

Pharmaceutical Standardization of Traditionally Prepared *Lauha Bhasma* Incinerated Iron, Characterised as Alpha Haematite

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Chavan *et al.*: Standardization of Incinerated Iron (*Lauha Bhasma*) as Alpha Haematite

Lauha Bhasma being incinerated metals, minerals and animal products attributed with specific therapeutic value needs standardization. Present study validates the pharmaceutical process of *Lauha Bhasma*/incinerated iron and characterizes it using traditional as well as modern techniques. *Lauha Bhasma* was prepared by traditional method. Pure iron turnings were sequentially quenched, 7 times each, into liquid media (1:4 w/v, for each) viz., sesame oil, cow's butter milk, cow's urine, sour gruel, *Dolichus biflorus* seed decoction and decoction of equally mixed pericarp of three myrobalans (*Triphala*). It was further mixed with sulphur, triturated with *Aloe barbadensis* leaf pulp and incinerated at 650°-850°, till the achievement of desired characters. The obtained reddish-violet powder contained 60 %-65 % of iron while characterized as crystalline ferric oxide, alpha haematite, on X-ray diffraction simulation. Vibrating sample magnetometer revealed hysteresis with less magnetisation saturation but high coercivity. Thermo-gravimetric analysis showed loss of ~3.5 % w/w between 300°-900°. Both, scanning and transmission electron microscopy visualised variably sized agglomerates of spherical to irregularly shaped particles with smooth edges. Dynamic light scattering analysis illustrated distribution of agglomerates as <250 nm (10 %), <515 nm (50 %) and <1050 nm (90 %) while negative zeta potential upto 14 mV. Elemental assay depicted heavy metals within permissible range and presence of trace elements up-to 2.75 %. Such *Lauha Bhasma*, characterized as alpha haematite, is traditionally prescribed in blood, liver, intestine and eye disorders. This study will help to explore this micro-nanomedicine as a therapeutic agent using *in vitro* and *in vivo* mechanistic studies.

Key words: Biomaterials, biomedicine, characterization, hematite, incinerated iron, *Lauha Bhasma*, standardization, quenching

Lauha Bhasma (LB) incinerated iron is one of the important ingredients in various Ayurvedic formulations like Arogyavardhini Gutika, Navayas Lauha, Tapyadi Lauha and Trailokyachintamani rasa^[1]. According to Rasashastra, the pharmaceutical branch of Ayurveda, Lauha is included in the metal group (dhatu varga) and more precisely as pure metal (shuddha lauha). Further, in Rasashastra, three broad types of iron (Lauha) are mentioned viz., Magnetite (kanta lauha), Wrought iron (teekshna lauha) and Pig iron (munda lauha). Though kanta lauha is mentioned as the best type, the latter two are also indicated for medicinal use^[2].

Classically, LB is attributed with bitter, sweet and astringent tastes; cold potency and eye tonic (chakshushya), scraping (lekhana), anti-aging (vayasya) and Kapha-Pitta pacifying properties. It is mainly recommended in inflammation (shotha), haemorrhoids (arsha), splenomegaly (pleeharoga), anaemia (pandu), obesity (medoroga), diabetes (meha), worms (krumi) and skin disorders (kushtha) with a therapeutic daily dose of 125-250

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mg^[3]. Recent trials in animals and humans with iron deficiency anaemia and obesity have shown significant effect of LB as compared to standard drug ferrous sulphate^[4]. However, if iron is not properly purified and incinerated as mentioned in classical texts, such LB can cause life threatening conditions like anaemia (pandu), skin disorders (kushtha), cardiac disorders (hrudroga), stomach ache (shoola), renal calculus (ashmari) and may also lead to death^[2].

In textual methods, all metals before their medicinal use are physically and chemically treated by a general purification process in a defined set of media (samanya shodhana)^[5]. Further, iron is specifically purified by quenching it into the juice of *Musa paradisiaca* rhizome/quenching in decoction of three myrobalans (*triphala*) or cow's urine or sprinkling decoction of three myrobalans on red hot iron (vishesh shodhana)^[2]. Further, LB preparation method i.e., incineration (Marana) is mentioned with various triturating media viz., three myrobalans/*Boerhavia diffusa* leaf juice/*Aloe barbadensis* juice/*Calotropis procera* latex/*Phyllanthus emblica* fruit juice/*Adhatodavastica* leaf juice, cow's urine, rice gruel and lemon juice with or without cinnabar/metacinnabar and sulphur^[2]. However, pharmaceutical standardization of a single preparatory method with comprehensive characterization is unavailable, even in Ayurvedic Formulary and Ayurvedic Pharmacopoeia of India (API).

Recent reports on pharmaceutical standardization of LB have focussed on manufacturing processes viz., quenching/purification followed by heating procedures viz., indirect heating under sun (bhanupaka), heating in a pan (sthalipaka) and heating with dried cow-dung cakes or muffle furnace (putapaka). So far, LB has been prepared either using decoction of three myrobalans or four different methods using combinations viz., iron, sulphur and Aloe vera; iron, cow's ghee and decoction of three myrobalans; iron triturated with pomegranate leaf juice; and iron, black sulphide of mercury and Aloe vera^[6-8].

In the present study, LB was prepared at industrial scale by upgrading the traditional method^[2]. Pure iron turnings were sequentially quenched, 7 times each, into liquid media viz., sesame oil, cow's buttermilk, cow's urine, rice gruel, *Dolichus biflorus* seed decoction and decoction of three

myrobalans. It was further mixed with processed sulphur, triturated with fresh *Aloe barbadensis* leaf pulp and incinerated, repeating the trituration and incineration cycles till the desired characters were achieved. The final product, LB, obtained through this standardized pharmaceutical process was characterized to develop a monograph. Advanced analytical techniques like scanning and transmission electron microscopy, X-Ray Diffraction (XRD), vibratory sample magnetometry and dynamic light scattering were used to study the physicochemical properties. Since, LB is widely used in Ayurvedic medicines, a comprehensive study conducted on industrial scale batches will help in quality control and quality assurance.

MATERIALS AND METHODS

Collection of raw material:

Iron turnings (Grade SAE1029) were purchased from Yukta Engineers, Pune, India while sulphur was obtained from S. V. Ayurvedic Bhandar, Navi Mumbai, India. Cow's ghee and sesame oil were purchased from B. V. Chitale Group, Pune and Pitambari Products, Thane, India respectively. Rice, turmeric powder and *Dolichus biflorus* Syn. *Vigna unguiculata* (Linn.) Walp. seeds were procured from local market while three myrobalans/*Triphala* i.e., pericarp of *Terminalia chebula* Retz./ Haritaki (TC), *Terminalia bellirica* Bibhitaki Willd. (TB) and *Phyllanthus emblica* Linn. Syn. *Emblica officinalis* Gaertn. (PE) from Manakarnika Aushadhalay, Pune. Cow's milk, cow's buttermilk, cow's urine and *Aloe barbadensis* mill. Syn. Aloe vera was obtained from Bharatiya Sanskriti Darshan Trust, Pune. Crude herbs were identified and authenticated at Indian Drug Research and Laboratory, Pune.

Preparation and storage of media:

All the processes were carried out in Atharva Nature Healthcare Pvt. Ltd., Pune; in triplicate. Buttermilk was prepared by adding 28 l of water in 14 kg of cow's curd, churned it to de-fat and strained to get 28.5 l. Sour gruel (28 l) was prepared by boiling 3 kg rice in 42 l of water and reduced to 30 l of starchy water. This water was self-cooled; turmeric (150 g), rock salt (75 g) and sesame oil (300 ml) were added and allowed to ferment in a tightly closed porcelain jar for 15 d to get rice gruel. Decoctions of crushed *Dolichus biflorus* seeds and coarse powder of *Triphala*, were

prepared separately from 7 kg of material, each, by adding 112 l of water and boiled to get 28.5 l of decoction, each^[2]. Crude sulphur was purified (shodhana)^[3] wherein sulphur (total 10 kg, 250 g aliquot) admixed with cow's ghee (total 2.5 kg, 62.5 g aliquot) were melt in iron pan and poured through a muslin cloth into cow's milk (total 40 l, 1 l aliquot) kept in a stainless-steel vessel. The solidified lump was cooled, crushed, washed with hot water, dried and powdered to get purified sulphur. Fresh leaves of *Aloe barbadensis* were cut, the pulp scrapped and ectoderm was discarded. The pulp was grinded and strained through #22 (710 µm).

Purification (Shodhana):

In common purification process^[2], iron turnings (1 kg) were heated for 5-10 min till they turned red hot (900°-1000°) and then dipped into sesame oil kept in a stainless-steel container (304 Grade, 6 l capacity; 19 cm×21 cm). The process of heating and quenching was repeated for 6 more times in sesame oil. Then, these turnings were further heated and quenched for 7 times in each of the media viz., cow's buttermilk, cow's urine, rice gruel and *Dolicus biflorus* decoction, sequentially. Further, iron turnings obtained after quenching from the last cycle of *Dolicus biflorus* decoction, were heated and quenched 7 times in the *Triphala* decoction. For each quenching, 4 l of fresh media was used. Organoleptic and weight change of iron turnings were recorded while temperature and physicochemical change in the medium before and after each quenching.

Incineration of iron (Marana):

The process of incineration was carried out after initial grinding and trituration, sequentially^[2] with some modifications like mechanized grinding and trituration techniques. For the first incineration cycle, equal quantity of purified iron turnings (~800 g) and purified sulphur (~800 g) were mixed thoroughly in a stone mortar (8 l capacity, ovoid, maximum inner dimensions as 13 cm×24 cm×51 cm). For 2nd and 3rd incineration cycles, 200 g of purified sulphur was added to the mixture obtained from previous incineration. The mixture was grinded each time for 3 h using a stone pestle (weight-2 kg, length-25.5 cm, basal circumference-22 cm and diameter-6 cm). The trituration (bhavana) of grinded iron and sulphur mixture with 200-400 ml of *Aloe barbadensis*

juice was carried out in the stone mortar manually, for the first four incineration cycles. Further, an electrically operated wet grinder (MK-GW200, Panasonic India Pvt. Ltd., Chennai, India) was used for both, grinding and trituration and 3 h duration was set in each case to maintain uniformity.

Coin like round, flat pellets (thickness-5 mm, diameter-3 cm) were manually casted out of the trituated material in earthen vessels (diameter-27 cm, inner height-4 cm, thickness-4-5 mm, capacity-1.2 l) and dried in shade at room temperature (18 h). The dried pellets were covered with another earthen vessel and their joints were sealed with wet clay smeared cotton cloth (width-8 cm, length-1 m) and kept in shade for drying. During every cycle, the trituated material was pelletized equally in 2 or 4 vessels. The assemblage of concealed earthen vessels was subjected to incineration as reported previously with slight modification^[9]. Here, four patterns as 15:7.5, 18:9, 20:10 and 24:12 kg of cow dung (below: above) were used. The product after each incineration was grinded to powder and sieved through #40, #150 and #200 mesh, sequentially. The incineration procedure was repeated till the desired form was achieved.

Organoleptic and physicochemical evaluation of raw, in-process and finished products:

Raw material, processing media, intermediates and finished products were subjected to organoleptic evaluation. Liquid media were evaluated for pH and specific gravity. Sesame oil was additionally assessed for refractive index while *Aloe barbadensis* juice for viscosity. Processed sulphur was analysed for loss on drying, bulk/tapped density, sieve analysis, oil and sulphur content. LB was evaluated for Fe content, Loss on Drying (LOD), loss on ignition, acid insoluble ash, pH, bulk/tapped densities and sieve analysis^[10,11].

Physical characterisation of LB:

Scanning Electron Microscopy (SEM) on Leica Cambridge 440 (Leica Cambridge Ltd., Cambridge, UK) and Transmission Electron Microscopy (TEM) on TECNAI 20ST with 200 kV, LaB6 source (FEI Company, Oregon, USA) were carried out to obtain the size and morphology of the LB particles^[9]. For TEM, sample was dispersed in acetone by ultrasonication, dropped onto a carbon coated copper grid (#200) and dried in air. The images were captured at magnification scales between 500-20 nm and micrometric measurements were recorded.

The particle size by Dynamic Light Scattering (DLS) technique and Zeta Potential (ZP) were measured on 90 Plus instrument (Brookhaven Instruments Corp., Holtsville NY, USA) equipped with 35 mW solid state laser and a highly sensitive avalanche photo diode detector^[9]. ZP was measured by taking sample in a square disposable cuvette and an electrode designated for aqueous suspensions was used for the electric field mobility measurements. Ten technical replicates were performed for each. Magnetic measurements were made on EG&G PAR 4500 Vibrating Sample Magnetometer (VSM-Princeton Applied Research, TN, USA) Magnetization as a function of applied magnetic field in the range ± 15 kOe was measured.

Chemical characterisation of LB:

Raw iron turnings and LB were analysed on PANalytical X'PERT Pro X-ray diffractometer (Lelyweg, Almelo, Germany) using Cu-K α radiation ($\lambda=1.5418$ Å) for XRD and Thermogravimetric Analysis (TGA)-Q5000 from TA Instruments Inc. (New Castle, DE, USA) as reported earlier^[9]. Elemental assay by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) was

performed using an established methodology^[9]. Samples weighing 100 mg were digested on Ethos Easy Advanced Microwave Digestion System (Milestone SRL, Milan, Italy) and diluted samples were aspirated on ICP-OES (Thermo Fisher Scientific, Waltham, MA, USA). The intensities for K, Li, Na, Ba, Ga, Sr, Ca, Ag, In, Cu, Al, Cr, Mg, Tl, Fe, Mn, B, Co, Cd, Bi, Ni, Pb, Zn, As, Hg and S were measured at wavelengths 766.490, 670.784, 588.995, 455.403, 417.206, 407.771, 393.366, 328.068, 325.609, 324.754, 309.271, 283.563, 279.553, 276.787, 259.940, 257.610, 249.773, 228.616, 226.502, 223.061, 221.647, 216.999, 213.856, 189.042, 184.950, 180.731 nm respectively. Fe and S were measured in radial view while others in the axial view.

RESULTS AND DISCUSSION

All the raw materials used for LB preparation are displayed in fig. 1. Raw iron was identified as bluish black metallic shavings of varying length (10-15 mm), having magnetic property and soluble in HCl. It contained 99.6 % w/w of Fe as measured by titration. The results of other raw materials are detailed in Table 1.



Fig. 1: Materials used during preparation of *Lauha Bhasma* (LB); (a) Iron turnings; (b) Sesame oil; (c) Cow's buttermilk; (d) Cow's urine; (e) Rice gruel; (f) Seeds of *Dolichos biflorus*; (g) Decoction of *Dolichos biflorus*; (h) *Terminalia chebula* pericarp; (i) *Terminalia bellirica* pericarp; (j) *Phyllanthus emblica* pericarp; (k) Decoction of three myrobalans; (l) Crude sulphur; (m) Processed sulphur; (n) *Aloe barbadensis* leaf and (o) *Aloe barbadensis* leaf pulp

TABLE 1: BASIC ANALYSIS OF RAW MATERIALS USED IN PREPARATION OF INCINERATED IRON/LAUHA BHASMA (LB)

Raw Material	Results
Sesame oil (n=8)	Refractive index 1.54 ± 0.002 , specific gravity 0.92 ± 0.002 , iodine value 80.97 ± 10.79 , acid value 3.14 ± 0.75 , saponification value 166.76 ± 12.35 ; unsaponifiable matter 4.58 ± 1.36 % and moisture content 0.17 ± 0.06 % w/w
Butter milk (n=8)	Specific gravity 1.03 ± 0.02 , pH of 3.44 ± 0.02
Cow's urine (n=3),	Specific gravity 1.03 ± 0.002 , pH 8.78 ± 0.79
Rice gruel (n=4)	Specific gravity 0.98 ± 0.001 , pH 3.26 ± 0.07
Decoction of <i>Dolicus biflorus</i> (n=3)	Specific gravity 1.05 ± 0.002 , pH 4.79 ± 0.32
<i>Terminalia chebula</i>	LOD 2.6, total ash 4.7, acid insoluble ash (AIA) 0.17, Alcohol Extractive value (AEV) 46.5, water Extractive Value (WEV) 57.6 (all % w/w); Thin Layer Chromatography (TLC) of ethanolic extract on mobile phase toluene: ethyl acetate:formic acid (5:4:1) showed R_f 0.08, 0.31, 0.43, 0.49, 0.91 (all grey) at UV 254 nm; 0.03 (yellow), 0.84, 0.91 (both greenish blue) at UV 365 nm and 0.09, 0.36 (both grey) on spraying 1 % Ferric chloride (FeCl_3)
<i>Terminalia bellirica</i>	LOD 5.3, total ash 4.6, AIA 0.4, AEV 27.4, WSE 43.0 (all % w/w); TLC of ethanol extract using mobile phase chloroform:ethyl acetate:formic acid (5:4:1) showed R_f 0.06, 0.20, 0.39, 0.73 (all black) at UV 254 nm; 0.06 (grey), 0.20, 0.39, 0.73 (all black) at UV 365 nm and 0.03, 0.39 (both black) after spraying with 1 % FeCl_3
<i>Phyllanthus emblica</i>	LOD 6.9, total ash 3.1, AIA 0.7, AEV 30.2 and WEV 39.3 (all % w/w); TLC of ethanol extract on solvent system acetone:chloroform:formic acid:toluene (2:2.5:0.5:3) showed R_f 0.05, 0.36, 0.60, 0.69, 0.90 (all grey) at UV 254 nm; 0.05 (grey), 0.60, 0.90 (both blue) at UV 365 nm and 0.13, 0.20, 0.30 (all black) after spraying with 1 % FeCl_3
Decoction of three myrobalans (n=3)	Specific gravity 1.08 ± 0.002 , pH 2.85 ± 0.01
Purified Sulphur (n=3)	Light yellow coloured, oily powder, with characteristic odour, soluble in carbon disulphide; LOD 0.29 ± 0.03 %, bulk density 0.70 ± 0.01 g/cc, tapped density 0.88 ± 0.05 g/cc, oil content 8.97 ± 0.8 % w/w, sulphur content 90.52 ± 0.7 % w/w, retention of 93.35 ± 1.41 % on passing through #100 sieve
Fresh <i>Aloe barbadensis</i> (AB) leaves (n=4)	LOD 96.62 ± 0.72 , total ash 0.53 ± 0.1 , AIA 0.16 ± 0.05 , AEV 1.85 ± 0.14 , WEV 1.51 ± 0.07 (all % w/w); TLC of ethanol extract using solvent system toluene:ethyl acetate:formic acid (5:4:1), displayed R_f value of 0.05, 0.33, 0.81 (all blue) at 254nm; 0.33 (red) at 365nm and 0.33 (blue) after spraying with anisaldehyde H_2SO_4 reagent
Fresh AB pulp (n=3)	Specific gravity 1.002 ± 0.001 , pH 4.92 ± 0.32 , viscosity 3.31 ± 0.41 mP

The temperature records of media during quenching are displayed in Table 2 while change is illustrated in fig. 2. The changes in iron turnings after each purification and incineration cycle are displayed in fig. 3. The weight change during both the purification processes and incineration cycles is depicted in fig. 4a. The average weight loss during common and specific purification was 54 ± 1.73 (~5.4 %) and 51.33 ± 15.5 (~5.13 %) g, respectively while total yield after complete incineration was 94.07 ± 0.91 g (93 %-95 %) with a loss of 5.93 ± 0.91

g (5 %-7 %). A weight gain between 4 %-11 % was noted after I1 while a weight loss between 0.8 %-3.5 % was recorded during successive incinerations. During Incineration (I), sulphur was used in equal amount of (814 ± 19.64 g) for I1 and 200 g for next 3 successive cycles. Similarly, 366.67 ± 70.71 of AB juice was required for trituration. For cycles 1 to 5 (I1-I5) and 6 onwards (I6), 2 and 4 vessels, respectively, were required to accommodate the dried pellets of triturated material.

TABLE 2: TEMPERATURE RECORDED DURING PURIFICATION (QUENCHING) OF IRON

Quenching cycle	Sesame Oil		Cow's buttermilk		Cow's urine		Sour gruel		Decoction of <i>Dolicus biflorus</i>		Decoction of three myrobalans	
	B	A	B	A	B	A	B	A	B	A	B	A
1	36	83	28	55	29	64	30	59	27.7	56	33	48.8
	32	80	29	68	28	61	29	62	33.7	55	30	70.8
	32	81	30	61	27	57	29	67	33.4	60	25	57.7
Mean	33	81	29	62	28	61	30	63	31.6	57	29	59.1
SD	2.2	1.3	1.2	6.2	1.2	3.5	0.4	4	3.38	2.4	4.3	11.1
2	37	89	27	57	30	65	30	65	28.2	61	32	47.9
	33	89	30	66	29	64	29	58	33.1	54	29	57.7
	31	93	30	57	27	68	31	62	30.9	57	30	50.3
Mean	34	90	29	60	29	66	30	61	30.7	58	30	51.9
SD	3.1	2.3	1.5	4.9	1.5	2.1	0.8	3.6	2.45	3.5	1.9	5.11
3	36	92	30	57	30	59	31	57	29.7	57	27	44.2
	33	87	30	74	30	62	30	58	32.3	54	30	62.2
	31	98	30	72	29	60	31	62	29.5	59	27	51.1
Mean	34	92	30	68	29	61	31	59	30.5	57	28	52.5
SD	2.4	5.2	0.2	9.1	0.6	1.4	0.9	2.7	1.56	2.6	1.6	9.08
4	40	89	29	59	32	57	33	53	33.2	56	26	55.2
	34	88	30	72	28	63	30	65	31.2	51	29	60.1
	33	95	30	63	27	65	31	68	30.4	61	26	48.2
Mean	36	91	30	65	29	61	32	62	31.6	56	27	54.5
SD	3.8	3.7	0.5	6.8	2.4	4	1.7	8.3	1.44	5	1.9	5.98
5	42	89	29	65	30	67	31	57	34.1	60	28	48.2
	34	81	30	74	28	59	30	69	33.4	57	29	76.9
	34	95	30	65	28	60	31	67	30.7	61	26	57.4
Mean	36	88	30	68	29	62	31	64	32.7	59	28	60.8
SD	4.6	7.5	0.6	5.1	1.5	4.6	0.5	6.7	1.8	2.3	1.6	14.7
6	40	88	30	62	31	65	32	60	31.2	52	28	56.3
	34	80	31	71	29	61	29	63	32.4	59	30	58.5
	36	81	30	77	29	65	32	64	30.8	58	27	54.1
Mean	37	83	30	70	30	64	31	62	31.5	56	28	56.3
SD	3.3	4.3	0.6	7.3	1.1	2.4	1.5	2.2	0.83	4.1	1.8	2.2
7	41	88	29	61	31	66	33	61	31.5	52	27	48.7
	34	90	31	68	28	62	29	64	32.5	61	29	52.3
	34	91	30	64	30	64	32	70	30.5	63	26	56
Mean	36	90	30	64	30	64	31	65	31.5	59	27	52.3
SD	4.3	1.7	1.2	3.4	1.7	2.3	2.2	4.5	1	5.7	1.7	3.65

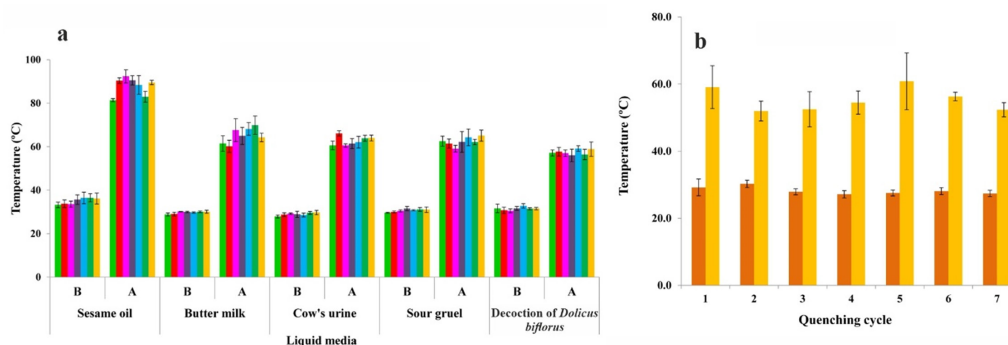


Fig. 2: Temperature of each medium recorded before (B) and after (A) quenching iron turnings, (a) Quenching cycles in common purification; (b) Stages of quenching in specific purification

Note: (■) 1; (■) 2; (■) 3; (■) 4; (■) 5; (■) 6; (■) 7 cycles; (■) before quenching and (■) after quenching in specific purification (in decoction of *Triphala*)

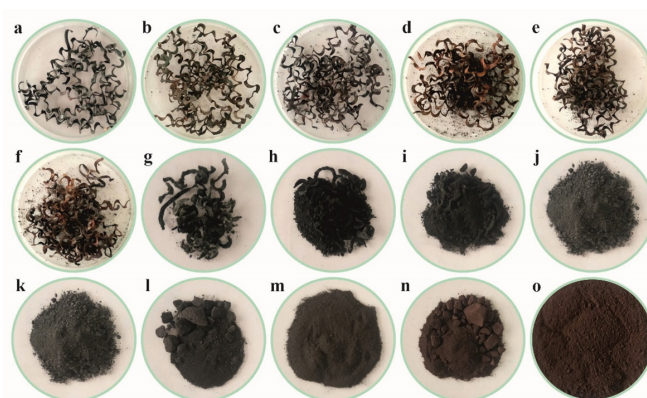


Fig. 3: Changes in iron turnings during LB (Batch 1) preparation, (a-f) Iron turnings after 7th quenching in sesame oil, cow's buttermilk, cow's urine, rice gruel, decoction of *Dolicus biflorus* and decoction of *Triphala*, respectively; (g-o) Conversion of purified iron turnings into powder form after each incineration i.e. from I1-I9 respectively

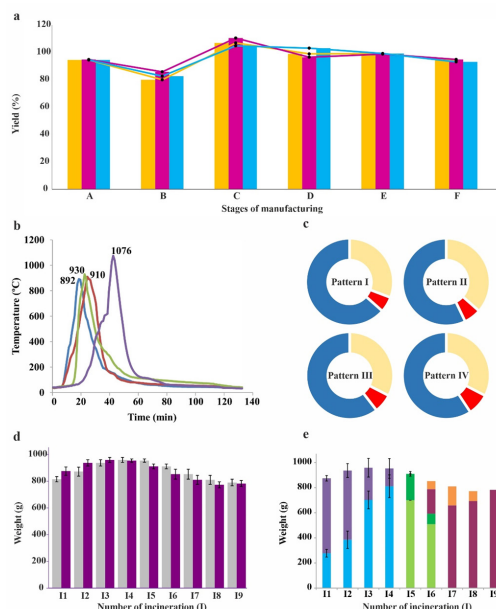


Fig. 4: In-process analysis during LB manufacturing; (a) Weight changes during manufacturing process; (b) Temperature recorded during incineration of iron using cow-dung cakes (kg); (c) Time (h:min) vs. temperature (°C) values in each pattern; (d) Weight changes; (e) In-process sieve analysis after each cycle of incineration

Note: (a): (A) After common purification; (B) After specific purification; (C) After 1st incineration (I); (D) After I4; (E) After I9; (F) Raw to finished product change; (■) LB1; (■) LB2; (■) LB3; (b) (■) [7.5:15]; (■) [9:18]; (■) [10:20]; (■) [12:24]; (C): (■) Phase I: 2:40, 3:50, 3:30, 3:40 vs. 36-745, 36-855, 39-853, 41-903; (■) Phase II: 0:30, 0:40, 0:45, 0:55 vs. 746-892-765, 856-910-834, 854-930-793, 904-1076-958; (■) Phase III: 5:30, 6:00, 6:30, 6:40 vs. 764-34, 833-41, 792-40, 957-32; (d): (■) before; (■) after incineration; (e): (■) Retention on #200; (■) passed through #200; (■) retention on #150; (■) passed through #150; (■) retention on #40 and (■) passed through #40 mesh

Incineration of iron was carried out in four heating ranges (low to high), based on the quantity of cow-dung cakes and the amount of sulphur. The graphical presentation of the temperature patterns recorded is given in fig. 4b (representative batch, LB1) while the duration and the range of temperature observed in all the patterns (of LB1) in fig. 4c. Maximum temperature reached during Pattern-1 (used in I2) was 892° while 910° reached in Pattern-2 (used for I1 and I3). Maximum temperature recorded during Pattern-3 (for I4 and I5) was 930° while in Pattern-4 it raised up to 1076° (used from I6 to I9). Hence, for all the 4 patterns, the maximum temperature during incineration ranged between 892° - 1076° . Each pattern used in this traditional method of incineration of iron can be divided into three phases with respect to the highest temperature (T_{\max}). Phase I as the duration required to attain temperature equivalent to ($T_{\max}-150^{\circ}$) from the point of ignition, Phase II as the duration for which the highest temperature range (from ascending and descending $T_{\max}-150^{\circ}$) was sustained while Phase III as self-cooling duration. T_{\max} is the temperature attained outside the vessel and $T_{\max}-150^{\circ}$ is the assumed temperature reached inside the vessels for the chemical changes. Therefore, the maximum temperature for each pattern at which the conversion might occur in vessels can be deduced as 742, 780, 760 and 926° inside the vessels i.e., T_{\max} ($892, 910, 930$ and 1076)- 150° , respectively.

The weight variation before and after incineration during each incineration cycle is illustrated in fig. 4d. During each cycle of incineration (I1-I9), in-process material was sifted through #40, #150 and #200 sieves. It was observed that 811.7 ± 90.9 g and 142.3 ± 78.3 g of material passed and retained, respectively, through #40 sieves, till I4; while 509.3 ± 255.8 g and 83.3 ± 52.4 g passed and retained, respectively, through #150 sieves, after I5. Similarly, 196.3 g and 63.7 g passed and retained, respectively, through #200 sieve after I6. Finally, 782.5 ± 18.37 g of LB passed through sieve #200 after I9, with negligible residue (fig. 4e).

After common purification, bluish tinge faded and black to dark grey colour appeared on iron turnings. Further, after specific purification, reddish brown tinge was noticed, sharp edges became blunt as well as brittleness increased.

Classical characteristics of LB were evaluated as lustreless (nishchandra), soft in touch (shlakshnata, mruduta), entered into the crevices of finger tips (rekhapurna, sukshmata); tasteless (swadahina); reddish brown (colour like pakva jambuphala-i.e ripen fruit of *Syzygium cumini*) in colour and floats on stagnant water surface (varitara).

Incinerated iron was a fine powder, insoluble in water but soluble in dilute HCl. It showed 0.08 ± 0.05 % w/w LOD, loss on ignition of 0.33 ± 0.2 % w/w, acid insoluble ash as 5.77 ± 2.4 and pH value as 5.87 ± 0.7 in 1 % distilled water. The bulk and tapped densities were 1.36 ± 0.29 g/cc and 1.68 ± 0.06 g/cc respectively. The Fe content was found to be 64.86 ± 2.29 % w/w and sulphur (as SO_4) was absent as assessed by gravimetric method^[10]. It passed 100 % through mesh size of #100 ($150 \mu\text{m}$) while showed retention of 2.7 ± 0.6 % and 98.1 ± 0.4 % through mesh #200 ($75 \mu\text{m}$) and #300 ($53 \mu\text{m}$), respectively.

LB examined under SEM showed distinct morphology and uniform pattern at all the magnifications (representative images of 1000X magnification in fig. 5a- fig. 5c). The LB particles were irregularly shaped, in cluster form wherein smaller particles were seen adhered to the larger ones. The approximate size of the agglomerates ranged from 2-5 μm where smaller particles were less than 2 μm . TEM depicted irregularly shaped smooth-edged agglomerates of LB (comparative images illustrated in fig. 5d-fig. 5o). The smaller particles were seen to be spherically shaped and adhered to the bigger particles. The size of the agglomerates was up-to 1 μm but the smaller particles were in nano-range, smallest up-to 20 nm.

The measurements of DLS and ZP of LB are enumerated in Table 3 and Table 4, respectively, while representative graphs are illustrated in fig. 6a and fig. 6b, respectively. The finished products showed effective diameter ranging 800-1200 nm while polydispersity and span from 0.30-0.38 and 1.35-1.55, respectively. The particle size distribution of LB showed that 10 % particles were below 260 nm ($<0.26 \mu\text{m}$), 50 % of particles below 500 nm ($<0.5 \mu\text{m}$) and 90 % portion of particles were below 1000 nm ($<1.0 \mu\text{m}$). LB 1, 2 and 3 showed ZP of -11.32, -15.9 and -15.59 mV, respectively, with an average of -14.27 ± 2.56 mV. Summary of DLS and ZP readings are depicted in fig. 6c and fig. 6d.

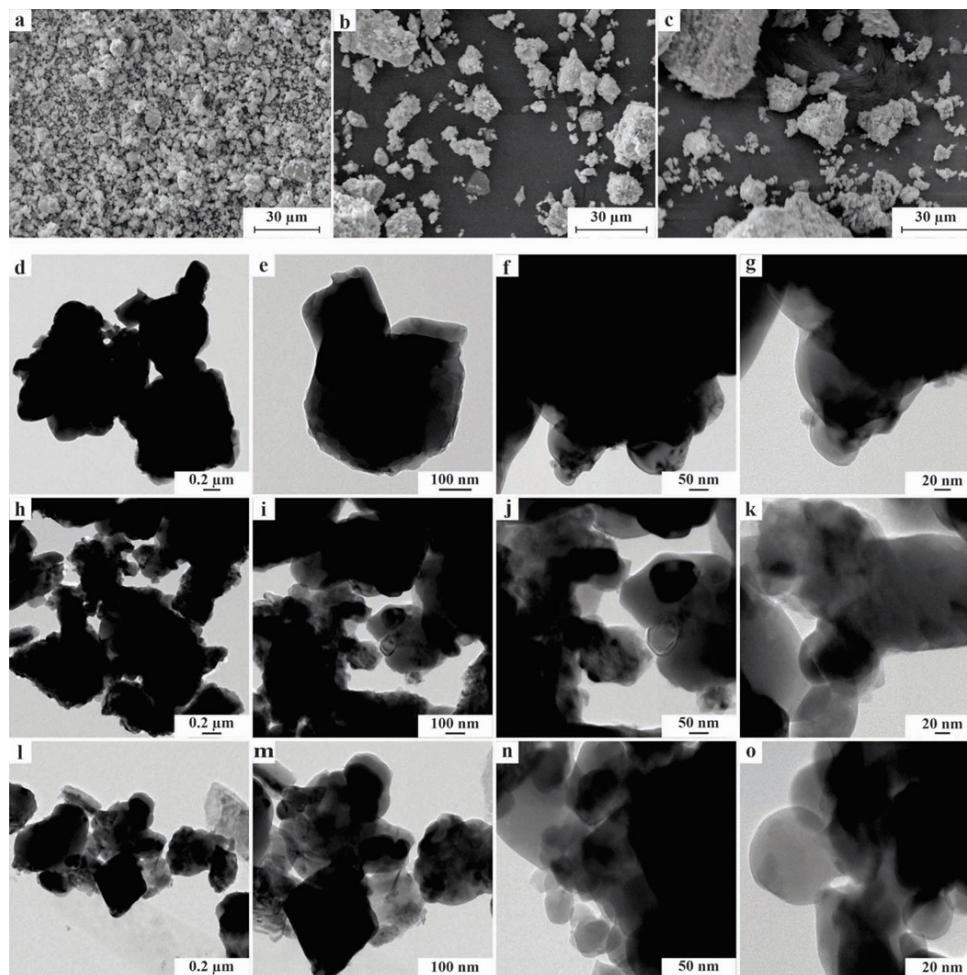


Fig. 5: Microscopic study of LB; (a-c) Scanning electron microscopy of LB1, LB2 and LB3, respectively; (d-o) Transmission electron microscopy of LB1, LB2 and LB3, respectively

TABLE 3: DYNAMIC LIGHT SCATTERING ANALYSIS OF LB

Batch	Run	ED (nm)	PD	Span	D10 (nm)	D50 (nm)	D90 (nm)
LB1	A	1110.2	0.39	1.54	255.4	518.3	1051.8
	B	1054.4	0.38	1.55	237.7	485.4	991.4
	C	981.8	0.39	1.56	220.4	450.8	921.8
Mean		1048.8	0.38	1.55	237.83	484.83	988.33
SD		64.38	0	0.01	17.5	33.75	65.05
LB2	A	857.6	0.33	1.43	225.7	438.2	850.8
	B	846.23	0.32	1.41	230	443.2	853.9
	C	741.43	0.33	1.43	189	367.8	715.9
Mean		815.09	0.32	1.42	214.9	416.4	806.87
SD		64.04	0.01	0.01	22.53	42.16	78.79
LB3	A	1364.33	0.32	1.39	379.5	724.7	1384
	B	1208.37	0.3	1.35	347.7	655.5	1235.8
	C	1002.73	0.29	1.36	287.9	543.1	1024.6
Mean		1191.81	0.3	1.37	338.37	641.1	1214.8
SD		181.37	0.02	0.02	46.51	91.65	180.62
LB (Mean±SD)	1018.57±190.2	0.34±0.04	1.45±0.1	263.70±65.7	514.11±115.2	1003.33±204.4	100

Note: ED: Effective Diameter; PD: Polydispersity and D10-D50-D90: Percentile values

TABLE 4: ZETA POTENTIAL MEASUREMENTS OF LB

Run	Mean mobility			Mean zeta potential (mV)		
	LB1	LB2	LB3	LB1	LB2	LB3
1	-0.82	-1.08	-1.46	-10.55	-13.79	-18.74
2	-1	-1.52	-1.3	-12.76	-19.43	-16.68
3	-0.83	-1.53	-1.21	-10.63	-19.55	-15.52
4	-0.84	-1.32	-1.43	-10.81	-16.94	-18.25
5	-0.97	-1.21	-1.04	-12.41	-15.48	-13.3
6	-1.05	-1.28	-1.31	-13.49	-16.41	-16.71
7	-0.91	-1.03	-1.26	-11.69	-13.24	-16.13
8	-0.87	-1.01	-1.33	-11.13	-12.91	-17.08
9	-0.82	-1.16	-0.94	-10.45	-14.84	-12.02
10	-0.73	-1.28	-0.9	-9.3	-16.41	-11.51
Mean \pm SEM	-0.88 \pm 0.03	-1.24 \pm 0.06	-1.22 \pm 0.06	-11.32 \pm 0.40	-15.90 \pm 0.74	-15.59 \pm 0.79

Note: One-way Analysis of variance (ANOVA) test performed if $p < 0.05$ showed significant difference in LB1 when compared with LB2 and LB3

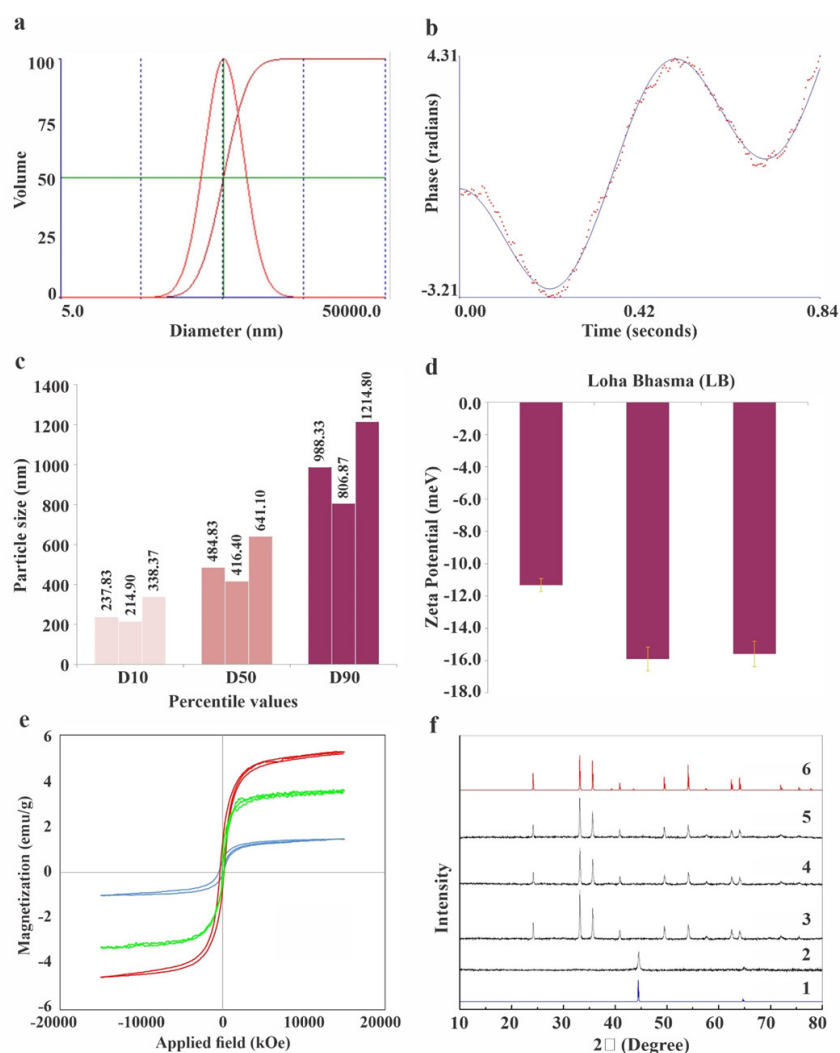


Fig. 6: Characterization of LB, (a) Particle size distribution obtained in Dynamic Light Scattering technique; (b) Phase of zeta potential measurement; (c) Percentile distribution of particle size; (d) Zeta potential values; (e) Vibratory sample magnetometry analysis; (f) X-ray diffraction simulation of compounds

Note: (c): () LB1; () LB2; () LB3 batches; (e): () LB1; () LB2; () LB3 batches; (f): (1) standard iron; (2) raw iron; (3) LB1; (4) LB2; (5) LB3 and (6) α -Fe₂O₃

Magnetization vs. applied field is graphically presented in fig. 6e. Magnetic hysteresis loop was observed for all the LB samples while the magnetization was almost saturated at the highest field of measurement (15 kOe). The values of saturation magnetization (M_s), remnant magnetization (M_r) and coercivity (H_c) were extracted from the graph. LB1, LB2 and LB3 exhibited M_s (emu/g) as 1.45, 5.27 and 3.56 (3.43 ± 1.91); M_r (emu/g) as 0.31, 1.02, 0.57 (0.63 ± 0.36) while H_c as (Oe) 290, 257 and 117 (221.33 ± 91.85).

A direct comparison of the XRD pattern of raw iron with simulated pattern showed that iron turnings were in pure form with characteristic peak at $\sim 45^\circ$, without any oxide content and adulterants. On the other hand, incinerated iron revealed presence of iron oxide content in the form of alpha hematite/ $\alpha\text{-Fe}_2\text{O}_3$ having characteristic peak at $\sim 33^\circ$ and without any presence of metallic iron or sulphides (fig. 6f).

Raw iron revealed no change in the mass while the three batches of LB recorded mass loss against the increase in temperature up-to 900° . The total average mass loss for all the three batches of LB

was 3.3 ± 0.36 % w/w. The losses (% w/w) up-to various temperatures deduced from the graphs were 0.58 ± 0.14 up-to 200° , 1.17 ± 0.57 up-to 400° , 1.75 ± 0.75 up-to 600° and 2.35 ± 0.38 up-to 800° . The percentage of mass loss vs. temperature was plotted graphically (fig. 7).

ICP-OES revealed raw iron containing 98 % Fe with minor impurities of Ag, Cr, As, Fe, Mn and Cu (0.033, 0.075, 0.089, 0.217, 0.484 and 96.33 ppm, respectively) while traces of Pb, K, Co, Zn and Bi (0.002-0.009 ppm) were also detected. LB showed 59.1 ± 0.52 % w/w of Fe and 1.7 ± 1.34 % of S content. Heavy metals viz., Hg was absent while 0.01 ppm of Cd was detected in all the batches of finished product. Arsenic (0.01 ppm) was found in only LB2 while <0.03 ppm of Pb was present in all the batches. LB also contained essential and non-essential elements at minor levels (% mean \pm standard deviation); viz., Al (0.17 ± 0.05), B (0.06 ± 0.001), Ca (1.5 ± 0.05), Cr (0.17 ± 0.06), Cu (0.17 ± 0.05), K (0.11 ± 0.05), Mg (0.1 ± 0.02), Mn (0.32 ± 0.03), Na (0.02 ± 0.006), Ni (0.04 ± 0.00) and Zn (0.01 ± 0.00) while negligible Bi, Ga, In and Tl (<0.03 %) were detected and Ag, Ba, Co, Li and Sr were not detected (Table 5).

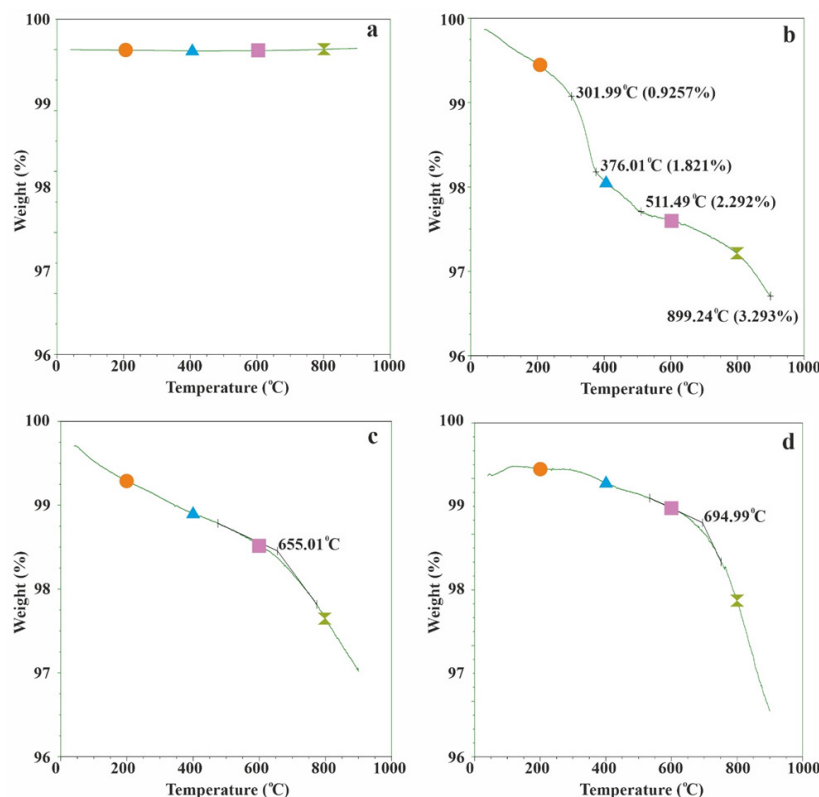


Fig. 7: Thermogravimetric analysis of LB, (a) Raw iron and (b-d) LB1, LB2 and LB3 respectively

Note: The degradation values at temperatures (●) 200° , (▲) 400° , (■) 600° and (✕) 800° for raw iron was nil while for LB1, LB2 and LB3 were 0.5, 1.8, 2.5, 2.75; 0.75, 1.0, 1.75, 2.3 and 0.5, 0.7, 1.0, 2.0 % respectively

TABLE 5: INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROPHOTOMETRY OF LB

Element	Raw iron			LB1			LB2			LB3		
	Concentration	Result	Mean	Concentration	Result	Mean	Concentration	Result	Mean	Concentration	Result	Mean
Al	-0.4	-3.54	0.00	2328.6	0.12	0.12	4152.7	0.21	0.21	3786.8	0.19	0.19
	-0.4	-3.49		2574.3	0.13		4343.2	0.22		3773.3	0.19	
As	0.01	0.09	0.09	-42.2	0	0.00	167.7	0.01	0.01	-44.6	0	0.00
	0.01	0.088		-50.9	0		167.4	0.01		-45	0	
B	2452.9	0.122	0.12	1065.1	0.05	0.06	1214.2	0.06	0.06	1246	0.06	0.06
	2532.9	0.123		1278	0.06		1260.5	0.06		1274.1	0.06	
Ca	-0.8	-8.26	0.00	0.3	1.51	1.52	0.3	1.48	1.44	0.35	1.72	1.54
	-0.8	-8.07		0.3	1.53		0.28	1.4		0.27	1.35	
Cd	-28.5	-0	0.00	103.1	0.01	0.01	118.7	0.01	0.01	123.3	0.01	0.01
	-29.8	-0		126.2	0.01		123.7	0.01		123.4	0.01	
Co	89.8	0.004	0.01	62.8	0	0.00	65.1	0	0.00	56.8	0	0.00
	94.9	0.005		64.5	0		68.5	0		55.9	0	
Cr	1502.1	0.075	0.08	1861.1	0.09	0.1	3813.9	0.19	0.19	4368.6	0.22	0.22
	1543.9	0.075		2178.3	0.11		3940	0.2		4361.2	0.22	
Cu	0.02	0.219	0.22	0.04	0.19	0.2	0.04	0.18	0.19	0.02	0.11	0.11
	0.02	0.214		0.04	0.2		0.04	0.2		0.02	0.11	
Fe	10.1	98.19	96.3	11.8	59	59.5	11.4	56.8	58.5	11.8	58.8	59.2
	9.7	94.47		12.1	60.1		12.1	60.3		11.9	59.7	
Hg	-847.3	-0.04	0.00	1.01	0	0.00	-4.12	0	0.00	-9.7	0	0.00
	-1106	-0.05		0.7	0		-4.23	0		-9.7	0	
K	39	0.002	0.00	1167.4	0.06	0.06	2852.8	0.14	0.15	2703	0.13	0.13
	36.5	0.002		1249.3	0.06		3005.4	0.15		2685	0.13	
Mg	-57.7	-0	0.00	1776.4	0.09	0.09	2284.3	0.11	0.12	1896.3	0.09	0.09
	-3.8	0		1932.1	0.1		2399.4	0.12		1861.5	0.09	
Mn	9631.9	0.48	0.48	6200.1	0.31	0.32	6616.7	0.33	0.34	5815	0.29	0.29
	10030.9	0.489		6688.6	0.33		6951.2	0.35		5787	0.29	
Na	-32.4	-0	0.00	219.8	0.01	0.01	367.3	0.02	0.02	353.4	0.02	0.02
	-38.1	-0		243.8	0.01		394.7	0.02		350.1	0.02	
Ni	1258	0.063	0.06	787.6	0.04	0.04	710.1	0.04	0.04	709.9	0.04	0.04
	1356.8	0.066		830.9	0.04		744.7	0.04		716.1	0.04	
Pb	34.1	0.002	0.00	410.6	0.02	0.02	478.5	0.02	0.02	506.8	0.03	0.03
	35.6	0.002		516.3	0.03		512.9	0.03		499.8	0.02	
S	-0.01	-0.12	0.00	0.9	4.7	2.99	0.3	1.24	1.87	0.08	0.41	0.33
	-0.01	-0.13		0.3	1.27		0.5	2.5		0.05	0.25	
Zn	153.9	0.008	0.01	191	0.01	0.01	175.6	0.01	0.01	154.4	0.01	0.01
	156.8	0.008		212.4	0.01		181.7	0.01		156.2	0.01	

Iron, the fourth most abundant element in the earth's crust is greyish, lustrous, soft, malleable and ductile but a strong metal. In nature, it occurs in minerals, herbs and animal origin products. It reacts readily with oxygen and water to give brown-black hydrated iron oxide called as rust. Several types of irons like pig iron, wrought iron, malleable iron and grey iron are in use for several purposes^[12].

In Rasashastra, types of iron viz., munda lauha, teekshna lauha and kanta lauha described with specific physical, chemical and medicinal properties are correlated with cast/pig iron, wrought iron and magnetite, respectively^[2,5]. Munda lauha is used for making daily household items like vessels, agricultural tools etc.; teekshna lauha (Faulad) is meant for armory equipment^[5] and kanta lauha is magnetite^[10], wherein the latter two are indicated for *Bhasma* preparation^[5]. Kanta lauha as specific characteristics of destroying the fragrance of asafoetida and bitterness of *Azadirachta indica* leaves when their paste is applied to kanta lauha vessel^[2]. Though kanta lauha is the best variety for medicine, it is not readily available^[5]. In this study, industrial waste iron in the form of turnings was used for the preparation of LB and can be considered as wrought type. LB, an official medicine^[3], is used in several ailments by Ayurvedic physicians. It is an important ingredient in numerous dosage forms like powders, tablets, confectioneries and hydro-alcohols^[1] as well as special formulations viz., Lauha kalpa (iron formulations) and Ayaskruti (quenched dosage form of iron)^[2,3,5]; but requires proper procedures for its synthesis. Different media are used to attain the desired form for easy absorption/assimilation in the body without causing toxicity^[2].

In this study, red hot iron quenched in sesame oil increased the temperature of sesame oil (90°), thus rendering brittleness due to faster cooling as well removal of associated greasy matter that cannot be removed by aqueous media. Organic liquids are preferred over aqueous media, since they cause insufficient heat transfer due to excessive air bubble formation on the surface^[13]. In addition, sesame oil may remove ferric ions by forming metal soaps and the repetitive thermal stress causing particle size reduction^[14]. Similarly, removal of other oxide impurities may be explained due to the aqueous media viz., butter milk, urine,

rice gruel and *Dolicus biflorus* decoction wherein temperature of all these also increased (55°-65°). Quenching in butter milk may help to remove the rust (Fe_2O_3) as reported earlier, where butter milk treated samples reduced Fe_2O_3 while used butter milk showed absence of free Fe^{3+} ^[15]. Ammoniated acid in cow's urine is known to dissolve Fe_2O_3 to form water soluble $\text{Fe}^{2+}/\text{Fe}^{3+}$ complexes whereas uric acid also forms ureate- $\text{Fe}^{3+}/\text{Fe}^{2+}$ complexes, hence removal of Fe oxides in this step can be considered^[16]. Rice-gruel may be helpful to remove Fe^{3+} through the formation of co-ordination compounds since it contains high level of phytic acid, a chelating agent reacting with $\text{Fe}^{2+}/\text{Fe}^{3+}$ salts to give iron complexes^[17]. Similarly, *Dolicus biflorus* decoction, rich in gallic acid forms complex with iron chloride and water soluble gallic acid-metal complexes may get formed/detached during repeated washing^[18]. Further, quenching in the *Triphala* decoction containing gallic acid may maintain iron in Fe^{2+} form, without getting oxidized. Thus, after quenching, bluish-black raw iron turned reddish-brown due to partial oxidation while sharpness of the edges reduced. The attained brittleness can be strongly corroborated with stress corrosion cracking theory^[19].

In the next step, iron was triturated with *Aloe barbadensis* juice with/without sulphur and incinerated. No quantitative data is so far available; hence the quantities of iron, sulphur, *Aloe barbadensis* juice and heating were standardized. *Aloe barbadensis*, water rich plant containing polysaccharides and saponins, used in the present study probably helped to wet grind the particles smoothly, bind them easily during pellet formation, desiccate in a short duration and leave behind negligible inorganic residue after incineration. It possibly also acts as a natural surfactant, stabilising agent^[20], solvent, bio-template for nano-metal oxide synthesis and preventer of particle agglomeration; thus, highlighting the importance of green biosynthesis against harmful chemicals as precipitating and reducing agents^[21].

Initial three cycles of grinding iron and sulphur mixture, trituration with *Aloe barbadensis* juice followed by incineration was an important step. The temperature was gradually increased from I2 to I9 cycles by increasing the fuel, except for I1 and I3, where it was kept higher to oxidize the sulphur. The gradual chemical transformation of iron from

sulphide to oxide form is associated with variables such as temperature, atmosphere and particle size in this case. The gradual temperature increase in the pit, sulphur dioxide atmosphere inside the earthen vessels, and gradual decreasing trend of particle size i.e., iron turnings into fine powder are observed during this entire manufacturing process. A study reports that, pyrite (>0.045 mm grain size) gets directly and completely oxidized to hematite at $\sim 500^\circ$ in air whereas for 0.09 - 0.125 mm size, hematite is formed at temperature $>515^\circ$ while pyrrhotite (FeS) is formed at temperature $<515^\circ$. Thus, a complex route reaction of $\text{FeS}_2 \rightarrow \text{FeS}$ (intermediate) $\rightarrow \text{Fe}_3\text{O}_4/\text{Fe}_2\text{O}_3$ during pyrite roasting is reported^[22]. Similar situation is possible during the incineration of iron in the concealed vessels. The assumed highest temperature inside the vessels ($\sim 750^\circ$ to $\sim 900^\circ$) and SO_2 atmosphere might be facilitating the gradual conversion of FeS (formed in the ascending temperature range) to oxide initially, followed by complete conversion due to *Aloe barbadensis*.

LB manufactured by this method was alpha hematite ($\alpha\text{-Fe}_2\text{O}_3$) (fig. 6f) which purports similar findings on LB analysed by Mössbauer parameters (stoichiometrically pure and chemically stable)^[23]. Various methods of $\alpha\text{-Fe}_2\text{O}_3$ nanoparticles synthesis such calcinating precursor ferric nitrate non-ahydrate at 450° in air and multi-step dehydration of ferrihydrite under specific conditions have been reported but with drawbacks of aggregation and phase impurity^[24]. Thus, the present method of preparation of LB also provides specific physicochemical properties as discussed further. The final colour of the product; negligible losses on drying and ignition; absence of SO_4^{4-} ions as well as passing through mesh of $53\text{ }\mu\text{m}$ indicated complete incineration in our method and further confirmed by advanced techniques. Other reports on LB prepared with *Triphala* decoction have shown Fe_2O_3 phase, calculated particle size in nano range by XRD^[6] and elemental composition as Fe ($\sim 52\%$), Ca ($\sim 2.5\%$) with particle size up-to $1.8\text{ }\mu\text{m}$ ^[7]. In another study^[8], LB prepared in muffled furnace at 650° when analysed by X-Ray Fluorescence technique revealed Fe up-to 70% , but its oxide status was not reported. However, SEM showed aggregates of irregularly shaped and variedly sized particles (100 - 500 nm).

In the present study, the size and surface morphology

determined with SEM and TEM revealed batch-to-batch consistency. TEM confirmed that this method of LB formation yielded powder containing nano- to micro- sized particle agglomerates. It also depicted nearly spherical shaped particles comparable with the reported study on $\alpha\text{-Fe}_2\text{O}_3$ ^[25]. However, the irregularities in the shape of particles may be attributed to the grinding process.

The particle size range (0.25 - $1\text{ }\mu\text{m}$) and negative ZP (-14 mV) of LB, both are unique for this method. ZP ranging from 5 - 20 is considered fairly stable. Moreover, negatively charged higher sized nano-particles (150 nm) accumulating more efficiently in tumours, are recognized by scavenger receptors facilitating uptake by the reticulo-endothelial system and pharmaco-kinetically cleared faster^[26]. It could be expounded that Bhasma particles colloid in aqueous medium and make a suspension of clustered negatively charged hydrophobic particles as in the present study, possibly due to the repeated incineration. Detailed ZP analysis on LB can further help to understand the ionic properties that are responsible for its efficacy.

Further, the VSM study showed magnetic behaviour but the mean M_s value (3.4 emu/g) was much less as compared to the previous reports (58.6 and 65.2 emu/g) on porous $\alpha\text{-Fe}_2\text{O}_3$ nanostructures synthesized by rapid combustion^[27]. The mean M_r value was also in accordance with some reports (0.13 and 0.73 emu/g)^[28] whereas the H_c value was either higher or lower^[29]. Magnetic property depends on size, shape, crystallinity, surface structure and porosity of the material. From XRD studies, the phase content of LB was identified as hematite. Therefore, the differences in the magnetization and coercivity values could be due to the difference in the particle size. Though hematite is a very weak ferromagnetic material, a relatively larger magnetization observed in our case could be attributed to the incineration at higher temperatures^[30]. In addition, the effect of anions (Cl^- , SO_4^{4-} , NO_3^-) acting as structure and surface directing agents in the synthesis of different shaped and larger particle sized red coloured $\alpha\text{-Fe}_2\text{O}_3$ nanoparticles might throw light on the optical and magnetic properties of LB as reported in chemical synthesis of $\alpha\text{-Fe}_2\text{O}_3$ ^[31]. Generally, Fe_2O_3 has less magnetization and coercivity as compared to Fe_3O_4 ^[32] indicating ferromagnetic and superparamagnetic natures, respectively;

irrespective of the method of preparation of iron oxides.

It is also possible that LB may contain minor amounts of ferromagnetic iron oxides like magnetite (Fe_3O_4) or maghemite ($\gamma\text{-Fe}_2\text{O}_3$) that can be ruled out by phonon confinement effects using Raman or Mössbauer Spectroscopy^[33]. These were not detected in the present XRD studies because they might be present in the samples probably beyond the detection limit of XRD. The XRD pattern of raw iron turnings showed metallic Fe without any oxides while LB demonstrated $\alpha\text{-Fe}_2\text{O}_3$ without metallic iron/other oxides or sulphide/sulphate forms of Fe indicating purity. This is also in accordance with the recent XRD studies on $\alpha\text{-Fe}_2\text{O}_3$ wherein higher crystallinity and greater particle size was observed at higher annealing temperature (700°)^[30].

The chemical status of finished product was also confirmed by decomposition pattern obtained in TGA (fig. 7). The first mass loss (<1 %) occurring up-to 200° may be attributed to the moisture and residual sulphur while the second loss (~1 %) observed up-to 500° may be due to the elimination of carbon residues. Finally, the third mass loss at temperatures $600^\circ\text{-}900^\circ$ (~2 %) may indicate phase transformation of iron oxides. Thus, the total loss in the product and the pattern of TGA is in accordance with synthesized $\alpha\text{-Fe}_2\text{O}_3$ ^[30]. Moreover, the elemental Fe in the present preparation (60 %-65 %) analysed by titration and spectrophotometric methods is comparable with the Pharmacopoeia. Heavy metals were within permissible limits^[10] while trace elements viz., Na, K, Mg, Zn, Ca and Cu were detected.

There are various reports on $\alpha\text{-Fe}_2\text{O}_3$ regarding therapeutic uses such as anti-bacterial, anti-cancer and anti-tumour due to its good bio-compatibility, bioavailability, biodegradability, ease of synthesis and differential magnetic behaviour^[34]. Iron Oxide Nano-Particles (IONP's) have also been studied as targeted drug delivery system with anticancer drugs, silica coated IONP's as alternative immunosuppressants, anti-interleukin- 1β monoclonal antibody functionalized IONP's as anticonvulsants and neuroprotectives, antifungals and antibiotics^[35] as well as specific targeting to red blood cells and cancer cells^[34] and cytotoxic to liver cancer cells^[36]. Yakrut Plihari Lauha containing LB as major ingredient when administered along

with *Picrorrhiza kurroa* showed symptomatic relief in liver disorders including liver cancers patients^[37]. Moreover, iron deficiency anaemia as well as inhibition of iron absorption due to specific dietetics and disease conditions has been recognised as an important health issue. Furthermore, the large dose of iron becomes toxic due to formation of free radicals in circulation, causing corrosion in the gastrointestinal tract, thus penetrating the cells resulting in damage to important organs. Therapeutically, iron oxide in high doses has been found similar to that of ferrous sulphate (FeSO_4), but the latter has several side effects like black stools^[38]. Animal experiments using iron deficit diet and phlebotomy induced anaemia has proved that LB is more bioavailable and useful in increasing the hemoglobin than FeSO_4 , probably due to the particle size and solubility of ferric ions more soluble in neutral pH^[39,40]. Therefore, extensive bioavailability studies of oxide form compared to salt counterparts needs to be conducted since the proper absorption, metabolism and its recycling is essential for good health condition.

$\alpha\text{-Fe}_2\text{O}_3$ is reported to be a stable n-type semiconductor used for biomedical application due to its haematinic, anti-tumour, anti-cancer and anti-bacterial activities^[33]. LB prepared in the present study having chemical nature of $\alpha\text{-Fe}_2\text{O}_3$ fulfils the standards required to be therapeutically used in anaemia and malignant conditions.

Thus, in the present study LB ($\alpha\text{-Fe}_2\text{O}_3$) was manufactured from raw iron by traditional method of incineration. Final product assessed by quality control measures for standard operating procedure generated here can be followed for consistency. Such prepared LB has irregularly shaped agglomerates, particle size from 250-1000 nm, negative zeta potential and magnetic behaviour. Incinerated iron, one of the potential ingredients of various Ayurvedic herbo-metallic mineral formulations, if prepared by these guidelines, can be further studied to understand the mechanistic aspects of its varied activities.

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Conflict of interest:

The authors declare no conflict of interest.

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