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A COMPARATIVE ANALYTICAL STUDY OF MANDURA BHASMA (IRON OXIDE-BASED NANO MEDICINE) PREPARED BY TWO DIFFERENT TRITURATION MEDIA (BHAVANA DRAVYA)

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ABSTRACT

Bhasmas are unique Herbo-mineral dosages form in ayurvedic system of medicine. It plays an important role in disease condition even in smaller dose irrespective of body constitution (prakriti). The preparation of Bhasma involves series of process in specific order namely Purification (shodhana), trituration (bhavana), pelletization (Chakrika Nirmana) and Incineration process (marana). Mandura is an iron oxide-based mineral drug obtained as a byproduct during the extraction of iron ores in the metallurgical process. The chemical substance called FeSiO4 functions as slag material (Mandura). Mandura having alternative names such as kitta and lohabhava and lohamla together with lohakitta and so on. It is widely used in management of rasa and rakta pradoshaja vikara like Pandu (Anemia) kamala (Jaundice) etc. Ancient scholars developed several tests which was mainly based on organoleptic properties of substances. Such as Varitar (lightness), Rekhapurnata (fineness), Apunarbhava (irreversibility), varna (colors) etc. which was not sufficient for proper analysis of Bhasma therefore, modern, advanced tools and techniques such as SEM analysis, chromatography, EDX, bioassays, XRD was used in the current study for the qualitative and quantitative analysis of both raw drugs and finished products.

Keywords: Ayurveda, Bhasma, Mandura, Shodhana, Bhavana, Marana, Pandu, SEM, EDX

INTRODUCTION

Rasa-ausadhi are an important herbo-mineral compound formulation in ayurvedic alchemy. It is taken in minimal dosage; the other herbal ingredients present in formulations helps in better assimilation of mineral drugs that results in rapid action of drugs. These metals-mineral drugs having no taste therefore, it not causes any nauseate sensation **[1]**. Rasa-ausadhi serve as traditional medicinal compounds that help heal both acute and chronic illnesses. The primary substances used in Rasashastra are Parthiv Dravyas, extracted from the earth because they provide enhanced therapeutic effects at lower doses compared to preparations containing only herbal (Audbhida) or animal (Jangama) products.

Metals and minerals have a particular importance in the framework of Ayurvedic medicine. These natural metals and minerals stay unused in their original state and converted in assimilable forms after several process of Bhasmikarana which is purification, Trituration and Incineration. Analytical study is an only way to standardized raw drugs and finished products in terms of quality, safety and efficacy. It also helps us to know pharmacodynamics and pharmacokinetic of drugs. Many analytical parameters have been developed by ancient scholars for quality assurance of bhasma, which are mainly based on organoleptic characters of bhasma and presently not sufficient to standardized drugs on global parameters. Hence modern analytical technique such as XRD, SEM, TEM, EDX, Chromatography etc. are necessary to make standard protocol for the testing of Raw drugs and finished product. This helps in producing safe, cost-effective and good quality of ayurvedic medicines. The present study focused on analysis of crystalline structure, particle size, composition and other physio chemical parameters of two differently prepared mandura Bhasma through both traditional and modern analytical techniques.

MATERIALS AND METHODS

Materials

Raw mandura and other raw material were procured from Rasa shastra and Bhaishajya Kalpana department, Govt. Ayurvedic College, Patna, Bihar. Identification and authentication of genuine raw mandura was done by experts of Rasa shastra department, GACH, Patna, Bihar. In this study, both ancient and modern testing methods was employed for the comprehensive analytical evaluation of raw drugs and prepared Mandura Bhasma. Ancient analytical technique used in this study was based on

references given in different Classical text of Rasa shastra, explaining different methods of Bhasma Pariksha while Modern analytical parameters would be applied as outlined in the protocol for testing Ayurvedic, Siddha, and Unani drugs, published by the Ministry of AYUSH, Government of India, in collaboration with the Pharmacopoeial laboratory for Indian Medicine, Ghaziabad. In this study Scanning Electron Microscope (SEM) and Energy dispersive X-ray spectroscopy (EDXS) was required, which is done at Central Discovery center, Banaras Hindu University, Varanasi, XRD was done at department of physics, Institute of sciences, BHU, Varanasi, UP.

Methods

Ancient (classical) analytical techniques:

Rekhapurnata (Fineness Test)

A little amount of mandura bhasma was taken in between index finger and thumb and then rubbed it, Bhasma easily enters into the furrows (lines) of thumb and index finger. This indicates Micro fineness of Bhasma **(Figure 1) [2]**.

Figure 1: Rekhapurnatva Test

Varitara (Lightness Test)

The Varitara property of Bhasma refers to its ability to float on the surface of stagnant water. This characteristic can be based on the principle of surface tension. The particles of *Bhasma* become so fine and light that they do not break the surface tension of the water, allowing them to remain on the surface of water. In this method water was taken in transparent glass jar then small amount of mandura bhasma was sprinkled over the surface of stagnant water. It was observed that Bhasma floats on the surface of water indicates lightness and fineness and ideal character of Bhasma **(Figure 2) [3]**.

Unama (Lightness Test)

In this method, A rice grain was put on the floated Bhasma over stagnant water in a glass beaker. Here bhasma does not sink which indicates lightness **(Figure 3) [4]**.

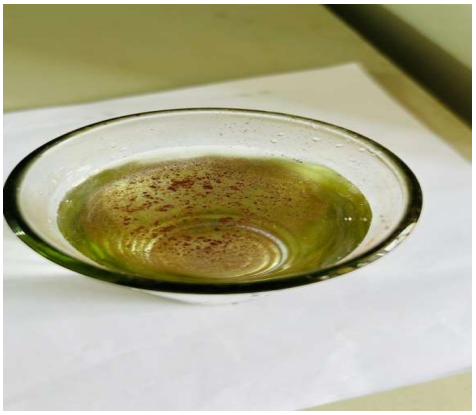

Figure 2: Varitara Test

Figure 3: Unama Test

Shlakshnatvam (Smoothness Test)

A little amount of Bhasma was rubbed between the index finger and thumb and it is Observed that bhasma does not produce any irritation. It shows prepared bhasma is smooth in nature.

Varna (color Test)

A specific color was mentioned for each Bhasma and this specific color suggests that the Bhasma is converted into desired metallic compound form because every chemical compound possesses specific color. Two different color was observed in two different prepared mandura Bhasma.

Avami

The Bhasma not producing any nauseates sensation.

Apunarbhava (Irreversibility)

Apunarbhava means irreversible state. This test is indicated for metallic Bhasma. If the Bhasma is prepared properly, it does not convert again into its original metallic form. In this method equal quantity of Bhasma(50mg) and Mitra Panchaka (Seeds of Abrus precatorius, honey, Ghrita, borax and jaggery) was taken and grounded. Then pellets were prepared and kept in a sharava samputa for incineration at same temperature (650^0C) for same duration, after self-cooling earthen pot, pellets were collected and marked for any lustrous particle or accumulated masses. It was observed that sample not containing any lustrous particles or free metals **(Figure 4) [5]**.

MODERN ANALYTICAL TECHNIQUE:

Scanning Electron Microscope (SEM)

SEM is a versatile instrument to investigate the surface of solid materials by scanning it with a fine focused beam of high energy electrons which provokes a number of signals. These signals are useful in characterizing the sample, at least regarding the outer surface properties, including texturing, chemical make-up and the crystal structure, and preferred orientation of the material phases

being built up in the sample. Conventionally, data is obtained on a surface of interest in the sample creating a two-dimensional map of the distribution of these parameters across the region. As in scanning mode, with conventional SEM techniques, one can image areas from about 1 cm to 5 microns in width with magnification that ranges from 20X to 30,000 X and spatial resolutions ranging from 50 to 100 nanometers. SEM can also analyze specific points on the sample, which is particularly useful for qualitatively or semi-quantitatively determining chemical compositions (through EDS), crystalline structure, and crystal orientations (using EBSD) **[6]**.

SEM of Mandura Bhasma

The Mandura Bhasma prepared through multiple cycles of trituration and incineration, resulting in a very fine Bhasma therefore there is no need of further grinding. A small amount of moisture-free Mandura Bhasma was used and coated with a thin layer of gold. This coating enhances contrast and helps protect the sample from excessive heat. The gold-coated Mandura Bhasma sample was placed on stub, which was then positioned on the sample stage inside the sample chamber. The chamber was evacuated to create a vacuum, as the presence of air could cause errors during scanning and image formation. Once the stage was properly aligned, the electron beam was activated. After focusing and adjusting the magnification on the SEM machine, high-resolution images of the Mandura samples from both batches was captured **(Figure 5, 6)**.

Energy-dispersive X-ray spectroscopy (EDS)

Energy dispersive x-ray spectroscopy (EDS) is also referred to as Energy dispersive analysis by X-ray or Energy x-ray microanalysis, is an analytical technique that applied for elemental quantification and chemical identification of the sample. This is achieved through examining the phenomena of interaction between an X-ray excitation source and the sample. The strength of the technique is that each of the element components of the material has a specific atomic number and the electromagnetic emission spectrum exhibited by the material consists of individual peaks. The outlet of this principle is that element identification is made with a high level of accuracy. These peaks can be predicted with extended accuracy using Moseley's law, and may even excel over the resolution constraints of traditional EDX systems **[7]**.

Figure 4: Apunarvbhava Test(Materials)

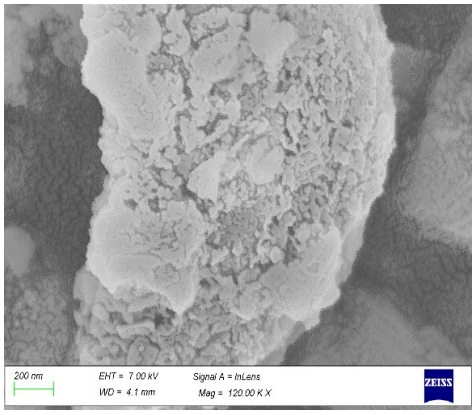

Figure 5: SEM of Batch 1 at 120 KX

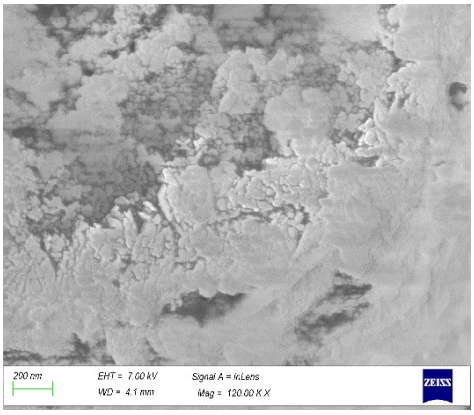

Figure 6: SEM of Batch 2 at 120 KX

EDXS analysis of Mandura Bhasama

EDXS analysis of mandura bhasma sample was done by EDS enabled scanning electron microscope. Here in this electron beam was bombarded over sample that causes excitation of inner shell electron which results in positive charged hole formation. The vacant space is filled by outer shell electron, during jumping of electron from outer shell to inner shell there are releases of energy in the form of X-ray signal, which is measured by detector. The signal produce is unique for each and every element. Elemental analysis was done using EDS software.

X-ray diffraction (XRD)

X-ray diffraction (XRD) is a fast analytical technique used to detect material crystalline structures and their unit cell dimensions. The small-sized granules go through detailed examination for determining the overall composition of the substance. XRD works on the principle of constructive interference between monochromatic X-rays and the crystal structure of the sample. The diffracted

X-rays result from Bragg's Law when the crystal satisfies $n\lambda = 2d \sin\theta$. Scientists monitor scattered X-ray light at various observation angles (2θ). Scientists use the obtained diffraction pattern to determine d-spacings then match them against standard patterns for mineral identification since minerals possess distinctive d-spacing combinations **[8]**. The linear plot draws by plotting $4\sin\theta$ with relation to $B\cos\theta$ gives W-H plots, which is used for determination of crystalline size and other crystalline parameters **(Figure 7, 8)**.

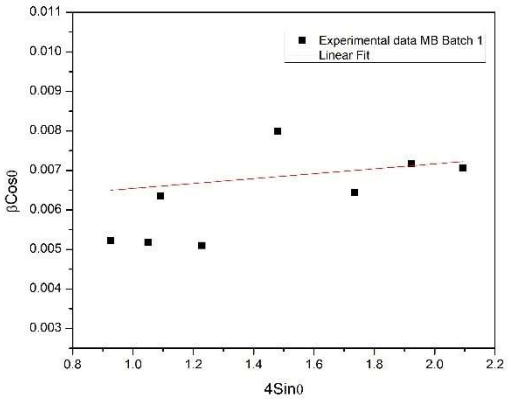

Figure 7: Linear fit plot of MB Batch1

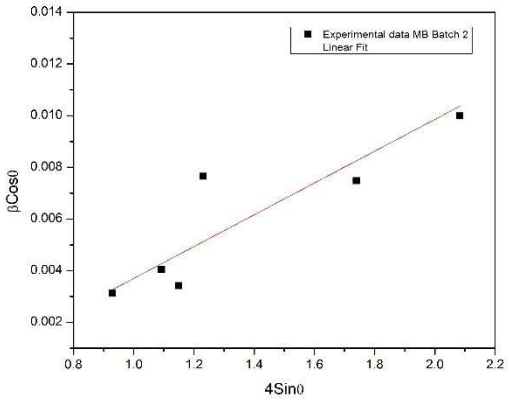

Figure 8: Linear fit plot of MB Batch 2

OBSERVATION AND RESULTS

The observation based on organoleptic character of prepared MB batch1 and MB batch 2 was detailed given in **Table 1 and Table 2** respectively **(Figure 9, 10)**.

The prepared Mandura Bhasma from both batches—Batch 1 (Aloe vera-processed Mandura Bhasma) and Batch 2 (Triphal kwatha-processed Mandura Bhasma)—successfully passed all traditional analytical tests for bhasma pariksha. These tests includs Varitar (floating on water), Rekhapurnata (filling fine lines), Unama (lightness), Shlakshnatvam (smoothness), Avami (non-reactivity with acids), Apunarbhava (irreversibility to the metallic state), and the prescribed color criteria mentioned in classical texts **(Table 3 and 4)**. These results confirm that both prepared bhasma are of ideal quality.

Table 1: Physical properties of Mandura Bhasma Batch 1

Texture	Color	Smell	Taste
Soft & smooth powder	Reddish-Brown	Not any	tasteless

Table 2: Physical properties of Mandura Bhasma batch 2

Texture	Color	Smell	Taste
Soft & smooth powder	Brick Red	Not any	Tasteless

Figure 9: Mandura Bhasma Batch 1

Figure 10: Mandura Bhasma Batch 2

Table 3: Classical test (Bhasma Pariksha) of Mandura Bhasma Batch 1

Test Parameters	Test Results	Lab Ref.
Rekhapurnata	+ve	CML/STP/037
Nischandrica	+ve	
Varitar	+ve	
Unam	+ve	
Apunarbhava	+ve	
Niswadu	+ve	
Nirdhoom	+ve	

Table 4: Classical test (Bhasma Pariksha) of Mandura Bhasma Batch 2

Test Parameters	Test Results	Lab Ref.
Rekhapurnata	+ve	CML/STP/034
Nischandrica	+ve	
Varitar	+ve	
Unam	+ve	
Apunarbhava	+ve	
Niswadu	+ve	
Nirdhoom	+ve	

SEM analysis showed that, at a magnification of 120 KX, with an EHT (electron high tension) of 7.00 KV and a working distance (WD) of 4.1 mm, the grain size of Mandura Bhasma of Batch 1 ranged from 48.76 nm to 199.79 nm, with an average particle size of 115.47 nm **(Figure 11)**.

While, the grain size of Mandura Bhasma of Batch 2 under the same conditions ranges from 16.26 nm to 115.11 nm, with an average particle size of 73.45 nm **(Figure 12)**. This finding indicates that Batch 2 underwent a more significant reduction in grain size compared to Batch 1. Additionally, SEM analysis also revealed that the particles size not uniform in both Batch of mandura bhasma.

Graphical representation SEM and EDS data: (Figure 11, 12)

EDXS analysis revealed that iron percentage in terms of weight is 40.7% and 36.3 % in mandura Bhasma of batch 1 and batch 2 respectively **(Table 5, 6)**. EDX analysis also revealed that atomic size of iron batch 2 mandura Bhasma is smaller than batch 1 in terms of atomic percentage which is 14.5% and 17.4% respectively **(Table 5, 6)** Iron intensity of the Fe Kα1 peak in the EDX spectrum of Mandura Bhasma Batch 2 is higher compared to that of Batch 2 which is 2.97K 6.5K respectively **(Figure 13, 14)**.

Elemental analysis through EDX prove that no heavy metals found in both batch of Mandura Bhasma **(Table 5, 6)**.

The test results of microbiological study revealed that sample of both batch of mandura bhasma free from yeast and molds while total bacterial count was 600 CFU/gm present in MB batch 1 and count 400 CFU/gm present in MB batch 2, Both batches having bacterial count under maximum acceptable limit **(Table 7, 8)**.

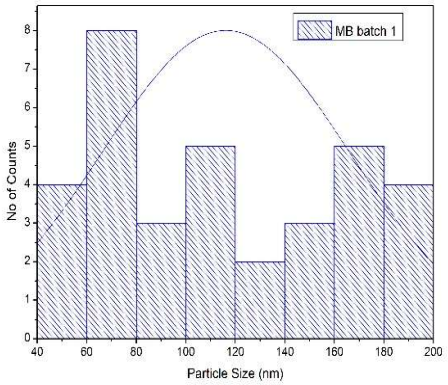

Figure 11: Particle size of MB batch 1(nm)

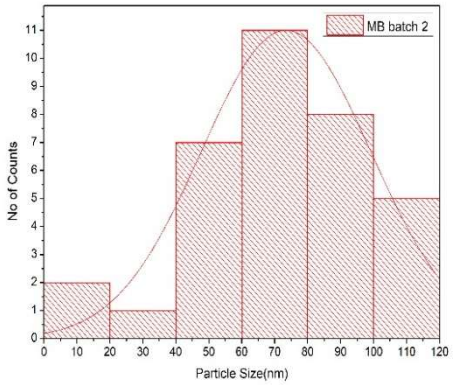

Figure 12: Particle size of MB batch 1(nm)

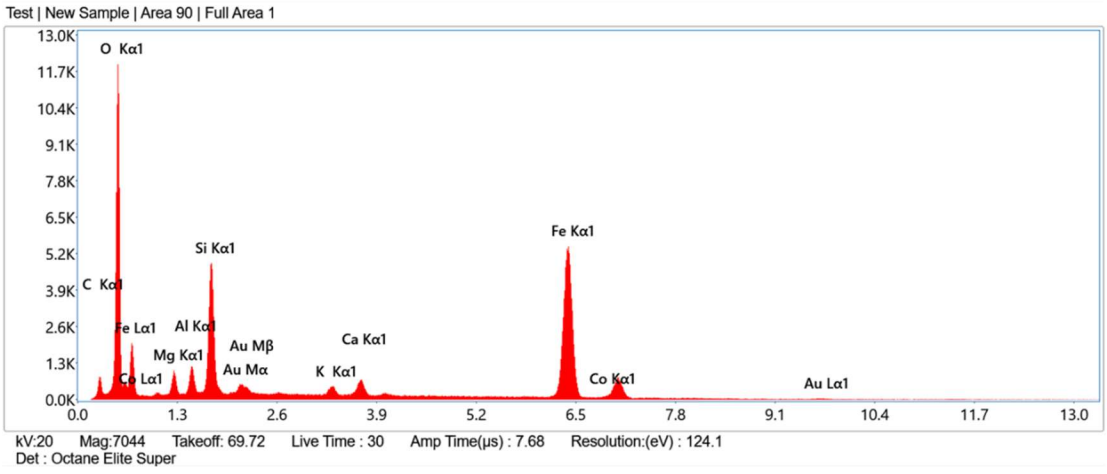

Figure 13: Graphical representation of Elements present in MB (Batch 1) by EDS

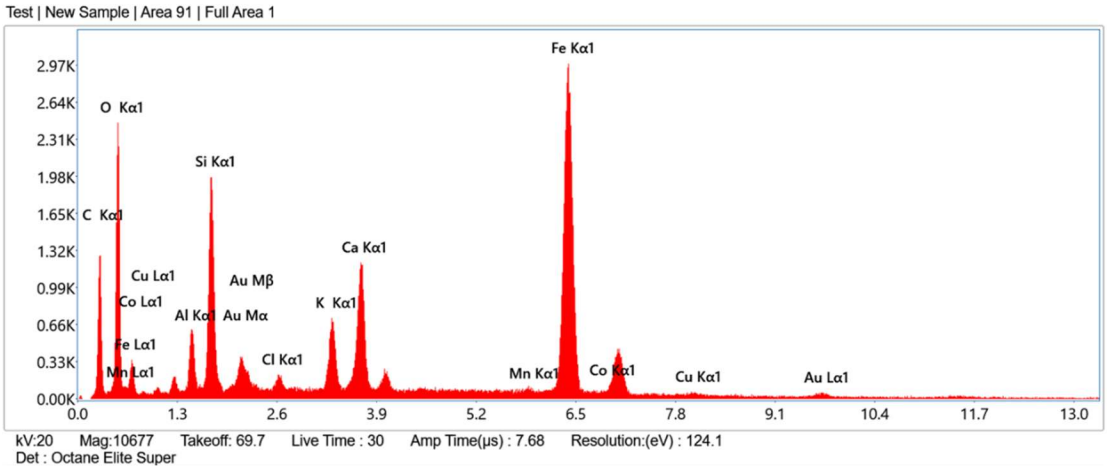

Figure 14: Graphical representation of Elements present in MB (Batch2) by EDS

Table 5: Elemental percentage presentation of Mandura Bhasma (Batch 1) By EDS

Element	Weight %	Atomic %
C K	9.2	18.3
O K	35.7	53.3
Mg K	1.7	1.7
Al K	1.6	1.4
Si K	7.3	6.2
K K	0.8	0.5
Ca K	1.6	0.9
Fe K	40.7	17.4
Co K	0.6	0.2
Au L	0.9	0.1

Table 6: Elemental percentage presentation of Mandura Bhasma (Batch 2) by EDS

Element	Weight %	Atomic %
C K	26.3	48.8
O K	19.5	27.1
Al K	1.1	0.9
Si K	4.2	3.4
Cl K	0.2	0.1
K K	2.3	1.3
Ca K	5.5	3.1
Mn K	0.5	0.2
Fe K	36.3	14.5
Co K	0.7	0.3
Cu K	0.4	0.1
Au L	3.1	0.3

Table 7: Microbiological Limit Test: MB batch 1

Sr. no.	Microbes	Units	Limit	Test Result	Test Ref.
1.	Total Bacterial count	CFU/gm	NMT 1,00000 CFU/gm	600	CML/STP/033
2.	Total yeast & mould count	CFU/gm	NMT 1,00000 CFU/gm	Absent	CML/STP/033

Table 8: Microbiological Limit Test: MB batch 2

Sr. no.	Microbes	Units	Limit	Test Result	Test Ref.
1.	Total Bacterial count	CFU/gm	NMT 1,00000 CFU/gm	400	CML/STP/034
2.	Total yeast & mould count	CFU/gm	NMT 1,00000 CFU/gm	Absent	CML/STP/034

XRD analysis was done by drawing graph using OriginLab software and obtained data was matched with JCPDS database to know chemical name and chemical formula. On XRD data analysis it was found that each sample was chemically iron oxides in Hematite (Fe_2O_3) and Magnetite (Fe_3O_4) forms, both samples are nano crystalline structure having crystal size 27.19nm in Mandura Bhasma batch 1 medicines and 18.12846 nm in Mandura Bhasma batch 2 medicines **(Figure 15)**.

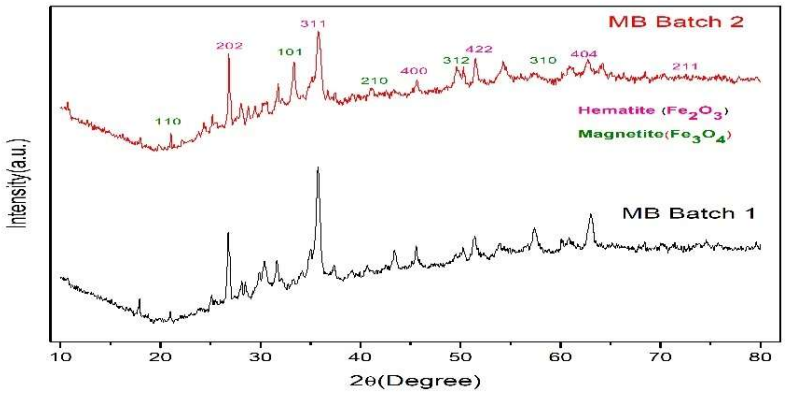

Figure 15: XRD peaks Pattern of Mandura Bhasma Batch1 and 2

DISCUSSION

Mandura Bhasma was prepared as per method described for the preparation of mandura Bhasma in Rasamrita (3/149) and Rasa Tarangani (20/129-130). In present study purification of mandura was done by using Nirvapa process seven times in cow urine. After purification (Sodhana) of mandura, Levigation process was done using Kumari swarasa for the preparation of batch 1 and Triphala kwatha for the preparation of batch 2 medicines **[9]**, **[10]**. Chakrika nirmana (pellets preparation) and sarava samputikarana was done. After proper drying of Earthen pot enclosed mandura pellets, it was subjected for incineration process (Marana) in electric muffle furnace at 650^0 temperatures. Mandura Bhasma batch 1 was prepared by using seven incineration cycle while Mandura Bhasma batch 2 was prepared by using 30 incineration cycle. Particles that are 100 nm or smaller are absorbed and translocated more efficiently than those between 500 nm and 6 µm. Particles larger than 5 µm are generally not absorbed through the gastrointestinal tract **[11]**. The particle size of bhasma prepared in present study also lies in this absorbable nano range, which was achieved by multiple trituration and incineration process during bhasma preparation. The other key aspect of

current study was to know the effect of different media on elemental constituent of bhasma, which was confirmed by EDS analysis. The Physio-chemical study, Microbiological study, classical and modern pharmaceutical study revealed that the prepared mandura bhasma was in standards quality and guarantee the medicine's safety, ensuring that it was free from heavy metals, microbes, and other harmful substances.

According to the 2021 Global Nutrition Report (GNR, 2021), India has made little progress in addressing anemia and childhood wasting diseases. A review of the Global Burden of Disease (GBD) survey indicates that iron deficiency anemia has remained a significant health burden in the country over the past decade. Consequently, there is a high demand for iron-based medicines both in India and internationally, emphasizing the need to adopt the best practices for producing affordable and high-quality iron-based medicine.

SEM analysis showed grain size and surface phenomenon of mandura Bhasma.SEM analysis proved that the prepared mandura Bhasma are in nano size. The average particle size of batch 1 mandura Bhasma was 115.47 nm while average particle size of mandura bhasma batch 2 was 73.45nm. The reason behind decrease particle size of batch 2 was due to number of trituration and incineration process which is approximately 4 times more than the trituration and incineration process of batch 1 medicine.

The elemental composition of mandura Bhasma was studied by EDX analysis. The succeeding increase in incineration cycle increases both quality and quantity of trace elements **[12]**. EDS confirmed that the both batch of mandura bhasma having iron in significant amount along with other element in trace amount like gold, cobalt, potassium, calcium, magnesium, carbon, oxygen, copper. some elements are micro and macro element while some elements present in both Bhasma may act as pro-enzyme in our body system for activation of different enzymes**.**

X-Ray Diffraction is one of the most advance technologies which is widely used in identification of unknown crystalline solids as well as to know unit dimension structures and crystal size. XRD analysis revealed that the both Bhasma are crystalline compound in Ferric oxide forms, Both Hematite (Fe_2O_3) and Magnetite (Fe_3O_4) forms of iron oxide found in both bacth of mandura Bhasma. But XRD graph shows sharp peak for hematite then Magnetite. MBB 2 having more smaller crystalline structure then MBB1, which may due to more cycles of Marana process in MBB2.

The Microbes, water vapors and other foreign body present in air and non-sterilized packaging material may cause contamination of the products. Therefore, aseptic condition was employed throughout the process. Microbiological study of both batch of medicine was done to know the total count of common microbes like Bacteria, Molds and yeast and it was found that molds and yeast are absent while total bacterial count was in acceptable limits in both batch of MB**.** Further, Human trials are necessary to know the effectiveness of mandura Bhasma which was prepared by using two different methods. There is also need of further study to know the pharmacodynamics and pharmacokinetics of Mandura Bhasma, as well as application other modern tools for analysis of mandura bhasma. There are also needs of study to compare mandura with other iron based ayurvedic medicine in terms of safety, efficacy. This helps in developing cost-effective treatments for the benefit of humanity, as well as establishing standardized methods for both drug preparation and analysis, ensuring the production of high-quality medicines on a global scale**.**

CONCLUSION

The present study confirms that the process described in the classical texts Rasamrita (3/149) and Rasa Tarangini (20/129–130) can be effectively utilized for the preparation of Mandura Bhasma. But the process described in Rasamrita text was more easy, convenient and economic than the process explained in Rasa Tarangani. SEM analysis confirmed that the average particle size of MB batch 2 medicine was 73.45nm, which was in size range having better absorption and translocation properties, hence drugs with better bioavailability and therapeutics benefits. EDS analysis shows that iron was significantly present in both batch of mandura Bhasma. XRD showed that the both Bhasma are chemically Ferric oxide in nano crystalline forms which proof that mandura Bhasma are Nanomedicines. The present study also suggested that media (Bhavana Dravya) directly affects the elemental constituent of Bhasma as iron content was more in MB batch 1 which was levigated with media of aloe vera juice. Both batch of medicine are safe for human use as both batch of mandura Bhasma not contained any heavy metals and microbial contents. There is Further need of study to revalidate the procedure and results for standardization of medicine in term of quality, safety and efficacy.

ACKNOWLEDGMENT

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Self-finance.

CONFLICT OF INTEREST

Authors declare that There is no conflict of interest

ETHICAL APPROVAL

Approval of Institutional Ethical Committee, Memo no.-351, dated-20/02/2023.

SUMMARY

Mandura is an iron oxide based ayurvedic formulation primarily used in treatment of anemia .In current comparative study, two methods were adopted for the preparation of mandura Bhasma .Preparation of batch 1 medicine was done by trituration of purified mandura with aloe vera juice and by using seven incineration cycles , which is described in text book of Rasa-amrita while batch 2 medicine was prepared by triturating with triphala decoction and by using thirty incineration cycles, The method described in text book of Rasa Tarangani .The analytical study of prepared mandura Bhasma can be conducted using various methods for comprehensive evaluation in terms of physical and chemical parameters. Both batches of medicine are of ideal quality, safe and efficient.

ABBREVIATIONS

R.T.-Rasa Tarangani, R.R.S- Rasa ratna samuchaya , SEM-scanning electron microscope, EDS- Energy-dispersive spectroscopy, NMT -Non-invasive micro-test, MB- Mandura Bhasma, EMF- Electric muffle furnace.

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