pack together in the crystalline state and what the inter-atomic distance and angle etc. Size and the shape of the unit cell for any compound can be detected most easily using the X-ray diffraction. Major peaks of Fe_2O_3 and a minor peak of K_2SO_4 were seen in XRD reprint. Mandura is considered as rusted iron, its chemical formula is $Fe_2O_3.H_2O$. During shodhana and Marana process the water portion of Mandura may get evaporated and only Fe_2O_3 (ferric oxide) remains. XRD analysis in the present study confirms the presence of Fe_2O_3 in Mandura Bhasma. Sharp peaks of Fe_2O_3 indicate the high crystallinity of Mandura Bhasma with Rhombohedral structure. Presence of K_2SO_4 as minor peak may be due to Bhavana with Kumari Swarasa which contains Potassium and Sulphur as major element.

Scanning electron microscopy (SEM) is an analytical procedure performed to understand the surface morphology of the drug. It uses electron beam rather than light to form a figure. It is capable of producing high resolution figures of a sample surface at a high magnification. SEM figures have a characteristic three dimensional appearance and are useful for determining the surface structure of the sample i.e. topography. Irregular distribution of more or less Rhombohedral particles of Mandura Bhasma may be due to the presence of Rhombohedral structures of Fe_2O_3 and K_2SO_4. Surface of Mandura Bhasma was appeared to be smooth due to the involvement of procedures like Nirvapa, Bhavana and Marana.

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. EDS of Mandura Bhasma confirmed the significant presence of elements like Iron and oxygen. The presence of other elements like Calcium, Aluminium, Potassium and Silica may be due to the addition of herbal ingredients during Shodhana and Bhavana.

The Particle size of the drug has a major role in therapeutic action, absorption and efficacy. The mean particle size of Mandura Bhasma is 3.2 nm. The particle size of drug indicates regarding absorption and dispersion into body resulting in better therapeutic efficacy. Zeta potential is a degree of the magnitude of the electrostatic or charge repulsion or attraction between particles, and it is one of the basic parameters identified to affect stability. The Zeta Potential (mean) value of Mandura Bhasma was found to be -32.7mV which indicates high colloidal stability. High zeta potential indicates easy dispersion towards target site, whereas less zeta potential indicates strong aggregation of particles in suspension.

UV-Spectroscopy mentions to absorption spectroscopy in the ultraviolet-visible spectral region. Radiation of different wavelengths are absorbed by different molecules. An absorption spectrum will display number of absorption bands matching to structural groups with the molecule. Electromagnetic spectrum of U.V region ranges from 190 to 400 nm and for visible region it is 400-800 nm. UV- Spectrum of Mandura Bhasma showed maximum absorption at 300 nm in the UV region.

FTIR was performed to detect the presence of functional groups or organic legends in Mandura Bhasma. Infrared spectroscopy deals with the infrared region of the electromagnetic spectrum that is light with a longer wavelength and lower frequency than visible light. When infrared light or radiation hits a molecule, the bonds in the molecule absorb the energy of the infrared and respond by vibrating. Mandura Bhasma showed 26 peaks between the wave length 3696.60cm^{-1} to 632.67cm^{-1}. O-H stretching vibrations peaks were observed between 3600-3700 cm^{-1}, 3500 -3560 cm^{-1} and 3200 to 3400 cm^{-1} wave lengths. Alcohols, phenols and carboxylic acids were observed in the peaks. Multiple peaks with N-H stretching vibrations containing primary amines and ammonium ions were observed at the wave lengths 3400 to 3500cm^{-1} and 2400 to 3200 cm^{-1}. A single peak containing C-C bond and C-X bond of medium intensity was found at 1659.29 cm^{-1} and 632.67cm^{-1} respectively. Alkyl and

Vinyl bonds of strong intensity were observed at 1479.61cm^{-1} and 959.69 cm^{-1} wave lengths.

CONCLUSION

The present study confirms the complete transformation (Structural and Chamical) of Mandura into bioabsorbable compound form (Mandura Bhasma). Complete transformation of base metal/mineral into Bhasma form is primary requisite in preparation of any metal/mineral formulation to avoid any adverse effects. Proper formation of Bhasma is checked through various Bhasma parikshas as mentioned in the Rasashastra texts. As these Bhasma parikshas are mainly qualitative in nature, to understand their characterization modern analytical parameters like XRD, SEM, EDS, PSA, ZP and FTIR are very helpful. Hence both the ancient and modern parameters must be used for the justification of the formulation, for a safe therapeutic approach.

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