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PHYSICO-CHEMICAL CHARACTERISTICS OF MUNDA LOHA AND MANDOORA BHASMAS AND UNDERSTANDING THEIR HAEMATINIC EFFECT IN ALBINO RABBITS

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ABSTRACT

Rasasastra practitioners are well-versed in the processing of metals and minerals before making it applicable to the human body as medicines. South Indian Physicians flawlessly and keenly use some loha preparations for many ailments. One among them is bhasmam made from Munda loha which is used in place of Mandoora for making bhasmam. The present study was carried out to identify the physicochemical nature of Munda loha and Mandoora and the changes associated with the transformation of the same to the bhasmam state. Modern techniques like XRD, ICP-AES, SEM, FTIR and so forth are made use for the analysis. Munda loha (ML) is found to be fayalite-orthorhombic and the Munda loha bhasmam (MLB) obtained is αFe_2O_3. The Haematinic effect of the Bhasmam prepared from Munda loha and Mandoora are studied using Albino rabbits for comparing their respective activity. It is experimentally seen that Munda loha bhasmam has better action than that of Mandoora bhasmam (MB).

Keywords: Munda loha, Mandoora, Physicochemical, Haematinic effect, Animal study

INTRODUCTION

Rasousadhis are a group of potent herbo-mineral, metal and mineral preparations used since the evolvement of Rasasastra from the medieval period[1]. Even though Rasousadhis shows wide clinical applications, its safety and efficacy have been a topic of discussion for a long time[2].

Ayurveda, the age-old renowned medical wisdom of the world, uses many metal-based compounds as effective therapeutic agents[3]. In Ayurveda, Loha is used for eradication of many diseases of which iron deficiency anemia or 'Kaphaja Pandu' is a most important disease. Iron deficiency anemia remains one of the most severe and important nutritional deficiencies. It affects more than 200 million people in the worldwide. About 88% of pregnant and 74% of a nonpregnant woman is affected by anemia in India[4]. Anemia is caused by defective synthesis of haemoglobin, resulting in smaller than normal red cells and containing reduced amounts of haemoglobin[5-7]. A total of 293 formulations are based on iron oxide nanoparticles (Loha bhasmam) and 85 formulations contain iron compounds such as iron pyrite, ferrous sulphate,and ochre. Before the prevalence of Rasasastra, iron was administered for internal use as Ayaskruti or Loha Rasayana. The preparation involved heating thin plates of iron to red hot condition and dipping in cow's urine or Amalaki juice. Later a unique method involving purification and calcination steps was developed to convert iron to a therapeutic form, called Loha bhasmam[8]. The use of iron as a supplement in modern medicine began in the 16th century. The existence of iron in blood was discovered only in 1713[9].

Bhasmam preparations have a specific role in Ayurveda[10]. However, very few studies intended to know what happens to the metal when it is subjected to Bhavana and in the subsequent calcination processes. It is interesting to note that the same metal is treated with different sets of herbs and are used for various indications. In this context, the present work attempted to understand the physico-chemical changes that happen during the various stages of bhavana (quenching) and subsequent calcination process with a view to ascertain whether it acquires a non-toxic, therapeutically efficacious form.

Munda lohabhasmam (MLB) is widely used by South Indian Physicians in place of Mandoora bhasmam (MB) as an active Haematinic agent. Some authors have opined that the bhasmam of Munda loha should not be used as a medicament. However, Acharya Vagbhata postulated that the properties of Munda loha bear very much similar to that of the Mandoora bhasmam. Traditional Vaidyas use Munda loha instead of Mandoora, because the collection of Mandoora is tedious as per classical references, whereas Munda loha is easily available.

Various researchers have reported the toxic and therapeutic efficacies of loha bhasmam. Pandit *et al*. studied the hematinic activity of loha bhasmam in Phlembotomy-induced iron deficiency anemia in test animals and found that the efficacy of loha bhasmam was comparable to that of ferrous sulphate[11]. In 2016 Joshi and coworkers studied the toxicity of loha Bhasmam in albino rats and found to be safe at the therapeutic dose and also at five times the therapeutic dose levels[12]. Singh and Reddy conducted the pharmaceutical study of loha Bhasmam[13]. Sarkar *et al*. carried out the toxicity studies of loha Bhasmam and Mandoora Bhasmam and reported that they are safe at five times the therapeutic effective dose[14].

There are three kinds of Munda loha according to Rasaratna Samutchaya and other scholars. These are Mridhu, Kunda and Kudaaram differentiated by the response produced on being beaten by a heavy object. In the present study, the Munda lohabhasmam and Mandoora bhasmam are prepared according to

the method mentioned in Ayurvedic literature Rasaratna samuchaya. The prepared bhasmam is characterized by a traditional Ayurvedic method and by using various analytical techniques such as X-ray diffraction technique (XRD), Fourier transform Infra red spectroscopy (FTIR) and Inductively coupled plasma atomic emission spectroscopy (ICP-AES). The outcome of this study can be used as standards for evaluating the quality and efficacy of Munda loha bhasmam as well as Mandoora bhasmam.

MATERIALS AND METHODS

Purification (Sodhanam)

The General purification is intended to eliminate dosha from metals and minerals. For the purpose, Munda loha is heated to red hot and dipped in media like TilaTaila (sesame oil), Takra (buttermilk), Gomutra (cow urine), Aranaala/kaanjika, (fermented cereals) and Kulatwaqwatha, (horse-gram decoction) for seven times.

Specific purification is a plan to induce certain therapeutic values in a particular drug. A sample of Triphala amounting to 16 Pala after the removal of the seeds (1 Pala = 48 g), is boiled in eight times of water till the volume is reduced to one-fourth. To the resulting Triphala qwatha sufficient amount of red hot mundaloha was dipped. The process is repeated for seven times, till the desired result is achieved.

Incineration (Maranam) of Munda Loha

Maranam is a process by which the treated metal based material loses its original metallic structure on prolonged heating and gets converted into a biologically acceptable form for developing medicinal values. The process consists of two stages such as bhavana or mardanam of the drug with some Drava dravya for a particular period and Putapaka where the drug is subjected to agni paaka at a different temperature.

After iron is heated to red hot and hammered in smiting places to make different utensils, weapons and other metallic equipment, blacksmiths usually discard the remaining molten residues in certain parts of neighboring areas. These buried parts remain in the soil for many years and get converted into Mandoora. Rasayana Sara explains the formation of Mandoora as a process by which natural forces play a significant role. There are two varieties of Mandoora based on the kind of loha from which Mandoora is originated and the time period of Mandoora remained in the earth. Mandoora Grahyatha; Which is rigid, devoid of any crackles in it, older than a hundred years and which is dark in colour is collected from four places.

The general purification of Mandoora

Samanya Sodhanam is a procedure for eliminating dosha from metals and minerals. Mandoora loha is heated to red hot and dipped in media like Tila Taila (sesame oil), Takra (Buttermilk), Gomutra, (cow urine) and Aranaala/kaanjika, (fermented cereals) Kulatwa kwatha, (horse-gram decoction) for seven times.

The specific purification method (Vishesha Sodhanam) of Mandoora

It involves the quenching in liquid media, commonly Gomutra, after heating up to red hot (Nirvapa).

Incineration method (Maranam) of Mandoora

Different thoughts about incinerations are mentioned in traditional text books. Some Rasasastra scholars like Madhavacharya; the author of Ayurveda Prakasha recommends that sodhita Mandoora itself can be used for therapeutic purposes. They opine that after sodhanam, Mandoora becomes a fine powder. If features of bhasmam are not attained, and then it should repeatedly be processed until it becomes finely powdered.

For Maranam of Mandoora in generic, drugs of Loha Maraka Gana are used. Triphala qwatha is the most commonly used liquid media for Bhavana and gajaputa is used mostly as a heating grade for Mandoora.

Evaluation of Bhasmam by Classical Methods

Nischandratwam

The bhasmam was taken in a petri dish and was observed for luster in daylight through a magnifying glass. Properly formed bhasma will be lusterless in its nature.

Rekhapoornathwam

A pinch of bhasmam was taken between the thumb and index finger and rubbed, the bhasmam entered into the lines of fingers and was not easily washed out from the cleavage of the lines. This property of the bhasmam is known as Rekhapoornathwam.

Nisvadutvam

The prepared bhasmam was found to be tasteless when a small amount was tasted by the tongue.

Varitaratvam

A small amount of the prepared bhasmam was sprinkled over the water in a beaker that kept undisturbed. It found that the bhasmam particle floated over the surface of the water.

Instrumental Analysis

A Bruker model S4 Pioneer sequential wavelength-dispersive x-ray spectrometer was used for XRF analysis of various samples. The X-ray powder diffraction pattern of Munda loha bhasmam and Mandoora bhasmam were collected in INEl make XRG 3000 diffractometer with monochromatic Cu Kαl (β=1.54056) equipped with a curved position detector (model CPS590). The X-ray data gathered in the range of 10 to 120 degrees of 2 that as with a step size of 0.012 degrees. ICP-AES is used for the detection of trace metals present in loha bhasmam. FTIR of the samples was recorded between 4000 and 350 cm^{-1}, using Bruker Alpha FT-IR Spectrophotometer with ZnSe ATR method (at two cm^{-1}resonance). Scanning Electron Microscope (SEM) was taken using JEOL JSM-5600LV. Energy dispersive analysis of X-rays (EDAX) was carried out using Bruker XSHLASH-6 I30.

Animal experimental study

The Animal experimental study was conducted at the Amala Cancer Research Centre, Thrissur with prior Animal Ethical Committee consent (Approval no ACRC/IAEC/16-05(6) dated 02/05/2016). The study on the Haematinic effect of Munda loha bhasmam and Mandoora bhasmam done at female New Zealand White Rabbit of 1000 g to 2500 g body weight with phenylhydrazine-induced Anaemia. Phenylhydrazine is the most suitable drug of choice to induce Anaemia orally as it required

minimal dose. It induces destruction of Red Blood cell by oxidation stress resulting in Haemolytic Anaemia.

The acclimatized New Zea Land White Rabbits were weighed and grouped into four with six animals in each cluster. The selection was made by equally distributing the weight into each group and were marked separately for proper identification. They were kept in a separate cage and supplied with adequate water for drinking which is devoid of other mineral substances. Group one is normal, Group 2: control, Group 3, treated with Munda loha bhasmam and Group 4 with Mandoora bhasmam.

Anemia induced with orally given Phenylhydrazine (1.85 ml to control group, 1.64 ml to group 3 and 1.68 ml to group 4) except in Group 1 animals. From the third day onwards after blood analysis; group 3 and four were treated with Munda loha bhasmam and Mandoora bhasmam respectively. On every 8th day, the blood collected by puncturing the right ear lobe Artery of Rabbits using an insulin syringe. Punctured blood gathered in an EDTA-coated blood vial. Immediately after taking the blood, parameters like Hb count, ferritin, MCH and MCHC were analyzed to avoid the interaction with EDTA in the vial.

Collected data were statistically analyzed through one-way ANOVA.

RESULTS AND DISCUSSION

Analytical study

A systematic study is mainly concerned with the fixation of standards, which help us to understand the quality of preparations. The analytical profile of Munda loha and Mandoora were done at Drug Standardisation Unit and Drug Testing Lab at Government Ayurveda College, Thiruvananthapuram.

The organoleptic evaluation of Munda lohabhasmam and Mandoora bhasmam

The color of Munda loha bhasmam is dark red whereas for Mandoora bhasmam the color is bright red. For both the samples, the taste is amlathwam. The density of Munda loha bhasmam and Mandoora bhasmam are 1.0216 and 1.0275 g/mL respectively which were correspondingly lesser compared to their respective raw materials where the values were 7.36 and 7.86 g/mL. The decrease in density indicates the particle size reduction of the finished products.

Classical evaluation of bhasmam

The Munda loha bhasmam and Mandoora bhasmam prepared as per classics were subjected to bhasma pareeksha at the end of each Puta. Its positive result confirms the safety and fineness of its nature. (Table 1)

From the analytical study, it is evident that the bhasmam had all the property of classical references. They attained each property during the different stages of bhasmeekaranam. Property of Nishchandratwam was attained after the first incineration itself, and that of Rekhapoornathwam was attained after the second Puta. The initial metallic taste of bhasmam decreased during each Puta. Varitharathwa was attained after the fourth puta.

There is a specific test for the examination of its chemical nature also, as listed below:

Apunarbhavathwam

The Marithabhasmam was mixed with marithapanchaka dravya (Gritha, Madhu Guggulu, Gunja and Tankana) enclosed in a saravasamputa and was heated at the temperature same as while preparing the bhasmam. The process did not yield the original metal taken, and hence the bhasma is considered to be Apunarbhava.

Niruthathwam

In this test, a specified quantity of pure silver and the respective lohabhasmam were placed in a crucible and subjected to agni-karma. (Figure 1) Since the bhasmam is not apakwa, no free particles got deposited, and silver did not gain weight.

Instrumental Analysis

Elemental analysis by AAS

The results of AAS estimation showed that Munda loha bhasmam and Mandoora bhasmam contain 28.0015 and 27.9457 % of iron as the main metal. The mass of lead in the two Bhasmas is 0.2771 and 0.4072 ppm respectively. Another heavy metal, cadmium is also found to be present with 0.0160 and 0.0204 ppm in Munda loha bhasmam and Mandoora bhasmam respectively. This much of the residual amount of cadmium and lead does not cause any adverse effect on the daily intake of the drug which is only 125 mg.

XRD analysis

Initially, two samples of Mandoora were taken, one from Muthiyavila of kattaykode region and another from Vilappilsala. The XRD patterns were found to be very much similar to one another, but the peak intensities of Mandoora from Muthiyavila was significantly higher than that of the other one. The former was thus considered to be much purer and was taken for the preparation of the medicine.

XRD patterns of Mandoora clearly identify the presence of an alpha form of silica (α SiO_2.) the prominent peak at 2θ values corresponding to 100 plane deflection (Figure 1). The most characteristic peak at 26.5 is due to the reflection from 011 plane; the other peaks are at 36.5, 42.5, 50, 55 and 60 corresponding to 110, 200, 112, 013 and 121 planes respectively. The other main phases present in raw Mandoora are Fe_2O_3 and Fe_3O_4. During the making of weapons and agricultural tools in traditional Ala, when hot raw spent iron is discarded into the nearby soil, it might have combined with silica. Gradually the surface part would have combined with oxygen, and after prolonged exposure, partially converted into Hematite (Fe_2O_3 variety) and Magnetite (Fe_3O_4).

In the purification process, when Mandoora under red-hot condition was plunged into Gomutra it was found to be powdered during the process. Cow's urine contains urea which in aqueous media under hot condition liberates NH_3. This ammonia dissolves in an aqueous medium and changes into ammonium hydroxide. It reacts with iron in Mandoora to form various hydroxides like FeO-OH (iron ox hydroxide), $Fe(OH)_2$, (ferrous hydroxide) and $Fe(OH)_3$ (ferric hydroxide) therefore on filtration, the precipitate or solid residue will encompass these new chemicals also. On heating, these hydroxides partially change to oxides of iron. During repeated heating and dipping in cow's urine ultimately changes the physicochemical characters of Mandoora. This slow transformation is visually experienced by the formation of a powdery form of Mandoora during each stage of calcination. The XRD spectra during the different stages revealed that up to the 2nd

cycle of quenching, no noticeable chemical changes happened (retaining the characteristic XRD pattern of raw Mandoora). From the 3rd stage onwards the characteristic peak of silica decreased, indicating the gradual removal of silicates.

New peaks are obtained at: 24.145(a), 33.135(a), 35.6(a), 40.762(a), 49.433(a), 54.015(b), 58.249, 62.45(a), and 63.9 (a) .a = Fe_2O_3, b = Fe_3O_4.

A prominent peak at (2θ) 32 indicates the formation of Fe_3O_4. It can be seen that this peak for the reflection in 202 planes is almost missing in the next cycle. The role of Amalaki juice in preventing the formation of Fe_3O_4 is thus established. In Mandoora, the final figure seems that the drug prepared is extremely pure and is free from either Fe_3O_4 or SiO_2. All the peaks correspond only to that of α-Fe_2O_3. The peaks obtained are narrow suggesting the highly crystalline andordered arrangement of α Fe_2O_3 lattice (222, 006, 200, 024, 242, 120, 214, and 300). The final product obtained is thus inconclusively proven to be αFe_2O_3.

The raw Munda loha mainly has the phase of Fe_2SiO_4 (jcpds-96-901-1591) belonging to Fayalite-orthorhombic (Figure 4). The characteristic peak for raw Munda loha corresponding to Fe_2SiO_4 is observed at 2θ values at 25, 32, 34, 35 and 36°. Repeated heating in the presence of ghee changes the hardness of the substances resulting in low stability and with high brittle nature. During the process, the iron ortho-silicate which has got ferrous and ferric iron, changes to the respective oxides such as Fe_2O_3 and Fe_3O_4. Fe_3O_4 is a mixture of FeO and Fe_2O_3. The oxidation of Fayalite is more complicated than the oxidation of iron oxides due to the presence of silica.

In figure 4, the MCB1 graph corresponds to the X-ray diffraction of the sample before incineration. A large number of peaks corresponding to 2θ values ranging from 25 to 55° indicate the presence of different crystal phases in comparison with free XRD pattern. It is seen that the 103, 040, 400, 140, 150 plane peaks correspond to SiO_2 and 113, 110, 311 peaks correspond to that of Fe_2O_3. Fayalite (Fe_2O_4Si) which is initially present in Munda loha after the first incineration decomposes and the peaks corresponds to silica; α Fe_2O_3 and β Fe_2O_3 become visible. The XRD pattern of the sample after the third incineration is examined. Most of the peaks corresponding to β Fe_2O_3 are found to have vanished; the only peak for β Fe_2O_3 is seen at a 2θ value of 24°. From the six[th] cycle onwards the oxidation product is formed into a paste with Amala juice. Since Amala juice contains ascorbic acid, there is a chance of iron complexes being formed during this process. The inference is supported by the fact that the resulting mixture has intense bluish black coloration. On drying it in sunlight, it is assumed that photochemical reduction of iron present in the complexes occurs[15].

On final incineration in a limited supply of oxygen by closing the earthen pot with clay-smeared cloth (sandhibandhana), the carbonaceous material will be incompletely oxidized preferably to carbon monoxide. Carbon monoxide is a good reductant so that the chance of formation of Fe_3O_4 will further be restricted. The XRD of the final preparation also corresponds to only sharp peaks of αFe_2O_3 and less prominent peaks of Fe_3O_4 are observed in the final product. It is also seen that the XRD spectra of raw Munda showed a peak corresponding to SiO_2, but in the last stage (Figure 3 and 4), no characteristic peaks corresponding to SiO_2 were observed.

Fourier-transform infrared spectral analysis

The FTIR spectrum of samples provided notable peaks in the region 350-4000 cm^{-1}. (Figure 5 and 6)

Though bhasmam preparations are widely used in Ayurveda, it knowledge regarding the chemical changes happening during the process of making bhasmam is rarely studied. It is interesting to note that the metallic substances are treated with different sets of liquid organic matter or herbal decoctions repeatedly before incineration. It would be very useful to study the mechanism by which the metallic compounds are purified during the preparation of bhasmam. Hot Mandoora, when dipped in cow's urine, undergoes some chemical change as is evident from FTIR studies. For a comparative analysis, samples of Mandoora under hot conditions are added to pure water also. Intensity distribution in various wavenumber bands is quite different for the two samples. It is seen that the samples have moisture content associated with them since prominent peaks are observed at a frequency of 3473 cm^{-1} for the samples, which correspond to the stretching mode of water molecules. It is supported by the presence of bending vibrations at a frequency of 1640 cm^{-1}. The intensity of Stretching mode of O-Si-O bonds is much lesser in the sample treated with cow's urine indicative of efficient removal of silica during the process. The peak values are obtained at 1080, 1198 and 1412 cm^{-1}. It is supported by the less intense peaks corresponding to the stretching mode of Si-O-Si bonds at 695, 780, 620 and 792 cm^{-1} and the bending mode of O-Si-O for the same sample is less intense at 524 and 460 cm^{-1}. The presence of Fe-O tetrahedral linkage and Fe-O octahedra are supported by the stretching mode of vibration seen at 566 and 396 cm^{-1} respectively. This fact clearly exemplifies the wisdom of ancient practitioners of Ayurveda in using samples of cow urine for the purification process.

The extreme stretching vibrations of different functional groups corresponding to organic moiety are absent showing that the samples are almost free from organic compounds due to the high temperature they are subjected to during incineration. So the presence of low organic matter can be explained as ample proof for proper cleanliness during the preparation of these medicines and confirms the absence of any external biological contamination.

Elemental analysis by ICP-AES

An ICP-AES study conducted revealed that the percentage of iron changed from 42.43 and 5.88 in raw materials to 35.84 and 19.79 in the final Munda bhasmam and Mandoora bhasmam respectively. (Table 2) 19.09 ppm of Arsenic was present in the raw Munda loha was found to be absent in Munda bhasmam. It substantiates the safety administration of the medicine internally. Mandoora does not have Arsenic in it. A great decrease in case of Mercury noticed from 40.17 ppm to 4.22 ppm in Munda loha. Thus all the heavy metals that are hazardous to the human body, exclusively are thrown away from the hard drug and made them extensively obedient to the Physicians intelligence. Increased percentage level of Barium and Chromium in Munda lohabhasmam and Mandoorabhasmam will facilitate the absorption of Iron by triggering the enzymatic mechanism in the human body. Reduction in weight, an increase in fineness and variation in color to "Pakwa jambu phala varna" (official description of a well-formed bhasmam) attained after four incineration. Zinc present in the samples is considered as an element which possibly enhances the absorption of iron and thus improves the utility of the bhasmam.

Table 1: Evaluation of Munda and Mandura bhasmam (physical nature)

Puta	Nishchandratwam	Rekhapurnathwa	Varitharathwa	Svadhu
1st	Present	To an extent	-	Metallic taste
2nd	Present	Present	35%	Started to diminish
3rd	Present	Present	70%	A little
4th	Present	Present	90%	little

Table 2: FTIR stretching vibrations of different functional groups

3473	Stretching mode of water
1640	Bending mode of water
1080, 1198, 1412	Stretching mode of O-Si-O bonds
695, 780, 620, 792	Stretching mode of Si-O-Si bonds
524, 460	Bending mode of O-Si-O
566	Stretching mode of Fe-O tetrahedra
396	Stretching mode of Fe-O octahedra

Table 3: ICP-AES analysis result

Elements measured	Sample Name			
	Raw Mundam (%)/ppm	MLB (%)	Raw Mandooram (%)	MB (%)
Fe 2395	42.43	35.84	5.88	19.79
Al 3082	2.72	1.31	2.52	0.26
Ca 3158	1.17	1.07	6.23	1.48
K 7664	0.44	0.61	3.19	0.64
Mg 2802	0.35	0.41	1.48	0.36
Mn 2576	0.08	0.09	0.10	0.13
Na 5895	0.20	0.33	0.31	0.35
Si 2124	0.13	0.13	0.18	0.13
Ti 3361	0.67	0.43	0.14	0.11
B 2089	BDL	0.58	32.61	4.89
Ba 4934	241.70	182.31	793.98	157.52
Bi 2230	5.25	13.79	0.12	9.91
Cd 2288	BDL	BDL	BDL	BDL
Co 2286	9.16	7.48	9.24	11.03
Cr 2677	47.83	100.41	74.94	239.90
Cu 3247	7.16	41.34	76.46	114.32
Li 6707	3.51	2.53	7.69	0.95
Ni 2316	BDL	7.43	69.80	5.22
Pb 2203	12.31	14.64	17.08	9.69
Sr 4077	92.37	112.65	602.46	321.49
Zn 2025	37.71	29.40	61.77	77.61
As 1890	19.09	BDL	BDL	BDL
V 3102	62.02	54.67	20.96	3.17
W 2079	5.04	8.21	3.42	6.53
Hg 1942	40.17	4.22	18.00	9.90

DL = below detection level; Fe, Al, Ca reported in %; Others in ppm

Table 4: Smart quant result of Munda bhasmam

Element	Weight %	Atomic %	Net Int.	Error %	K ratio	Z	R	A	F
O K	27.42	52.18	3390.92	6.75	0.1438	1.1539	0.9144	0.4546	1.0000
AlK	0.98	1.11	186.52	10.47	0.0043	1.0336	0.9615	0.4252	1.0074
SiK	14.26	15.46	3522.47	5.87	0.0840	1.0574	0.9694	0.5528	1.0070
FeK	57.34	31.26	5813.77	1.83	0.5322	0.8987	1.0389	1.0032	1.0296

Table 5: Observation on Average Body weight of Animals in each group

	1st day	3rd day	8th day	15th day	22th day	29th day	36th day	41st day
G 4	1.68 kg	1.59	1.66 kg	1.70 kg	1.69 kg	1.62 kg	1.67 kg	1.67kg
G 3	1.7 kg	1.61	1.69 kg	1.81 kg	1.82 kg	1.71 kg	1.72 kg	1.72kg
G 2	1.86 kg	1.79	1.84 kg	1.81 kg	1.86 kg	1.85 kg	1.86 kg	1.86kg
G 1	1.86 kg	1.86	1.86 kg	1.86 kg	1.86 kg	1.9 kg	2 kg	2kg

G4 = Group 4: treated with MLB.; G 3 = Group 3: treated with MBG ;G 1 = Group 1: Normal group.;G2 = Group 2: Control group

Table 6: Significant Date and Time for the comparison of Hb among experimental group during different treatment periods

	N	C	G3	G4	P-value
INITIAL	14.48 ± 1.27	11.88 ± 3.44[a]	11.60 ± 3.04	10.86 ± 3.43	0.192NS
8TH	14.49 ± 1.26	5.54 ± 0.31[a]	13.68 ± 0.10	8.62 ± 0.26	0.000*
15TH	14.49 ± 1.26	5.46 ± 0.09[b]	16.79 ± 0.61	12.22 ± 0.16	0.000*
22ND	14.53 ± 1.22	12.53 ± 1.02[c]	12.89 ± 0.90	12.51 ± 0.72	0.006*
29TH	14.39 ± 1.31	12.422 ± 0.78[d]	12.95 ± 0.63	13.01 ± 0.56	0.006*
36TH	14.47 ± 1.22	12.24 ± 0.50[e]	12.81 ± 0.59	13.40 ± 0.56	0.001*
41ST	14.47 ± 1.22	12.27 ± 0.52[f]	12.84 ± 0.60	13.41 ± 0.56	0.001*

NS = Not significant (p>0.05) * significant (p<0.05) * Means having different alphabets significantly differ for day-wise comparisons by PAIRED T-TEST

Table 7: Data and Test of significance for the comparison of MCH among experimental group during different treatment periods

	N	C	G3	G4	P-value
Initial	22.54 ± 1.05	22.72 ± 0.49	23.37 ± 0.43	23.01 ± 0.50	0.188NS
3rd	22.54 ± 1.05	15.78 ± 2.05[a]	14.77 ± 1.05	14.84 ± 1.04	0.00*
8th	22.55 ± 1.04	15.77 ± 2.03[a]	23.51 ± 0.35	15.24 ± 0.80	0.00*
15th	22..89 ± 0.75	17.14 ± 1.42[b]	23.55 ± 0.35	23.18 ± 0.95	0.00*
22nd	23.53 ± 1.02	21.96 ± 0.49[c]	23.60 ± 0.29	23.16 ± 0.91	0.00*
29th	23.71 ± 0.81	22.23 ± 0.21[d]	23.50 ± 0.18	23.79 ± 0.35	0.00*
36th	23.72 ± 0.80	23.15 ± 0.02[e]	23.52 ± 0.17	23.85 ± 0.33	0.069*
41st	23.83 ± 0.82	23.18 ± 0.02[f]	23.55 ± 016	23.90 ± 031	0.049*

NS = Not significant (p>0.05) * -significant (p<0.05) * Means having different alphabets significantly differ for day-wise comparisons by PAIRED T-TEST

Table 8: Data and Test of significance for the comparison of MCHC value among experimental group during different treatment periods

	N	C	G3	G4	P-value
Initial	30.87 ± 1.85	30.90 ± 1.87a	31.80 ± 0.63	31.90 ± 0.61	0.428NS
3rd	31.00 ± 1.7	22.06 ± 4.06b	21.31 ± 0.62	21.26 ± 054	0.000*
8th	31.20 ± 1.52	20.37 ± 0.12c	33.16 ± 0.49	26.65 ± 2.14	0.000*
15th	31.97 ± 1.03	29.42 ± 0.13d	33.18 ± 0.49	31.89 ± 0.47	0.000*
22nd	32.08 ± 1.01	30.65 ± 0.40e	32.79 ± 0.97	32.15 ± 0.44	0.001**
29th	32.11 ± 1.04	30.71 ± 0.40 f	32.49 ± 0.57	31.93 ± 0.58	0.002**
36th	32.83 ± 0.74	30.78 ± 0.47g	32.74 ± 0.63	31.97 ± 0.53	0.000*
41st	32.84 ± 0.74	30.77 ± 0.46h	32.75 ± 0.63	31.99 ± 0.53	0.000

NS = Not significant (p>0.05) * -significant (p<0.05) * Means having different alphabets significantly differ for day-wise comparisons by PAIRED T-TEST

Figure 1: Niruthathwa test

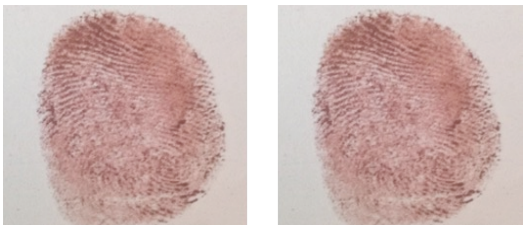

Figure 2: Rekhapoornathwam

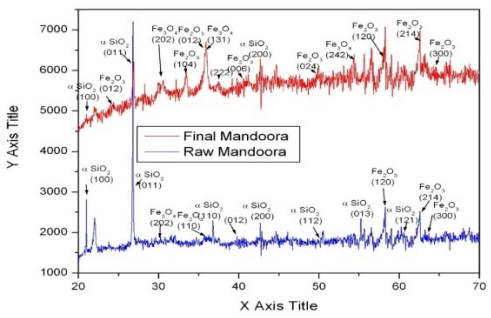

Figure 3:Comparison graph between raw mandura and final mandura bhasmam

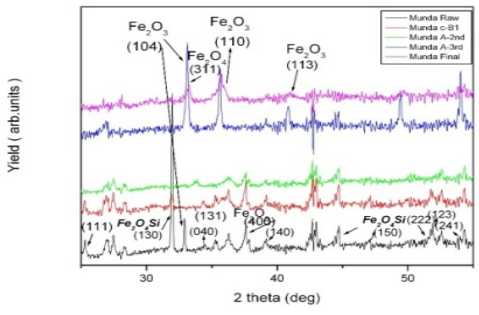

Figure 4:comparisons between raw Munda to Bhasmam

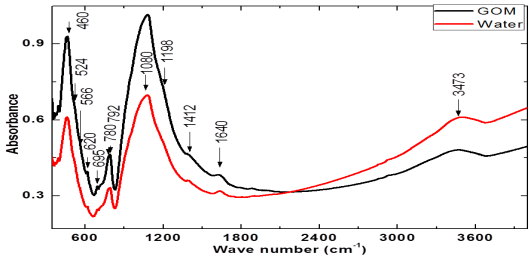

Figure 5: FTIR Spectra of Gomutra and Water quenched Mandura sample

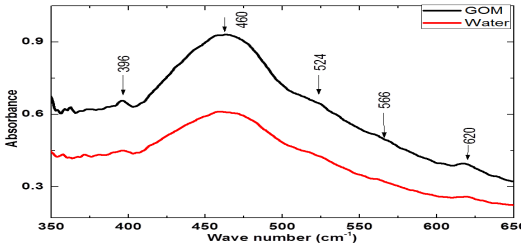

Figure 6: FTIR Spectra of Gomutra and Water quenched Mandura sample

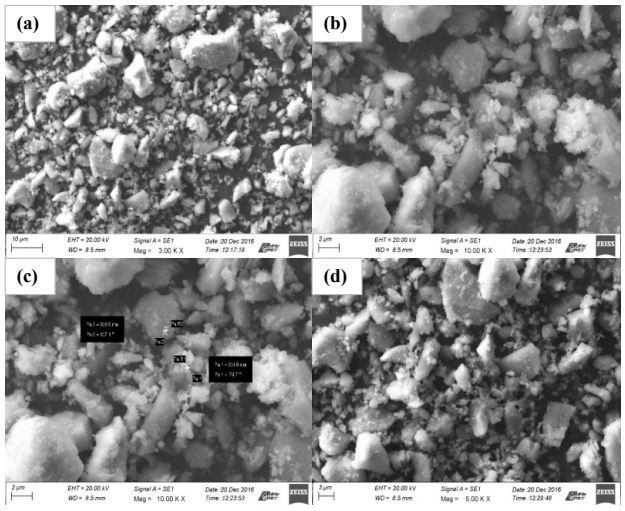

Figure 7: SEM results

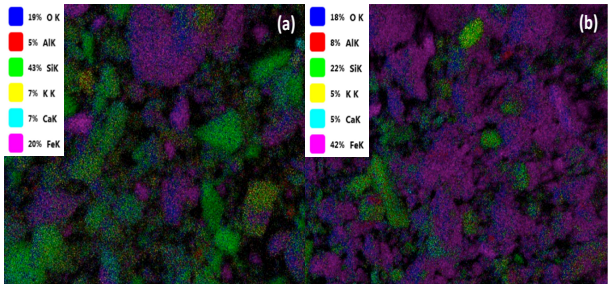

Figure 8: Elemental overlay of (a) MLB and (b) MB

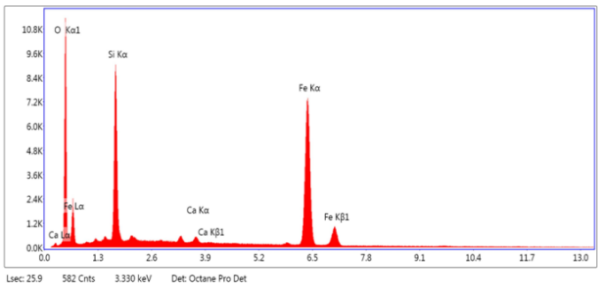

Figure 9: EDS spot graph of Mandura bhasmam

SEM-EDX results

The SEM graph in Figure 7 of 'Mandoora' and Mandoora bhasmam reveals the morphology, size and distribution of the core particle of finished Mandoora and Mandoora bhasmam respectively. It is seen that most of the particles are non-spherical in nature. The elemental analysis using SEM-EDX revealed about the percentage of Iron, Silicon, and Calcium by weight percentage. The presence of Silica indicates the α Fe_2O_3 are covered with a small layer of SiO_2. Thus the monodispersed core Fe_2O_3 particle has a surface coating and is thus a composite material.

The magnification is 1000 (10K x) to view the particle morphology clearly. The particle varies significantly with its size ranging from 4.827 µm to 9.089 µm. It can also be understood that there are many particles which have sizes considerable lesser and higher than this one, this indicates the rather in homogeneity of the particle, but it is true that all particles are existing in the micrometer range. This size distribution is quite homogenous, but the morphology is slightly irregular. A weak agglomeration of the primary particle is also visible owing to the particle sintering during the incineration and grinding process.

Haematinic effect of Munda loha bhasmam and Mandoora bhasmam in Albino Rabbits

One of the objectives of the study was to assess the Haematinic effect of Munda loha bhasmam and Mandoora bhasmam in Albino Rabbits were objectively done by observing clinical change, the difference in body weight and statistically analyzing the serum biochemical parameters like Hb count, ferritin level in blood. MCH and MCHC

Statistical analysis results

Observation on average body weight of animals in each group

The group contains six animals respectively. On the eighth day of the treatment, after phenyl hydrazine administration for three

days there noticed an average reduction of 0.02 kg of body weight in group 4 compared to the initial stage, while that of group 3 is 0.01 kg average. In Control group 0.02 kg mean weight loss occurred. Upon treatment with the particular drug in each cluster, they gained 0.04 kg and 0.13 kg respectively. From 22nd day of treatment to 41st day there occurred an average body weight decrease by 0.01 kg, 0.08 kg and 0.03 kg respectively compared to the 15th day of the treatment in group 4. In Group 3 on the 29th day of the treatment, they lost an average body weight by 0.11 kg of their body weight compared with their body weight on the 22nd day. On 29th-day Control group lost their body weight by an average of 0.01 kg of their body weight. During the study on the 29th day, Group 2 gained an average body weight by 0.01 kg. It remained unchanged until the 41st day of the treatment. From the initial stage to the 22nd day of the study period Group 1 animal nor lost or gained their body weight. On 29th-day of the treatment, Group 1 animals showed an average increase of their body weight by 0.14 kg. This increase in their average body weight continued until the 41st day of the treatment. On the 41st day, their average body weight increase seems to be 2 kg.

All the values were expressed as the mean ± standard deviation of six animals in each group. Group difference based on outcome variables are tested for significance using one-way analysis of variance (ANOVA) followed by a least significant difference (LSD) test for pairwise comparison between groups. An assessed P value less than 0.05 (5%) is measured to be statistically significant.

From Table 6 ANOVA followed by L.S.D. showed that the mean Hb level of all treatment groups is approximately the same ($P > 0.05$) and hence all the animals are eligible to perform experiments with them. ANOVA followed by L.S.D results showed that the mean Hb level of experiment groups differ significantly in the eighth day ($P < 0.05$) and it is seen that G3 animals almost doubled their mean reputed Hb level(13.68 ± 0.10) as compared to G4 (8.62 ± 0.26) and control group (5.54 ± 0.31).

During the 15th day of the medication the mean, Hb level significantly varied among experiment group ($P < 0.05$). As compared to the control group (5.46 ± 0.09) G3 reported three times increased Hb level(16.79 ± 0.61) and also more than double the level of Hb in G4. (12.22 ± 0.16).

On the 22nd day and 29th day of the experiment, 'N' group differ significantly with all other groups in Hb level. ($P < 0.05$). The control group (12.53 ± 1.02) took 22 days to get normal Hb value, more or less equal to G3 and G4. Hence proved the efficacy of Munda bhasmam and Mandoora bhasmam. Among them, Munda bhasmam plays a captain role to increase the Hb level remarkedly.

During towards the final stages of the treatment, Control group (12.24 ± 0.50) showed more or less equal Hb value compared to G3 (13.40 ± 0.59) and G4 (13.01 ± 0.56). Thus it evidently states that Anaemia when left untreated, it takes 41 days to get normalized with body functions and to happen the repairment eventually.

Mean corpus haemoglobin (MCH) and haemoglobin concentration (MCHC) are the red cell indices used to characterize the blood off Rabbits with anemia.

From Table 7 we can find that Initially, all the animals in the Normal group showed MCH mean calculation value as Not Significant ($P > 0.05$). It means all the animals are healthy in their nature. On the third day, all groups except Group 1 showed significant different values, proving they are Anaemic. ($P < 0.05$) ANOVA followed by L.S.D.showed that the MCH level of the experimental group differs significantly in the 8th day ($P < 0.05$) and it is seen that G3 reported almost double mean MCH value (23.51 ± 0.35) comparing to G4 (15.24 ± 0.80) and control (15.77 ± 2.03) (54.26%, 49.11%). While comparing the MCH value of G4 (15.24 ± 0.80) and control (15.77 ± 2.03) (-3.36%), we can see that there is no much effect of medicine happening in experimental animals.

During the 15th day of the treatment, the MCH level significantly varies among experiment group ($P < 0.05$) and reported that G3 (23.55 ± 0.35) and G4 (23.53 ± 0.95) are almost equal having a difference of 1.59%. During the 36th day of the Treatment, all the group reported more or less the same level of MCH ($P > 0.05$). Here G4 showed 1.04% more effect than G3.

From Table 8 ANOVA followed by L.S.D revealed that the mean corpuscular haemoglobin concentration (MCHC) of all treatment group are approximately same during the initial stage, hence all the Animals are eligible for the experimental study.

ANOVA followed by L.S.D. showed that the mean corpuscular haemoglobin concentration level of experiment group differ significantly in the 8th day ($P < 0.05$) and 24.45% of gain reported in the case of G3 compared with G4. It indicates the potency of Munda bhasmam is much higher than Mandoora bhasmam.

During the 22nd day of the experiment, the mean corpuscular haemoglobin concentration level achieved almost equal values in all experimental clusters, and it is highly significant ($P < 0.05$).

CONCLUSION

The ancient analytical approaches to understanding the characteristics of bhasma and that of the modern methodology have differences even though the objectives remain same. In the present study, a combination of the two approaches is implemented for analyzing the drug samples and their basic raw materials. Iron is biologically very important because of the fact it is involved in the structural and functional aspects of many bio-co-ordination compounds like haemoglobin, myoglobin, and cytochromes. The specimen of Munda loha used for the study is identified to be Fayalite-orthorhombic in its structure. Munda loha bhasmam is found to act more efficiently than Mandoora bhasmam. The physico-chemical analysis of Munda loha bhasmam revealed that αFe_2O_3 is one of its main constituents. Munda loha bhasmam contains αFe_2O_3 as well as Fe_3O_4. The SEM-EDX analysis finds all the Bhasmam possess the particle size ranging from 2 μ to 10 μ. From ICP-AES study it is evident that the arsenic and many toxic elements present in the raw samples are either absent or considerable lesser in the finished products. Heavy metals like Arsenic, Cadmium, and Lead, were found to be within the limited value, or absent which ensures the non-toxicity of the finished products. Iron and zinc being heavy metals are already present in the finished product has a high value in the administration of the drug. The ICP-AES study showed the presence of iron in Munda bhasmam and Mandoora bhasmam are different due to the fact that Mandoora bhasmam is a mixture of two oxides of Fe. FTIR study revealed the efficient removal of silica during the process.

The instrumental analytical methods ultimately revealed the chemical nature, compositional change of raw material to the products as well as their crystalline phases and morphological features. It is recommended that systematic clinical trials are to be conducted to generate more scientific data for further validating the claims of this study.

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