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Standard manufacturing procedure of *Teekshna lauha bhasma*Thakur Rakesh Singh ^{a,*}, Laxmi Narayan Gupta ^b, Neeraj Kumar ^b^a Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Raipur, Chhattisgarh, India^b Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

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ABSTRACT

Background: *Lauha bhasma* is one of the herbo-metallic preparations used in *Ayurveda*, a traditional Indian system of medicine for treating various ailments such as anemia, diarrhea, hyperlipidemia and diabetes.

Objective: To establish standard manufacturing procedure of *Teekshna lauha bhasma* and analyze its physico-chemical properties.

Materials and methods: The preparation of *T. lauha bhasma* (calx of iron [Fe] turning) involves *samanya shodhana*, *vishesa shodhana* followed by *bhanupaka*, *sthalipaka* and *putapaka* with *Triphala kwatha* as a medium under temperature of 650 °C in electric muffle furnace (EMF) and maintained for 1 h. *T. lauha bhasma* were subjected to different physico-chemical characterization using X-ray fluorescence spectrophotometer and scanning electron microscopy.

Results and discussion: The results suggest that these steps are necessary to obtain a good quality of *bhasma* and also make it acceptable for trituration during *Bhasmikiran* process. It is found that *T. lauha bhasma* was prepared properly in 20 *puta* at a temperature of 650 °C. The particle size of 20 *puta T. lauha bhasma* is 100–500 nm in range.

Conclusion: Pharmaceutical procedures given in *Ayurvedic* texts are necessary to prepare *pakwa jambu phala varna T. lauha bhasma* that complies with all the classical *bhasma pariksha* and modern analytical parameters in 20 *puta* at a temperature of 650 °C maintained for 1 h in EMF.

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Introduction

Ayurveda, the science of life, is a comprehensive medical system that has been the traditional system of healthcare in India for more than 5000 years. The basic aim of this science is to maintain healthcare by balancing the physical, mental, and spiritual functions of the human body [1,2]. *Rasashastra*, an integral part of *Ayurveda*, deals with the drugs of mineral origin, and details their varieties, characteristics, processing techniques, properties, therapeutic uses, possibilities of developing adverse effects and their management, etc. in a comprehensive way [3]. *Ayurvedic* experts have estimated that 35–40% of the approximately 600 medicines mentioned in the *Ayurvedic* formulary may contain at least one metal [4]. *Ayurvedic* medicines are mostly *Rasoushadhies* (herbo-mineral) and they play an important role in *Ayurvedic* therapeutics

because of their qualities such as *Alpamatropayogitvat* (low dose), *Arucher-aprasangata* (good palatability) and *Kshipramarogayadayitvat* (fast acting) [5]. *Rasayana* (immunomodulation and anti-aging quality) and *Yogavahi* (ability to target drugs to the site) are characteristics of a properly made herbo-mineral preparation, which is also nontoxic, readily absorbable, adaptable, and assimilable in the body [6]. *Bhasmas* are herbo-metallic ashes in which the metal is calcined along with various herbal ingredients to form organometallic complexes [7]. These complexes should neither contain free metal nor contain free organic constituents, whose presence in *bhasma* indicates improper calcination [8].

Iron (Fe) is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid synthesis, and electron transport [9]. The incinerated Fe preparations of *Ayurveda* are known as *lauha bhasma* (Fe calx) [10]. It is a herbo-metallic calx that has several therapeutic applications. Pandit et al. reported that, *lauha bhasma* for hematinic activity and hemoglobin regeneration efficacy on agar gel diet and phlebotomy induced Fe deficiency anemia in rats and reported significant hematinic and hemoglobin

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regeneration efficiency in comparison to control and standard ferrous sulfate containing drug [11]. Antibacterial activity of *lauha bhasma* was reported by Tambekar and Dahikar [12]. In the *Samhita* period Fe (*Ayas-Lauha*) was used in the form of fine powder. Later, *Rasashastra* classical texts explained the *shodhana* (purification) and *marana* (incineration) methods [13]. According to *Rasa Ratna Samuchchhaya*, *kanta lauha* (magnetite Fe ore) is considered as best raw material variety of Fe-for *lauha bhasma* [14]. However in the absence of *kanta lauha*, *Teekshna lauha* (Fe turning) is used for the preparation of *lauha bhasma*. Now-a-days, in many *Ayurvedic* pharmacies and industries *lauha bhasma* is prepared from *T. lauha* and the preparation protocol for *bhasma* varies from manufacturer to manufacturer; there are many *Ayurvedic* texts describing different methods of preparation of *lauha bhasma* [15,16] and it plays a major role in deciding the therapeutic efficacy, as well as the toxic effects of *bhasmas*. The conventional *puta* (using electric muffle furnace [EMF]) method of heating is very easy and convenient to regulate temperature in closed atmosphere as comparative to traditional *puta* (using cow dung). Hence, in this study standard manufacturing procedure of *T. lauha bhasma* (calx of Fe turning) was established by following the guidelines of *Ayurvedic* formulary of India by adopting various procedures such as *samana shodhana* (normal purification), *vishesha shodhana* (special purification), *trividh lauhapaka*, that is, *bhanupaka* (exposure to sunlight), *sthalipaka* (roasting in an Fe pan), and *putapaka* (calcination) using EMF. This study also attempts to characterize physico-chemical properties of *T. lauha bhasma* through conventional studies for studying the quality of *bhasma Nischandratvam* (lusterless), *Apunarbhava* (metal irreversibility test), *Varitaratvam* (floating test), and detailed information on elemental composition and particle size of *T. lauha bhasma* has been evaluated by Bhargava et al. using modern

analytical instruments like scanning electron microscope (SEM) and X-ray fluorescence (XRF) spectrophotometer [17,18].

Materials and methods

Procurement of raw material

The authenticated raw materials; *T. lauha* (Fe turnings) were collected from the Department of Metallurgy, IIT (BHU); *Tila taila* and *Triphala* were collected from the *Ayurvedic* pharmacy, BHU; *Kulattha* collected from local market, Varanasi and *Gomutra* (cow's urine) were collected from Dairy farm, Institute of Agricultural Sciences, BHU, Varanasi.

Materials

EMF – inner hearth (length: 15 cm, breadth: 19 cm, depth: 30 cm, and maximum temperature capacity: 1000 °C), *khalva yantra* (mortar – length: 36 cm, breadth: 21 cm, thickness: 3 cm, depth: 11 cm; pestle – length: 13 cm, and diameters: 7 cm).

Methods

The preparation of *T. lauha bhasma* (calx of Fe turning) was carried out in Laboratory of Department of Rasa Shastra, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India by following the procedure described in the *Ayurvedic* formulary of India [19]. It involves the following major steps; *samana shodhana* (normal purification), *vishesha shodhana* (special purification), *trividh lauhapaka*, that is, *bhanupaka* (exposure to sunlight), *sthalipaka* (roasting in an Fe pan), and *putapaka* (calcination) (Fig. 1).

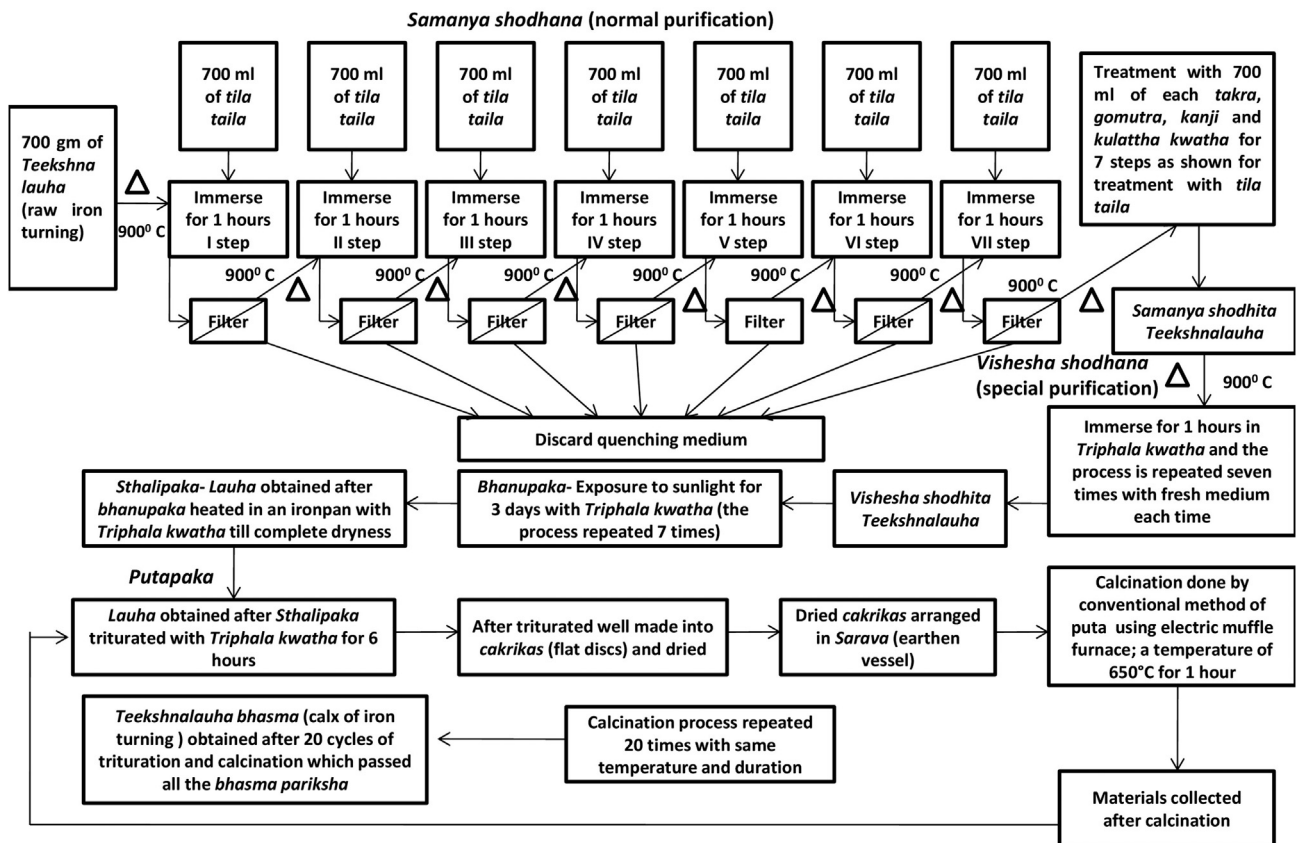


Fig. 1. Flow diagram for the preparation of Teekshna lauha bhasma.

T. lauha bhasma was subjected to various organoleptic and physico-chemical analysis such as color, taste, texture, loss on drying [20], ash value [21], acid insoluble ash [21], and water soluble ash [21]. Modern analytical instruments such as XRF and SEM were employed to determine the elemental composition and particle size respectively.

Samanya shodhana

In *samanya shodhana* process, 700 g of raw material (Fe turning) was heated in EMF till red hot condition (~875–900 °C) and immersed in 700 ml of each medium viz. *tila taila* (sesame oil), *takra* [22] (buttermilk), *Gomutra* (cow's urine), *kanji* [23] (rice gruel), and *kulattha kwatha* [24] (decoction of horse gram) and kept for self-cooling (approximately 1 h) at room temperature (Fig. 2). This quenching process was repeated for seven times consecutively in *tila taila* followed by seven times consecutively in *takra*, *gomutra*, *kanji*, and *kulattha kwatha* by using fresh media every time. After completion of the process, material was filtered by Fe mesh and dried under sunlight. The material obtained at this stage is called *samanya shodhita lauha*.

Vishesha shodhana

In this purification step, quenching was done in *Triphala kwatha*. It was prepared by taking coarse powders of three myrobalans, taken without seed: *Haritaki* (*Terminalia chebula* Retz.), *Bibhitaki* (*Terminalia bellirica* [Gaertn.] Roxb.), and *Amalaki* (*Phyllanthus emblica* L.) in equal quantity (each 1 kg) and boiled in 24 L of water till reduction to 1/4th of the original volume of water to obtain

Triphala kwatha. Using this, repeated quenching process of *samanya shodhita lauha* was done. This purification step was repeated seven times using freshly prepared *Triphala kwatha*. The *lauha churna* (coarse powder of Fe turning) obtained at this stage is called *vishesha shodhita lauha*.

Bhanupaka

Triphala kwatha was prepared by heating equal quantity of *Triphala* to *vishesha shodhita lauha* churna with two parts of water and reduced to 1/4th of original volume. This *Triphala kwatha* was added to *lauha* obtained after *vishesha shodhana* and allowed to dry under sunlight. It took a maximum of 3 days for complete drying of *Triphala kwatha*. This process was repeated seven times to yield intermediate after *bhanupaka* (Fig. 3a).

Sthalipaka

In this step, *Triphala kwatha* was prepared by taking *Triphala* 3 times of *lauha* obtained after *bhanupaka* and 16 times of water was added to it. The whole material was boiled in a stainless steel container to reduce the volume to 1/8th of the original volume of water. *Lauha* obtained after *bhanupaka* was washed with hot water and placed in a *sthali* (Fe pan), to which above freshly prepared *Triphala kwatha* was added and intense heating was given for complete evaporation of water contents of *Triphala kwatha*. On complete drying of the material, again *Triphala kwatha* was added and subjected to heat. This process required 4 h 30 min for complete drying of *Triphala kwatha*. The whole process was repeated seven times to yield intermediate after *Sthalipaka* (Fig. 3b and c).

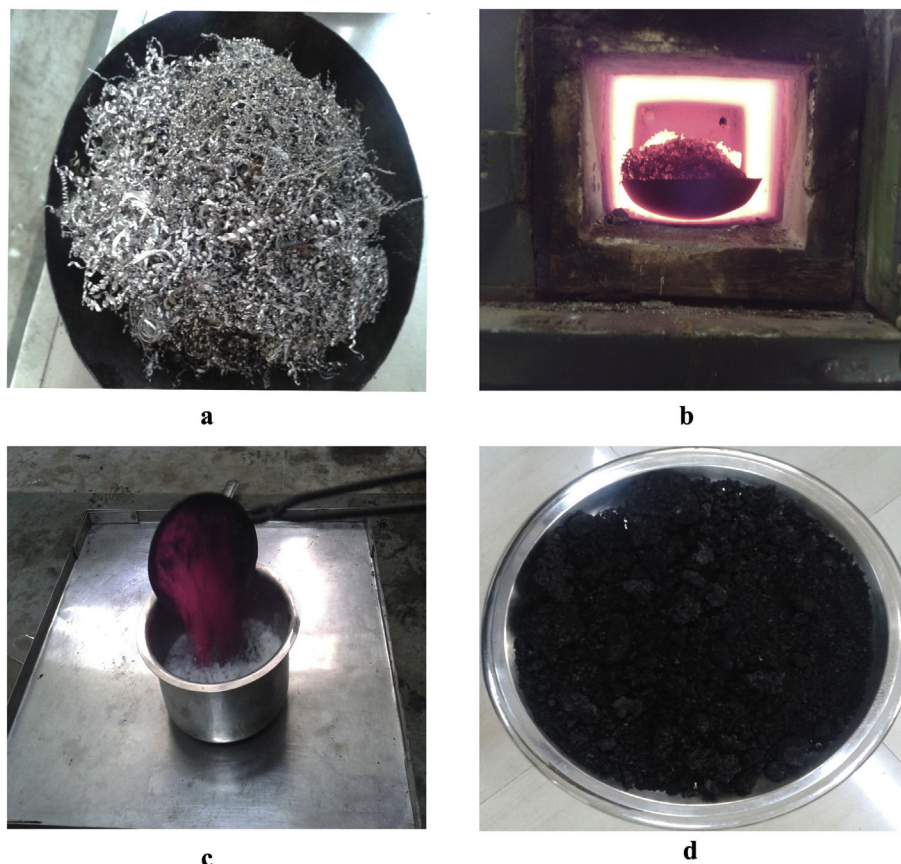


Fig. 2. Shodhana process of *Teekshna lauha* (iron turning). (a) Raw *Teekshna lauha* (iron turning), (b) red hot iron turning, (c) quenching process, (d) *Teekshna lauha* after shodhana.

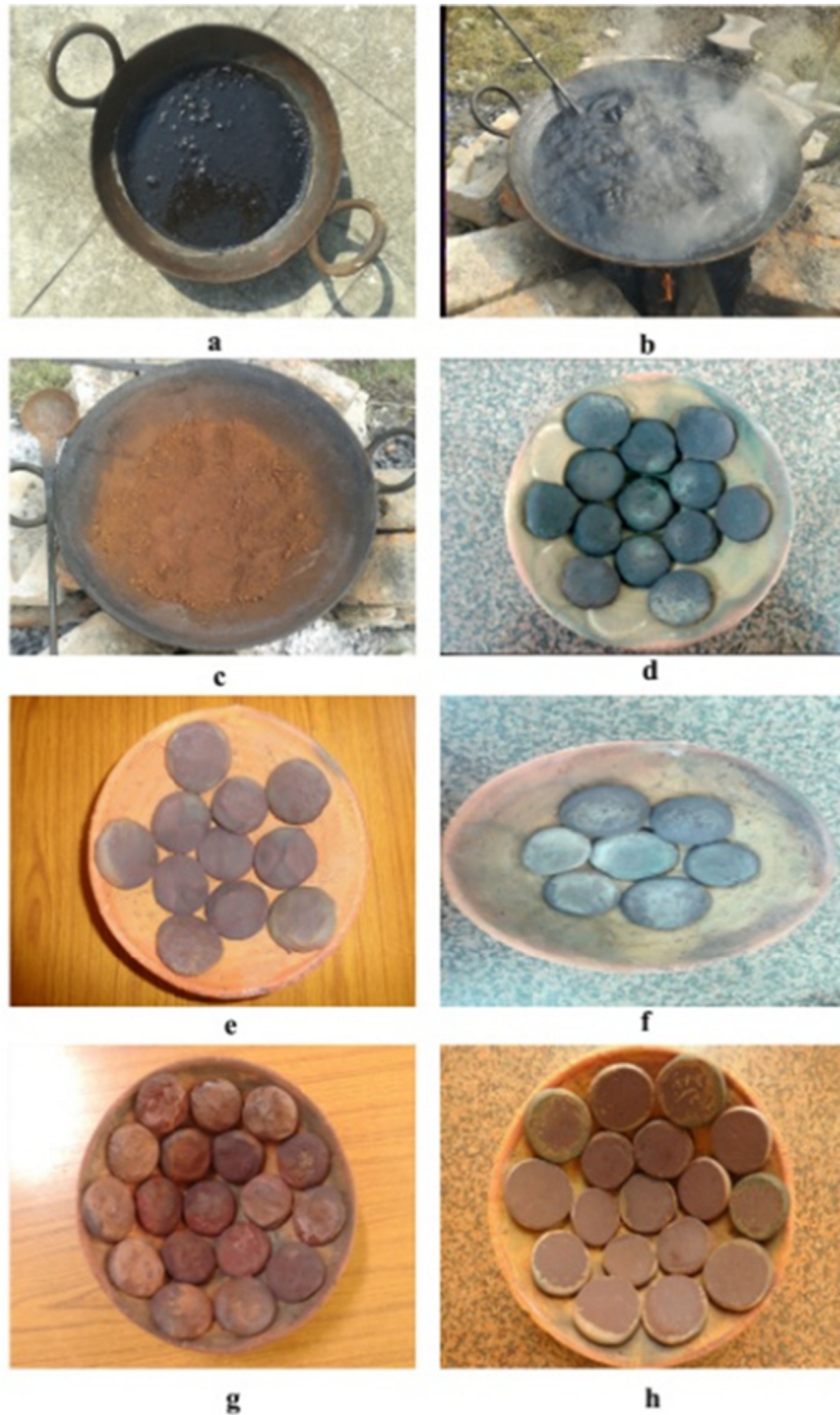


Fig. 3. Trividh lauhapaka. (a) Bhanupaka, (b) Sthalipaka, (c) after Sthalipaka, (d) after 1 puta, (e) after 5 puta, (f) after 10 puta, (g) after 15 puta, (h) after 20 puta.

Putapaka

In traditional literature, the process of *puta* (calcination) refers to controlled heating of herbo-mineral preparations and allowing the preparation to cool to room temperature. [25] According to *Rasa tarangini*, *lauha bhasma* should be prepared by triturating with

specific media according to disease and subjecting to *puta*, but in case of unavailability of the specific media the *bhasma* should be prepared by *Triphala kwatha*. [24,26] In this process, freshly prepared *Triphala kwatha* was mixed with *lauha* obtained after *sthalipaka* in mechanized *khalva yantra* and trituration was done with a frequency of 60 times/min. The paste formed during this trituration

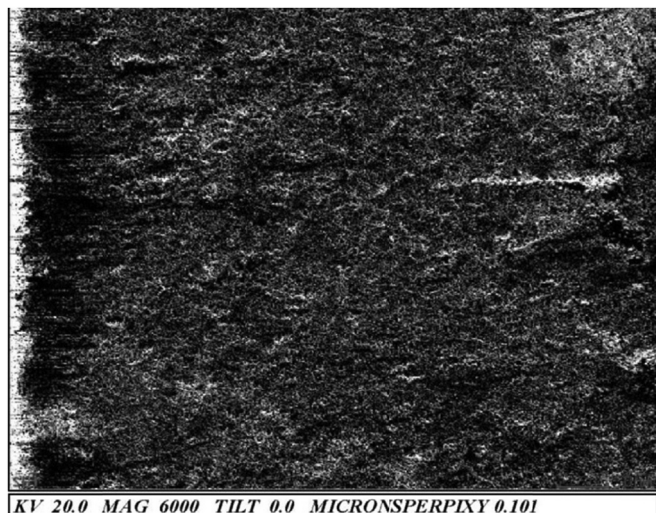


Fig. 4. Scanning electron micrographs of 20 puta Teekshna lauha bhasma.

was made into *cakrikas* (pellets) and dried under sunlight. After complete drying of *cakrikas*, it was taken in an earthen vessel (*sarava*) and covered with another inverted earthen vessel. The space between the two earthen vessels was covered with clay smeared cloth; this specific process is known as *Sarava samputi-karana* (sealed earthen pot). After this, it was subjected to *puta* in horizontal EMF and temperature was allowed to gradually rise to 650 °C in 2 h and maintained for 1 h [27]. The furnace was then switched off and allowed for self-cooling. The next day, pellets were collected from *sarava* and again triturated with *Triphala kwatha*. Same process of *puta* was repeated for 20 times to obtain *T. lauha bhasma* of desired quality.

Observations and results

Samanya shodhana of *T. lauha* (Fe turning) was carried out as per *Ayurvedic* formulary of India where the sequential quenching process was done in different media by following conventional order. Metallic luster was lost after quenching in *tila taila* and prominent cracks and flakes were seen after quenching in *takra* and *gomutra*. The maximum part was in powder form after quenching in *kulattha kwatha*. At the end of *samanya shodhana*, it was observed that, the hard shining Fe metal was converted into black colored lusterless powder of brittle material. After the process of *vishesha shodhana*, brittleness and weight of *T. lauha* increases and the dark black color powder was formed. It was observed that pH of media increases after every quenching of *shodhana* process (Tables 1 and 2).

During the process of *bhanupaka* while adding *Triphala kwatha* in *shodhita lauha churna*, color changes from greenish brown to black (Fig. 3a). After complete sun drying, *lauha churna* gets converted into a big cluster. The product obtained after *bhanupaka* was

Table 2

Changes in pH of media before and after quenching during *samanya* and *vishesha shodhana* of *Teekshna lauha* (iron turning).

Media	Before	After
<i>Takra</i>	3.5	4
<i>Gomutra</i>	7.5	8.3
<i>Kanji</i>	3	4
<i>Kulattha kwatha</i>	6.4	7.4
<i>Triphala kwatha</i>	2.8	3.3

more brittle in nature and big particles of *lauha churna* were converted into fine particles. In *sthalipaka*, during heating *lauha churna* was adhering to the surface of container and fumes increased with the duration of heating (Fig. 3b). At the end of *sthalipaka* the color changes from dark black to brick brown in color and *lauha churna* was converted into powder form completely (Fig. 3c). Various observations during *bhanupaka* and *sthalipaka* are tabulated in Table 3.

During *Putapaka* (calcination), *lauha churna* obtained after *sthalipaka* was triturated with *triphala kwatha*. In first *puta*, it was taking 8 h to get converted into paste like structure and also difficult to make pellets. After *putapaka* the color of pellets changed from brick brown to dark brown (Fig. 3d–h). After every *puta*, it was easy to make pellets due to reduction in particles size and also color changes. Physical characteristics of the material recorded down after every *puta* which was shown in Tables 4 and 5. *T. lauha bhasma* was passed by various classical *bhasma pariksha* (Table 6). The results of various physiochemical parameters color, taste, texture, loss on drying, ash value, acid insoluble ash, water soluble ash, particle size (Fig. 4), and elemental composition are tabulated in Tables 7 and 8.

Discussion

Bhasma preparations involve the conversion of the metal into its mixed oxides, during which, the zero valent metal state is converted to a higher oxidation state. The significance of this “*Bhasmikiranana*” is that the toxic nature of the resulting metal oxide is completely destroyed while introducing the medicinal properties into it [28]. A *bhasma* means a fine ash obtained through incineration [29]. Selection of raw material is the most important step in the *bhasma* preparation. Authentic raw material with high quality assures producing safe and efficacious finished product. In traditional literature, *kanta lauha* (magnetite Fe ore) and *T. lauha* (Fe turnings) is considered as best raw material for *lauha bhasma* preparation [30]. The availability of *kanta lauha* (magnetite Fe ore) is rare, that is why, in many *Ayurvedic* pharmacies and industries, *lauha bhasma* is prepared from *T. lauha*. Hence in this study, a step is made to find out the best quality of *T. lauha bhasma* using EMF. In this regard, raw material *T. lauha* was tested by classical method, as well as by modern analytical techniques [18,31]. In classical method, *Kalka* of *Kasisa* (ferrous sulphate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and *Amalaki* (*P. emblica* L.) fruit pulp was applied over the surface

Table 1

Observations made during *samanya* and *vishesha shodhana* of *Teekshna lauha* (iron turning).

Media	Quantity (ml)	Initial weight (g)	Final weight (g)	Gain/loss (g)	Actual percentage change
<i>Tila taila</i>	700	700	740	40 ↑	5.7 ↑
<i>Takra</i>	700	740	780	40 ↑	5.7 ↑
<i>Gomutra</i>	700	780	746	34 ↓	4.4 ↓
<i>Kanji</i>	700	746	740	6 ↓	0.9 ↓
<i>Kulattha kwatha</i>	700	740	740	No change	No change
<i>Triphala kwatha</i>	700	740	850	110 ↑	15% ↑

Table 3Observations made during *bhanupaka* and *sthalipaka* of *vishsha shodhita Teekshna lauha*.

Pharmaceutical procedure	Media (<i>Triphala kwatha</i> in ml)	Initial weight (g)	Final weight (g)	Gain/loss (g)	Actual percentage change
<i>Bhanupaka</i>	425	850	1250	400↑	47↑
<i>Sthalipaka</i>	7500	1250	885	365↓	29↓

Table 4Changes in weight of *Teekshna lauha bhasma* before and after *puta* during *putapaka*.

<i>Puta</i>	Media (<i>Triphala kwatha</i> in ml)	Initial weight (g)	Final weight (g)	Loss (g)	Actual percentage change
1st	325	885	839	46 ↓	5 ↓
2nd	325	839	836	3 ↓	0.35 ↓
3rd	325	836	832	4 ↓	0.47 ↓
4th	325	832	830	2 ↓	0.25 ↓
5th	325	830	828	2 ↓	0.25 ↓
6th	350	828	815	13 ↓	1.5 ↓
7th	350	815	813	2 ↓	0.25 ↓
8th	350	813	813	No change	No change
9th	350	813	810	3 ↓	0.35 ↓
10th	400	810	810	No change	No change
11th	400	810	790	20 ↓	2.4 ↓
12th	400	790	786	4 ↓	0.47 ↓
13th	400	786	783	3 ↓	0.35 ↓
14th	400	783	783	No change	No change
15th	425	783	780	3 ↓	0.35 ↓
16th	425	780	770	10 ↓	1.2 ↓
17th	425	770	769	1 ↓	0.1 ↓
18th	450	769	766	3 ↓	0.35 ↓
19th	450	766	765	1 ↓	0.1 ↓
20th	450	765	765	No change	No change

of *T. lauha* and after some time conical protrusion (*girisringa*) are seen over the surface of *lauha*, it shows the sample of *T. lauha* is genuine. The elemental analysis through XRF study also revealed that raw Fe turning contains 98.10% of Fe (Table 8) [18]. All the other necessary media for pharmaceutical procedure such as *kanji*,

takra, *Triphala kwatha*, etc., that were used at various stages were prepared under observation.

Shodhana (purification/detoxification) is the foremost step in the preparation of *lauha bhasma* because impure *lauha bhasma* on administration causes *Hritpida* (chest-pain), *Agnimandya*

Table 5Observations recorded before and after *puta* of *Teekshna lauha bhasma* during *putapaka*.

<i>Puta cycle</i>	Before <i>puta</i>	After <i>puta</i>
1st	Brick brown color, on trituration color changes to black	Dark brown color
2nd	Pellets were made easily and rough in consistency	Pellets were easily breakable by hand and color was bluish black but surface of pellets was brown
3rd	After trituration color turns to grayish black	Pellets become soft and color changes to more bluish black
4th	Particle size increases and soft in consistency	Pellets were fragile and color was same as previous <i>puta</i>
5th	Duration of trituration decreases and pellets were made easily	Color of pellets were nearer to blackish red, that is, <i>pakwa jambu phala varna</i> and soft in consistency
6th	<i>Lauha bhasma</i> was very soft and quantity of media increases for trituration	Color was blackish red and pellets were easily breakable
7th	Again on trituration, color was black and very soft in consistency	Color was very much similar as previous <i>puta</i> and 15% <i>rekhapurnatva</i> test positive
8th	Color was bluish black before trituration	Pellets were easily broken by mild pressure of finger and color was blackish red. 20% <i>rekhapurnatva</i> passed
9th	<i>Lauha bhasma</i> was very soft in consistency	25% <i>rekhapurnatva</i> and other findings are same as previous <i>puta</i>
10th	More quantity of liquid media required for trituration and it was easily to make pellets	Hardness of pellets was increased. Color of pellets was turned to blackish red and black spots were found over few pellets. 35% <i>rekhapurnatva</i> passed
11th	<i>Bhasma</i> was brownish red color and became more <i>rekhapurnatva</i>	Pellets were still very hard and color was blackish red and 45% <i>rekhapurnatva</i> test positive
12th	Color was same and after trituration material becomes stickier	Pellets were hard and <i>bhasma</i> achieved 50% <i>rekhapurnatva</i> and 30% <i>varitar</i>
13th	<i>Bhasma</i> was blackish red colored and metallic taste present	Color maintained and pellets were mild hard
14th	Same as previous	Color was blackish red and <i>bhasma</i> achieved 60% <i>rekhapurnatva</i> and 40% <i>varitar</i>
15th	Quantity of liquid media increases for trituration	Pellets were little hard and blackish spots were found on the surface. Color of <i>bhasma</i> was blackish red and 50% <i>varitara</i> test positive
16th	After trituration material were stickier in nature and difficult to make pellets	Color of <i>bhasma</i> was completely blackish red, that is, <i>pakvajambuphalavarna</i> . 65% <i>rekhapurnatva</i> and 60% <i>varitara</i> test positive
17th	On trituration gritty appearance of <i>bhasma</i> was observed. Color maintained	Color of <i>bhasma</i> maintained and metallic taste present. 70% <i>rekhapurnatva</i> . Pellets were now soft
18th	Color of <i>bhasma</i> maintained and soft in consistency. On trituration quantity of media increases	75% <i>rekhapurnatva</i> and 70% <i>varitara</i> test positive. Metallic taste present
19th	Color and softness of pellets maintained	90% <i>rekhapurnatva</i> and 75% <i>varitara</i> test positive. Metallic taste absent
20th	Same as previous	<i>Bhasma</i> passed all <i>bhasma pariksha</i> with 95% <i>rekhapurnatva</i> and 80% <i>varitara</i> test positive

Table 6
Analysis of *Teekshna lauha bhasma* by ancient methods.

Test	<i>Teekshna lauha bhasma</i>
<i>Rekhapurnatvam</i>	The <i>Lauha bhasma</i> was rubbed in between index finger and thumb. It enters into the ridges of the finger – positive
<i>Varitaratvam</i>	A small amount of <i>Lauha bhasma</i> was carefully sprinkled on the water. It was found that 80% <i>bhasma</i> was floating on the water surface – positive
<i>Nirdhumatvam</i>	The <i>Lauha bhasma</i> was sprinkled on red hot coal. It did not emit smoke – positive
<i>Nischandratvam</i>	It was not having any lustre found positive
<i>Apunarbhava</i>	The <i>Lauha bhasma</i> was triturated with <i>Gunja</i> (<i>Abrus precatorius</i> L.), <i>Goghrita</i> (cow ghee), <i>Madhu</i> (honey), <i>Tankana</i> (Borax), <i>Guggulu</i> (<i>Commiphora wightii</i> [Arn.] Bhandari), and made pellets. Then it was get subjected to <i>puta</i> on 650 °C in EMF maintained for 1 h. Next day after self-cooling pellets were triturated and not any agglomeration was found

EMF = Electric muffle furnace.

Table 7
Physico-chemical analysis of *Teekshna lauha bhasma*.

Test	Result
Color	<i>Pakva jambu phala varna</i>
Taste	Tasteless
Texture	Amorphous
Loss on drying (%)	0.31
Ash value (%)	98.15
Acid insoluble ash (%)	27.50
Water soluble ash (%)	30.26
Particle size	100–500 nm

(indigestion), *Apasmar* (epilepsy), *Shotha* (oedema), *Napunsakata* (impotency), *Prameha* (diabetes), *Ashmari* (renal calculus), and even death also [32,33]. For the process of *samanya* and *vishesha shodhana*, the process of *nirvapa* (quenching) was adapted. It is one of the foremost step in the purification process of *T. lauha* in which the metal is heated up to red hot condition and immediately immersed in various plants and animal media (sesame oil, butter milk, cow urine, cow milk, etc.) and kept for self-cooling [34]. It removes the inorganic impurities and incorporates beneficial organic moieties into the metal which render them suitable for further process of preparation of *bhasma* (grinding with plant drug and repeated calcination) [35]. In this study, *T. lauha* (Fe turning) was heated till they were red hot and poured in different media, that is, *tila taila*, *takra*, *gomutra*, *kanji*, *kulattha kwatha*, and *Triphala kwatha* (7 times each). At each time, sufficient quantity of media was taken which was approximately 700 ml. The average temperature of the heating device and surface of container was 1000 °C and 950 °C, respectively. The average temperature of red hot Fe turning recorded in EMF was 900 °C. The use of a particular media and particular sequence is notable. The probable concept behind using such variation may be removal of impurities from the drug in a particular acidic or alkaline media and also reduction in particle size of drug [36]. After heating, immediate cooling in liquid media leads to decrease in tension and increase in compression force. Repetition in heating and cooling causes disruption in compression tension equilibrium and leads to increased brittleness, reduction in hardness, and finally reduction in the particle size [37]. After removing the hydrophobic impurities using sesame oil (*tila taila*) treatment, oxide scales are formed due to atmospheric oxidation

of the raw material, are removed by treatment with aqueous media viz., butter milk (*takra*), cow urine, rice gruel (*kanji*), and horse gram decoction, which are traditionally known for this property [25]. The formation of oxides scales are also responsible for increase in pH after every purification steps. Oxides (Fe oxides) are mainly basic in nature, so it can raise the pH after every quenching. In each of the steps in *samanya shodhana* (normal purification), progressive increase in surface area and reduction in particle size, probably due to micro cracks formed during heat treatment is observed. At the end of *shodhana* process weight gain was observed, it may be due to addition of contents of quenching media (Tables 1 and 2).

During the process of *bhanupaka*, *Triphala kwatha* was added to *vishesha shodhita lauha* and allowed to dry under sunlight. The role of sunlight during *bhanupaka* has very specific reason. It has been widely established that the metallic Fe is toxic [38]. Hence, Fe supplements should contain Fe in the form of complex. The ultraviolet radiation present in the sunlight reduces the oxidation state of Fe in the presence of Vitamin C present in the *Triphala* decoction thereby improving the bioavailability [39]. In this process, more time is available for the reaction between *Triphala* and *lauha churna*, it causes an increase in the weight of *lauha churna*, which may be due to the addition of solid contents of *Triphala kwatha*. In *sthalipaka*, *lauha* obtained after *bhanupaka* was mixed with *Triphala kwatha* in an Fe pan and intense heating was given up to complete dryness. After *sthalipaka* weight of *lauha churna* was decreased due to burning of solid contents of *Triphala kwatha* (Table 3).

Triphala mainly consists of tannins and ascorbic acid. The absorption of food Fe can be greatly influenced by other constituents in the diet, such as ascorbic acid (Vitamin C) and phenolics. Ascorbic acid increases the bioavailability of Fe by converting Fe^{3+} to Fe^{2+} , while phenolics can reduce the bioavailability of Fe by binding to its phenolics (e.g., tannins). Excess of ascorbic acid and/or a lack of dietary tannins have both been suggested as contributing to clinical/pathological Fe storage disease [36]. Five hundred milligram of Fe causes severe toxicity, leads to shock, metabolic acidosis, and liver damage [38]. In other words, this may also be taken as the various constituents of *Triphala* have antagonizing activity. Thus, too much Fe absorption is prevented. *Triphala* is a mild laxative and thereby counteracts the constipating property of Fe and thus be

Table 8
Results of XRF analysis showing elemental composition.

Element	Raw iron turning (%)	After <i>sthalipaka</i> (%)	After 10 <i>puta</i> (%)	After 20 <i>puta</i> (%)
Fe	98.10	58.18	80.92	70.26
Si	0.40	3.74	1.44	0.96
Al	0.13	1.26	0.49	0.31
Ca	0.074	0.30	1.32	1.50
Mn	0.75	1.36	1.77	1.63
Others elements ^a	0.54	35.16	14.06	74.66

^a Other elements are P, Cl, Ni, Ar, S, K, Tb, Sm, W, Dy, Cu, Zn, Gd, Co, Rb, Sr, Ti, Er, Ga, Y, and Na. XRF = X-ray fluorescence.

beneficial due to which ancient scholars of *Ayurveda* might have mentioned *Triphala* in maximum *lauha bhasma* preparations [13]. For the *putapaka* process instead of traditional method, to provide controlled and regulated heat and with a view of standardizing a modified EMF was used. *Lauha bhasma* were prepared on same temperature, that is, 650 °C and the duration of peak temperature was 1 h for all *puta*. The amount of liquid media (*Triphala kwatha*) for trituration increases after every successive *puta*, this may be because of decrease in particle size causes increases in surface area which are responsible for more absorption of *Triphala kwatha* during trituration (Table 4). [40] During first few *puta*, metallic luster was observed on the surface of pellets, later on it disappeared. Luster is the physical character of metal, when the metal transforms to compound form then its luster is lost. Appearance of luster after first few *puta* indicates *lauha* was still persistent in metallic form, later on it completely transformed to lusterless compounds. After trituration *cakrikas* (pellets) were formed and *sarava samputikarana* was done by earthen vessels that are responsible for homogeneous distribution of heating and also does not react with the material because of inert property of earthen vessel. After every *puta*, weight of *lauha bhasma* decreases due to burning of solid contents of *Triphala kwatha* (Table 4). After 5th *puta*, color was nearer to blackish red and pellets were fragile and soft. In the 7th *puta* 1st time *rekhapurnatva* (the *bhasma* enter the ridges of finger) test presence which shows the reduction in particle size of *bhasma*. In 10th *puta*, pellets were hard and after 11th *puta* color was almost blackish red, that is, *pakwa jambu phala varna* which suggests the formation of an entirely new compound. On 15th *puta*, it was observed that hardness of pellets decreases and *bhasma* passed 50% *varitara* (the *bhasma* floats on the still water surface) test. After 20th *puta* *bhasma* passed all *bhasma pariksha* with 95% *Rekhapurnata* and 80% *varitara* test and color of *bhasma* was blackish red, that is, *pakwa jambu phala varna* (Tables 5 and 6). The color of *lauha bhasma* is purple (*Pakwa jambu phala varna*), it may be considered as a mixture of ferrous oxide, ferrous sulphide, ferric oxide and other trace elements. Ferrous oxide and ferrous sulphide are black in color, and ferric oxide is red in color. Combination of all these compounds makes the *lauha bhasma* purple in color. Several physical and chemical parameters have been described for ascertaining the purity of *lauha bhasma* [19]. Physical parameters include luster, color, fineness, floatability, etc., while chemical parameters include test of *lauha bhasma* for irreversibility to metallic state, floatability taste, etc. [19] In this study, after quality check done by classical parameters (Table 6), *T. lauha bhasma* were subjected to different physico-chemical characterization studies using modern analytical tools. Our results showed negligible moisture content (0.31% loss on drying), total ash value (98.15%), water soluble ash (30.26%), and lower solubility in acid (27.50%) (Table 7). The results are comparable to the reported values [41]. The total ash value is useful in determining the purity of *bhasma* and indicates the absence of free organic moieties. During the preparation of *lauha bhasma*, large quantity of *Triphala* were added resulting in the formation of complexes between the constituents of *Triphala* and the metal. The formation of coordination compounds will be precluded however, if the *bhasmas* are not prepared properly, resulting in lower total ash content [41]. Lower acid-insoluble ash indicates higher bioavailability of the drug [42] and lower value of loss on drying indicates the absence of moisture in the drug. The results of XRF study revealed that, Fe present in the elemental form along with its impurities in the raw Fe turnings is converted into different forms of Fe oxide in final *bhasma* were estimated by X-ray diffraction [18,43]. Other impurities such as Si, Al, etc., are also

get oxidized after repeated trituration and calcination. *Triphala* which is used extensively in preparation of the *bhasma* for *puta* and also for trituration between *puta* consist of a number of minor elements such as Na, K, Mg, Ca, Cl, and P; and 23 trace Al, Ba, Br, Cd, Co, Cr, Cs, Cu, Fe, Eu, Hf, Hg, La, Mn, Ni, P, Pb, Rb, Sb, Se, Th, V, and Zn elements [18]. These get oxidized during *puta* and remain as integral part of the final *bhasma*. The SEM of 20 *puta T. lauha bhasma* showed irregular aggregates of various sizes and shapes with nano structure on the surfaces (100–500 nm) (Fig. 4) [17]. The role of nano structured materials as therapeutic agents has been reasonably established [44,45] and we also presumed that the efficacy of *lauha bhasma* may be attributed to the presence of nanostructures.

Limitations of the study: Particle size after each *puta* was not measured. It can be analyzed at regular intervals to provide leads for further studies.

Conclusion

Lauha bhasma preparation includes major steps such as *samanya shodhana* (normal purification), *vishesh shodhana* (special purification), and *trividh lauhapaka*, that is, *bhanupaka* (exposure to sunlight), *sthalipaka* (frying in an Fe pan), and *putapaka* (calcination). It is essential to follow all these procedures as per *Ayurvedic* guidelines to get good quality of *bhasma*. It is also very important to understand each and every step to set up the standards for *bhasma* preparation. This work has resulted in establishing standard manufacturing procedure of *Teekshna lauha bhasma* (calc of Fe turning) by adapting conventional method of *puta* using EMF. This study revealed that a temperature of 650 °C with peak duration of temperature 1 h in 20 *puta* is sufficient to obtain purple colored, that is, *pakwa jambu phala varna Teekshna lauha bhasma* which passed the *bhasma parikshas*. Our results, also evaluated the physico-chemical properties of *Teekshna lauha bhasma*, based on AYUSH guidelines, as well as modern analytical tools.

Source of support

None.

Conflicts of interest

None declared.

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References

- [1] Bhanu P. Use of metals in ayurvedic medicine. *Indian J Hist Sci* 1997;32:1–28.
- [2] Sushruta. *Sushruta Samhitaa Sutra Sthana* (Doshadhatumalakshayavidhi Vigyaniya) Verse- 15/ 48 *Ayurved Tatva Sandipikahindi commentary* by Kaviraja Ambika Dutta Shastri. 11th ed. Varanasi: Chaukhamba Sanskrit Sansthan; 2010. p. 84.
- [3] Galib, Barve M, Mashru M, Jagtap C, Patgiri BJ, Prajapati PK. Therapeutic potentials of metals in ancient India: a review through Charaka Samhita. *J Ayurveda Integr Med* 2011;2:55–63.
- [4] Gogtay NJ, Bhatt HA, Dalvi SS, Kshirsagar NA. The use and safety of non-allopathic Indian medicines. *Drug Saf* 2002;25:1005–19.
- [5] Rasa Vagbhatta, Rasa Ratna Samuccaya, commentary by Siddhinandan Mishra, Verse 28/1. Varanasi, India: Choukhambha Orientalia; Reprint 2011. p. 633.

- [6] Sarkar PK, Chaudhary AK. Ayurvedic bhasmas: the most ancient application of nano medicine. *J Sci Ind Res* 2010;69:901–5.
- [7] Rajendran N, Pemiah B, Rajan KS, Krishnan UM, Sethuraman S, Krishnaswamy S. Role of gallic acid in the preparation of an iron-based Indian traditional medicine – Lauhabhasma. *Int J Pharm Pharm Sci* 2012;4:45–8.
- [8] Nagarajan S, Pemiah B, Krishnan UM, Rajan KS, Krishnaswamy S, Sethuraman S. Physico-chemical characterization of lead based Indian traditional medicine – Naga bhasma. *Int J Pharm Pharm Sci* 2012;4:69–74.
- [9] Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. *J Res Med Sci* 2014;19:164–74.
- [10] Sarkar PK, Prajapati PK, Choudhary AK, Shukla VJ, Ravishankar B. Haematinic evaluation of lauha bhasma and mandur bhasma on HgCl₂ – induced anaemia in rats. *Indian J Pharm Sci* 2007;69:791–5.
- [11] Pandit S, Biswas TK, Debnath PK, Saha AV, Chowdhury U, Shaw BP, et al. Chemical and pharmacological evaluation of different ayurvedic preparations of iron. *J Ethnopharmacol* 1999;65:149–56.
- [12] Tambekar DH, Dahikar SB. Screening antibacterial activity of some bhasma against enteric pathogens. *Recent Res Sci Technol* 2010;2:59–62.
- [13] Gupta KL, Pallavi G, Patgiri BJ, Galib, Prajapati PK. Critical review on the pharmaceutical vistas of Lauha Kalpas (iron formulations). *J Ayurveda Integr Med* 2012;3:21–8.
- [14] Vagbhatta Rasa. *Rasa Ratna Samuccaya*, commentary by Siddhinandan Mishra, Verse 5/96. Varanasi, India: Choukhambha Orientalia; 2011. p. 163.
- [15] Rasa Vagbhatta. *Rasa Ratna Samuccaya*, commentary by Siddhinandan Mishra, Verse 5/98–132. Varanasi, India: Choukhambha Orientalia; Reprint 2011. p. 164–168.
- [16] Sharma S. *Rasa Tarangini*, 20/15–44. 11th ed. New Delhi: Motilal Banarasidas; Reprint 2012. p. 494–500.
- [17] Bhargava SC, Prakash R, Sastry GVS, Reddy KRC. Pharmaceutical standardization and characterization of Lauha bhasma. MD Ayurveda thesis. Varanasi: Banaras Hindu University, Department of Rasa Shastra Institute of Medical Sciences; 2010. p. 143–5.
- [18] Bhargava SC, Reddy KR, Sastry GVS. Identification studies of Lauha bhasma by X ray diffraction and X ray fluorescence. *AYU* 2012;33:143–5.
- [19] Anonymous. *Ayurvedic formulary of India*. Department of Ayush, Ministry of H and FW; 2003. p. 624–8. Part 1. 2nd ed. India: Government of India.
- [20] Anonymous. *Ayurvedic pharmacopoeia of India*. Department of Ayush, Ministry of H and FW; 2007. p. 214. Part 2. 2nd ed., Vol. 1. India: Government of India.
- [21] Anonymous. *Ayurvedic pharmacopoeia of India*. Department of Ayush, Ministry of H and FW; 2007. p. 213. Part 2. 2nd ed., Vol. 1. India: Government of India.
- [22] Sushruta. *Sushruta Samhitaa Sutra Sthana (Dravdravya Vidhi) Verse- 45/ 85 Ayurved Tattva Sandipika Hindi commentary by Kaviraja Ambika Dutta Shastri*. 11th ed. Varanasi: Chaukhamba Sanskrit Sansthan; 1997. p. 227.
- [23] Anonymous. *Ayurvedic pharmacopoeia of India*. Department of Ayush, Ministry of H and FW; 2007. p. 249. Part 2. 2nd ed., Vol. 1. India: Government of India.
- [24] Sharangdhar, Sharangdhar Samhita, Madhyama Khanda, Verse 2/1 Jiwanprada Hindi commentary by Shailaja Srivastava. Varanasi: Choukhambha Orientalia; Reprint 2009. p. 135.
- [25] Krishnamachary B, Rajendran N, Pemiah B, Krishnaswamy S, Krishnan UM, Sethuraman S, et al. Scientific validation of the different purification steps involved in the preparation of an Indian ayurvedic medicine, Lauha bhasma. *J Ethnopharmacol* 2012;142:98–104.
- [26] Sharma S. *Rasa Tarangini*, 20/39. 11th ed. New Delhi: Motilal Banarasidas; Reprint 2012. p. 500.
- [27] Singh TR, Gupta LN, Kumar V, Kumar N. Characterization of an ayurvedic drug (Shilajatwadi Lauha): an approach to standardization. *Int J Res Ayurveda Pharm* 2014;5:424–7.
- [28] Wadekar MP, Rode CV, Bendale YN, Patil KR, Prabhune AA. Preparation and characterization of a copper based Indian traditional drug: Tamra Bhasma. *J Pharm Biomed* 2005;39:951–5.
- [29] Subbarayappa BV. Siddha medicine: an overview. *Lancet* 1997;350:1841–4.
- [30] Sharma S. *Rasa Tarangini*, 20/7. 11th ed. New Delhi: Motilal Banarasidas; Reprint 2004. p. 489.
- [31] Sharma S. *Rasa Tarangini*, 20/8. 11th ed. New Delhi: Motilal Banarasidas; Reprint 2004. p. 491.
- [32] Vagbhatta Rasa. *Rasa Ratna Samuccaya*, commentary by Siddhinandan Mishra, Verse 5/97. Varanasi, India: Choukhambha Orientalia; 2011. p. 163.
- [33] Mishra Siddhinandan. *Ayurvediya Rasashastra*. Varanasi, India: Choukhambha Orientalia; 2013. p. 453.
- [34] Sharma S. *Rasa Tarangini*, 2/40. 11th ed. New Delhi: Motilal Banarasidas; Reprint 2012. p. 19.
- [35] Devanathan R. Concept of Bhasmikaran. *Int J Res Ayurveda Pharm* 2011;2: 18–23.
- [36] Singh N, Reddy KR. Pharmaceutical study of Lauha Bhasma. *AYU* 2010;31: 387–90.
- [37] Sarkar P, Choudhary AK, Shukla VJ, Ravishankar B, Prajapati PK. A comparative pharmaceutico-pharmaco-clinical study of Lauha Bhasma and Mandoora Bhasma w.s.r. to its Panduhara effect. MD dissertation. Jamnagar, Gujarat: Department of RS and BK Including Drug Research; 2005. p. 234. IPGT&RA, G Ay U.
- [38] Goyer RA, Klaassen CD, Amdur MO, Doull J, editors. *Toxic Effects of Metals. Toxicology*. 3rd ed. New York: McGraw Hill; 1986. p. 842–3.
- [39] Krishnamachary B, Purushothaman AK, Pemiah B, Krishnaswamy S, Krishnan UM, Sethuraman S, et al. Bhanupaka: a green process in the preparation of an Indian ayurvedic medicine, Lauha Bhasma. *J Chem* 2013;2013: 1–8.
- [40] Singh TR, Gupta LN, Kumar V, Singh RS, Kumar N. Comparative pharmaceutical and pharmacological evaluation of Shilajatwadi Lauha and modified Shilajatwadi Lauha: a preclinical study. MD Ayurveda thesis. Varanasi: Banaras Hindu University, Department of Rasa Shastra Institute of Medical Sciences; 2014. p. 162–84.
- [41] Krishnamachary B, Pemiah B, Krishnaswamy S, Krishnan UM, Sethuraman S, Sekar RK, et al. Elucidation of a core shell model for Lauha bhasma through physio-chemical characterization. *Int J Pharm Pharm Sci* 2012;4:644–9.
- [42] Chaudhary A, Prakash B. Scientific validated approach for application of Mandura bhasma a review. *Electron J Pharmacol Ther* 2010;3:35–40.
- [43] Singh N, Reddy KR, Prasad NK, Singh M. Chemical characterization of Lauha bhasma by X ray diffraction and vibrating sample magnetometry. *Int J Ayurvedic Med* 2010;1:143–9.
- [44] Rathod KB, Patel MB, Parmar PK, Kharadi SR, Patel PV, Patel KS. Glimpses of current advances of nanotechnology in therapeutics. *Int J Pharm Pharm Sci* 2011;3:8–12.
- [45] Lakshmi NR, Swaminathan S, Udaykumar R, Uma MK. Development of a liposomal nanodelivery system for nevirapine. *J Biomed Sci* 2010;17:57.