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Original Research Article

The chemical role of natural substances used in *Lauha Bhasma* preparation process

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ABSTRACT

Lauha Bhasma (LB) is a prominent Ayurveda medicine and uses as an ingredient to prepare other indigenous medicines in Ayurveda. The outcomes of this study on chemical and physical changes during the preparation process of LB become significant to explore ancient knowledge of east within the modern context. The preparation process of LB was carried out under laboratory conditions; starting from the elemental form of the Iron sample to identify the chemical and physical changes. The metallic composition of the starting material and intermediate products formed during the LB preparation process was determined using the AAS technique. The variation of the amount of Fe^{2+} and Fe^{3+} throughout the process and formation of nanoparticles was identified using quantitative analysis. Even though the amount of heavy metals (Cr, Cd, Cu, Pb, Zn, and Mn) present in the starting material is low, the trace level of heavy metals in the iron sample significantly reduces during the LB preparation process. Irregular-shaped, agglomerated, blackish red (Pakvajambuphala varna) fine LB powder formed at the end of the Putapaka step. The value of ancient knowledge can be revealed using the chemical and physical changes identified throughout the study on the LB preparation process.

1. Introduction

Rasa Shastra is a specialized branch of *Ayurveda*. Metals, minerals, and plant parts were frequently used for medicine preparation in *Ayurveda*. Mineral and metal-based medicinal preparations are described under *Rasa Shastra* [1,2]. *Rasa Shastra* is dominated by descriptions of mercury processing techniques. Therefore, the word "*Rasa*" is considered as a synonym of "mercury" [1]. Apart from mercury the utility of metals such as gold, silver, copper, tin, lead and iron, along with salts, corals, seashells, and feathers is also described in *Rasa Shastra* [3,4]. These mineral and metals require specific preparation processes before they can be used for treatments because mineral and metal ingredients in their natural form can be harmful to human body. However, most of the animal and plant products that are used in *Ayurveda* can be used for human body treatments without following any specific preparation methods [5].

Lauha Bhasma (LB) is a nano iron particles based medicine used in *Ayurveda* [6]. Iron is a crucial element for human body, its key functions include, oxygen transport and electron transport. Apart from this iron is a component of many enzymes, and iron nanoparticles can actuate the human immune system [7]. *Lauha Bhasma* was used for the treatement of many diseases such as anemia, hematochezia, liver disorders, worm infestation, dry skin, irregular fat metabolism, heart disease, pica, rhinitis, etc. [4,8] *Lauha Bhasma* is utilized to burn excess fat in the human body, including belly fat and central obesity because it has *Lekhana* (scraping or corrosive action) [9]. Anemia can occur due to the lack of red blood cells adequacy in the body or due to bleeding. Iron deficiency anemia is a common type of anemia that can be related to the poor intake of iron-rich foods. *Lauha Bhasma* medicine was used to treat anemia in Ayurveda because LB contains highly absorbable nano-range iron particles [10]. *Lauha Bhasma* is recommended to utilize for bleeding with other remedies including Raktachandanadi Kwath, Mochras because LB can compensate for the iron loss during bleeding [11]. Antibacterial nature of LB

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medicine for several bacterial strains, including *Salmonella typhimurium*, *Pseudomonas aeruginosa*, etc. was published by Tambekar and Dahikar [12].

Kanta lauha or *Teekshana lauha* can be used as the main iron source for the LB preparation process. Among them, *K. lauha* has been reported as the best material for the LB preparation process. However, due to lack of its availability, *Teekshana Lauha* is commonly used for the LB preparation process. Iron sources would contain miniscule amounts of some other metals, and the presence of these metals (heavy metals) can cause harmful effects on the human body. Consequently, *Ayurveda* medicine preparations follow *Shodhana* methods (purification techniques) to eliminate or decrease the amount of disease-causing minor metals [13]. *Shodhana* is an important concept in *Ayurveda*. Approximate meaning of the *Shodhana* is purification. But, *Shodhana* process changes the starting materials beyond the purification. Ayurveda has mentioned several *Shodhana* methods that can apply in different conditions. These methods include *Prakshalana* (Washing), *Swedana* (Boiling), *Mardana* (Trituration), *Bhavana* (Levigation), *Nirvapa* (Heating and quenching in specific liquids), etc. Among all these *Shodhana* methods, the LB preparation process requires the *Nirvapa Shodhana* method [14,15].

The LB preparation process contains four main steps, the first step is called *Samanya Shodhana,* the second step is called as *Vishesha Shodhana*, the third step is called as *Bhanupaka Sthalipaka* and the final step is called as *Putapaka* [4,8]. *Samanya Shodhana* is a generally application procedure for ingredients that belong to a particular category. *Vishesh Shodhana* is a specific application procedure for specific drug materials, but not for a particular group [16]. *Bhanupaka* means exposure of the herbo-metallic mixture to the sunlight and *Sthalipaka* means roasting the herbo-metallic drugs in an iron vessel. Approximate meaning of *Putapaka* is calcination [17]. Traditional *Putapaka* process used cow dung containing holes for the controlled heating of herbo-metallic mixtures. Industries currently use electric muffle furnace for the controlled heating of herbo-metallic mixtures after the standardization of the traditional *Putapaka* process [18].

Ayurveda literature advocates the use of six different media for the *Samanya Shodhana,* and *Visesha Shodhana* steps in the LB preparation process. Sesame oil (*Tila Taila*), buttermilk (*Takra*), cow's urine (*Gomutra*), sour gruel (*Kanji*), and Horse gram (*Kulattha Kwatha*) decoctions were recommended to use respectively for the *Samanya Shodhana* step. *Triphala Kwatha* was specifically recommended to use at the *Vishesha Shodhana* step of the LB preparation process [19,20]. *Triphala* means three fruits and *Triphala Kwatha* contains an equal amount of three fruits. *Emblica officinalis*, *Terminalia bellirica*, and *Terminalia chebula* are the three fruits that use to make *Triphala Kwatha* [21]. In literature, *Triphala Kawatha* is recommended to be used as a mixing solution for *Bhanupaaka Sthalipaaka* and *Putapaka* steps.

Sesame seeds are used for Sesame oil preparation and Sesame oil contains 5.7% of moisture, 20% of crude proteins, 3.2% crude fiber, 54% fat (oleic, linoleic, palmitic, and stearic) and 13.4% carbohydrate [22]. Buttermilk is prepared using curd and water. Curd contains Carbohydrates (primary lactose, glucose, and galactose), inorganic ions (potassium and calcium), organic acid (citrate), and amine-containing compounds (Creatinine, choline). Additionally, minor quantities of vitamins and triglycerides also contained in the curd [23]. Cow's urine contains water 95%, salts 2.5% (sodium, potassium, magnesium and sulphate), and 2.5% urea (uric acid, uroporphyrin, leucocyte) [24]. Horse gram seed contains carbohydrate (57.2%), protein (22%), dietary fiber (5.3%), vitamins (thiamine, riboflavin, and niacin) [25]. *Triphala Kwatha* contains tannins, quinone, flavones, flavonoids, flavonols, gallic acid, vitamin C [26]. All different media used in the LB preparation process have coordinate covalent bond forming molecules that can form bonds with metals (metal complexes) [27].

All the previous studies of the LB preparation process have reported the characterization of the final product [28,29]. The importance of the *Shodhana* steps for the LB preparation process has not been studied till date. The aim of the present study was to identify the chemical changes that have taken place during the LB preparation process and to understand the chemical role of natural substances used in the LB preparation process.

2. Material and Methods

2.1. Materials

Teekshana lauha (Iron) sample (purity > 90%) was obtained from the D. Peiris & Co. Ltd., who is the main supplier of medicinal herbs, *Ayurveda* and *Unani* drugs to the University of Colombo, Institute of Indigenous medicine at Rajagiriya. Sesame oil, *Macrotyloma uniflorum* seeds for *Kulattha Kwatha* and *E. officinalis*, *T. bellirica*, and *T. chebula* seeds for *Triphala Kwatha* was collected from D. Peiris & Co. Ltd. Cow urine (*Gomutra*) was collected from a dairy farm. This research was completed at the University of Colombo, Institute of Indigenous medicine at Rajagiriya, and University of Sri Jayewardenepura, Nugegoda. All the other chemicals and reagents used for the experiment were of analytical grade.

2.2. Laboratory preparation of Lauha Bhasma

2.2.1. Preparation of thakra solution

An equal amount of curd and water was mixed using a mixer grinder. After proper mixing of the solution, the whole mixture was transferred into a separate container and used at the *Samanya Shodhana* step.

2.2.2. Preparation of kanji solution

Organic brown rice and Masha (Black gram) were cooked by adding a sufficient amount of water. Subsequently, small pieces of raddish were added to the cooked rice and Masha. Then, the whole material was transferred into a plastic container which can tightly close and kept 15–20 days for fermentation. After the fermentation process, completion tests were conducted to obtain whitish color Kanji, the pH of the solution was 4.10.

2.2.3. Preparation of Kulattha Kwatha solution

Around 2 Kg of *Kulattha* and 16 L of water were added into an iron container, and it was gradually heated and allowed to boil until volume of the medium reduced to 1/4th part of the original volume.

2.2.4. Preparation of Triphala Kwatha solution

Amla, Haritaki, and Bibhitaki (three fruits) were used for the *Triphala Kwatha* preparation. Around 660 g of each fruit (total mass of three fruits was around 2 Kg) and 16 L of water was added into an iron container and the mixture was boiled by gradual heating till around 4 L of mixture remained.

2.2.5. Lauha Bhasma preparation process

Teekshana lauha (iron) samples weighted 100 g were placed in the clay pots and heated up to 850 °C using an electric muffle furnace (EMF) until the iron samples were red hot [12]. Then the iron samples were quenched in *Tila Taila* solution (500 mL). Thereafter, the iron mixtures were filtered using iron mesh. Subsequently, the filtered mixtures were again heated up to 850 °C using EMF. After that, the same procedure was repeated six more times using new *Tila Taila* solutions (a total of seven times quenching in *Tila Taila*). Thereafter, the above procedure was repeated for buttermilk (*Thakra*) for seven times, Cow's urine (*Gomuthra*) for seven times, *Kanji* for seven times, and *Kulattha Kwatha* for seven times.

For the *Vishesha Shodhana* step, *Triphala Kwatha* (A mixture of three *Ayurveda* medicinal fruits) was used and the red hot herbo-metallic samples were again quenched seven times in new *Triphala Kwatha* solutions.

After completing the *Shodhana* steps, *Lauha Churna* (herbo-metallic iron mixture) collected after the *Vishesha Shodhana* step was kept in a tray and 100 mL of *Triphala Kwatha* was added until completely immersed in the *Lauha Churna*. Subsequently, the mixture was mixed together uniformly and dried in sunlight till the mixture was completely dry. The same procedure was repeated six more times (A total of seven times). For the *Sthalipaka* process, dried *Lauha Churna* was transferred into an iron container and 100 mL *Triphala Kwatha* was added into the same iron container. Subsequently, the iron container was kept on the heating device and heated till the complete evaporation of *Triphala Kwatha*'s liquid content. This procedure was repeated six more times (A total of seven times).

During the *Putapaka* step, *Lauha Churna* that was collected after the *Sthalipaka* process was triturated using *Triphala Kwatha*. Then, the triturated mixture was used to make pallets (*chakrika*) and prepared pallets were dried til the moisture content was completely evaporated. Thereafter, dried pallets were collected into a clay pot and placed uniformly. Subsequently, clay pots containing pellets were covered using another clay pot of the same size and sealed using clay and a cotton cloth. Finally, the sealed pots were placed in the Muffle furnace and the temperature was allowed to gradually rise to 600 °C in 2 hours and maintained at 600 for 1 hour [17,18]. Afterward, EMF was switched off and the pellets were allowed to come to room temperature. The above mentioned procedure for the *Putapaka* process was repeated up to 30 times to obtain *Lauha Bhasma* of the desired quality. At the end of 30 cycles, color test, floating test (*Varitaratvam*), and *Rekhapurnatava* test were conducted for the synthesized LB powder [17,4].

A control experiment was conducted parallel to the LB preparation process. *Teekshana lauha* (100 gm) was used as the iron source and deionized water was used at each step instead of different media.

The metallic composition of the starting iron sample, control samples, and intermediate products formed after every step was determined using the AAS technique [31]. The chemical composition of the intermediate products formed after every stage was determined using Powder X-Ray diffractometer (PXRD). Formation of nanoparticles at the final step was determined using SEM.

2.3. Powder X-Ray diffractometer (PXRD)

Physical and chemical parameters of intermediate products at every step were determined using PXRD. Rigaku X-ray powder diffractometer at the University of Sri Jayewardenepura was used for the purpose. The radiation source of it is Cu Kα radiation (wavelength -1.540 Å) over 2θ angle, of 20^0 to 80^0 with a step size of 0.02^0 [30].

2.4. Atomic absorption spectrometry (AAS)

Concentrations of major metal (Fe) and minor metals (Mn, Pb, Cu, Cd, Cr, and Zn) were analyzed using the Atomic Absorption Spectrometer with flame and graphite furnace mode (Thermo Scientific iCE 3000). Heavy metals and other disease-causing metals were considered during the determination of other metals in the iron sample [31].

2.5. Scanning electron microscope (SEM)

Hitachi SU 6600 scanning electron microscope at the University of Moratuwa was used for the analysis. Samples were analyzed in the secondary electron mode. All samples were coated with gold prior to analysis [32].

2.6. Determination of Fe^{2+} concentration in the intermediate solid iron samples

Exactly 0.5 g of intermediate solid iron sample was measured and it was dissolved in10.00 cm^3 of conc. H_2SO_4. The mixture was transferred to a 25.00 cm^3 volumetric flask and volume was adjusted to the full capacity with distilled water. The above solution was transferred to a 250.00 mL Erlenmeyer flask. Followed by addition of 50.00 mL of distilled water and 12.00 mL of 6.0 mol dm^{-3} H_3PO_4 to the Erlenmeyer flask. Lastly, iron samples were titrated using a standardized $KMnO_4$ solution, until a faint pink color was observed at the endpoint. Above mentioned procedure was repeated twice [33,34].

3. Result

3.1. Determination of metallic composition of Teekshana lauha using AAS technique

The average metallic composition (w/w %) of the starting iron sample (*Teekshana lauha*) that used at the LB preparation process is summarized in Table 1.

3.2. Determination of metallic compositions of the intermediate products using the AAS technique

The metallic compositions of the intermediate products formed at the *Samanya Shodhana*, *Vishesha Shodhana* and *Bhanupaka Sthalipaka* processes are summarized in Table 2. Relative metals reductions at the *Samanya Shodhana*, *Vishesha Shodhana* and *Bhanupaka Sthalipaka* process are summarized in Table 3. Sample digestion was conducted for all the intermediate samples because all samples contained organic materials. Final product obtained from the *Putapaka* step wasn't dissolve in the aqueous acid media.

3.3. PXRD analysis of intermediate iron samples at the Samanya Shodhana step

Chemical compositions of the starting iron sample (Teekshana Lauha) were determined using PXRD technique. The spectrum of starting iron sample was shown in Fig. 1 (A). The intermediate iron samples after have been quenched in different five media, were analyzed using the PXRD technique and its PXRD spectra are shown in Fig. 1 (B–F).

3.4. PXRD analysis of intermediate iron samples at the Vishesha Shodhana and Bhanupaka Sthalipaka processes

Fig. 2 contains the chemical compositions of intermediate iron products formed in the *Vishesha Shodhana* and *Bhanupaka Sthalipaka* steps in the LB preparation process.

3.5. PXRD analysis of the synthesized LB powder at the putapaka step

The PXRD spectrum of the final product (Synthesized LB powder) has shown in Fig. 3 (A). A synthesized product was obtained at

Table 1
AAS analysis data of raw iron sample.

Fe %	Cu%	Cr%	Cd% 10^{-4}	Mn%	Zn%	As%	Pb% 10^{-3}	Acid insoluble mass %
91.33	0.103	0.0985	8.43	0.642	0.01	Not present	1.17	7.52

Table 2
Average mass percentages of metals contained in the *Teekshana lauha* sample and intermediate solid products.

Raw/After 7 times Quenched in media	Average mass percentage of metals						
	Fe (%)	Cu (%)	Cr (%)	Cd (%) 10^{-4}	Mn (%)	Zn (%) 10^{-2}	Pb (%) 10^{-3}
Teekshana lauha sample	91.33	0.103	0.0985	8.438	0.642	1.0	1.17
Tila Taila	91.32	0.074	0.0812	6.196	0.549	0.92	0.874
Thakra	91.31	0.052	0.0796	5.546	0.505	0.823	0.761
Gomuthra	91.29	0.048	0.0766	4.052	0.483	0.811	0.666
Kanji	91.26	0.044	0.0747	0.568	0.478	0.762	0.592
Kulattha Kwatha	91.25	0.042	0.0720	0.417	0.464	0.688	0.476
Triphala Kwatha	91.22	0.039	0.0711	0.391	0.459	0.647	0.464
Bhanupaka	91.22	0.038	0.0711	0.390	0.457	0.644	0.461
Sthalipaka	91.21	0.037	0.0708	0.387	0.455	0.637	0.459

Table 3
Relative metals reductions at the LB preparation process.

After 7 times Quenched in media	Reduced metal percentages						
	Fe (%)	Cu (%)	Cr (%)	Cd (%)	Mn (%)	Zn (%)	Pb (%)
Tila Taila	0.011	28.15	17.56	26.57	14.48	8.0	25.29
Thakra	0.021	49.51	19.18	34.27	21.33	17.70	34.95
Gomuthra	0.043	53.39	22.23	51.97	24.76	18.90	43.07
Kanji	0.077	57.28	24.16	93.26	25.54	23.80	49.40
Kulattha Kwatha	0.087	59.22	26.90	95.05	27.72	31.20	59.31
Triphala Kwatha	0.120	62.13	27.81	95.36	28.50	35.30	60.34
Bhanupaka	0.120	63.11	27.82	95.37	28.82	35.6	60.60
Sthalipaka	0.131	64.08	28.12	95.41	28.82	36.3	60.77

the end of all steps called a *Lauha Bhasma* (LB) and synthesized LB powder was compared with the commercially available LB powder. The spectrum of the commercially available LB powder is shown in Fig. 3 (B).

3.6. SEM analysis at the putapaka step

Nature and particle size of the fine powder which formed at the *Putapaka* process were determined using SEM technique. Magnified SEM image of the synthesized LB powder has shown in Fig. 4 (A) and the magnified SEM image of commercially available LB powder has shown in Fig. 4 (B).

3.7. Quantitative analysis of iron oxidation (oxidation of Fe^{2+}) at the LB preparation process

Table 4 shows the variation of Fe^{2+} percentage throughout the LB preparation process.

4. Discussion

The metallic iron sample (*Teekshana lauha*) was used as the major ingredient for the LB preparation. Weight of the intermediate products slightly increases during the samanya shodhana and vishesha shodhana. However the weight of intermediate increases significantly during the Bhanupaka Sthalipaka process, it may be due to the incorporation of organic materials. Most significant observation was the reduction of the mass of heavy metals during the major steps. Therefore, the metallic composition of the starting iron turnings was determined using Atomic Absorption Spectrometry (AAS). According to AAS results in Table 1, iron turnings have shown to contain Cu, Cr, Cd, Mn, Zn, and Pb metals as minor metals and As was not present in the iron turnings. Among these minor metals, Mn was present in the highest percentage and Cd in the the lowest percentage. Metallic compositions of the intermediate products formed during the LB preparation process were determined using AAS (Table 2). AAS results have confirmed a significant reduction of the minor metal concentrations during the first two steps of the LB preparation process. Using these values, relative reduction percentages of metallic impurities were calculated concerning the w/w% of the metallic components in the starting iron sample (Table 3). Cd has shown the highest reduction during the LB preparation process. The reduction percentages of Cu and Pb were similar. Reduction percentages of Mn, Zn, and Cr were reasonably low compared to the reduction percentages of Cu and Pb. The chelation effect of organic solutions and sudden changes in temperature are the potential causes for the efficient elimination of minor metals during the *Shodhana* steps. The quenching process can generate sufficient (free energy) driving force for the massive structural transformation of the iron sample [35]. Assuming, organic solutions which were used at the first two steps may accelerate the transformation and it may also cause for the removal of metallic impurities from the iron sample. Besides, micro-cracks can form on the metal surfaces during the sudden heat change (during the quenching) and these cracks can increase the surface area for reaction with organic solutions.

The chemical and physical nature of the *Teekshana lauha* sample was determined using powder X-Ray diffraction spectroscopy (PXRD). Peaks of the PXRD spectrum of the iron sample appeared at 30.092°, 35.37°, 42.5°, 44.65°, 56.968° 2-theta (deg). PXRD spectrum of the starting iron sample is shown in Fig. 1 (A)

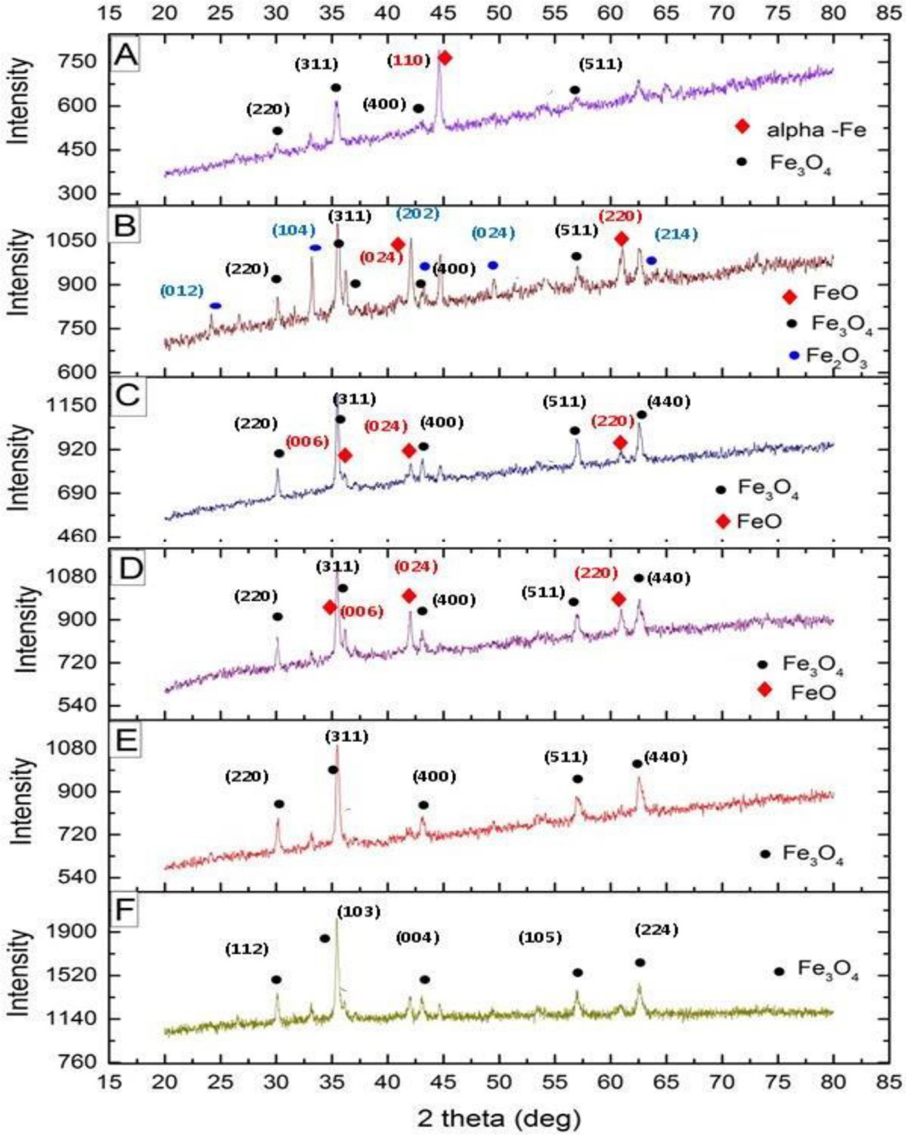

Fig. 1. PXRD spectra of solid iron samples after being quenched in each organic solutions at *Shodhana* step (A) Starting iron sample (B) Solid iron sample after been quenched in *Tila Taila* (C) Solid iron sample after been quenched in *Thakra* (D) Solid iron sample after been quenched in *Gomuthra* (E) Solid iron sample after been quenched in *Kanji* (F) Solid iron sample after been quenched in *Kulattha Kwatha*.

and it contains high and low intense sharp peaks. It confirmed that the iron sample is in a crystalline form. Furthermore, the raw iron sample was not in a fine powder form and its physical nature may have caused an ascent of the PXRD spectrum [36]. PXRD spectrum of the starting iron samples has shown that it contains a mixture of magnetite (Fe3O4) and alpha- Fe. Peaks appeared at 30.092°, 35.37°, 42.5°, and 56.968° matched with magnetite (ICDD card number is 01-089-4319) and peak appeared at 44.65° matched with alpha- Fe (ICDD card number is 03-065-4899). Alpha-Fe is an allotrope of iron at low temperature and it has a body-centered cubic (BCC) lattice structure [37].

When comparing the PXRD spectra in the *Samanya Shodhana*, the alpha-Fe peak contained in the *Teekshana lauha* sample at 44.65 ^{0} has disappeared after the quenching in *Tila Taila* solution. Moreover, the spectrum of the intermediate iron sample after being dipped in sesame oil was found to contain a mixture of FeO (compound ICDD card number 01-089-0690), Fe_3O_4 (compound ICDD card number 01-079-0417), and Fe_2O_3 (compound ICDD card number 01-071-5088) (Fig. 1). Quantitative analysis of PXRD results has confirmed that the intermediate iron sample after being dipped in *Tila Taila* contained 19% of hematite, 26% of iron oxide, and 55% of magnetite. The intermediate iron sample after dipping in the *Thakra* solution was shown to contain a mixture of iron oxides. It contained 65% of magnetite (compound ICDD card number 01-074-1910) and 35% of iron oxide (compound ICDD card number 01-073-2145). The spectrum obtained of the solid iron sample which was dipped in *Gomuthra* solution is shown a mixture of iron oxide (compound ICDD card number 01-089- 0689) and magnetite (compound ICDD card number 01-074-0748). But the spectrums of solid iron samples after being dipped in *Kanji* and *Kulatta Kwatha* were confirmed to be magnetite by the chemical nature (compounds ICDD card numbers were 01-086-1341 and 01-077-1545 respectively).

When comparing the PXRD spectra at the *Vishesha Shodhana* and the *Bhanupaka* steps, solid iron samples were confirmed to be magnetite by the chemical nature (Fig. 2) (Compounds ICDD card numbers were 01-086-1341, 01-086-1340 respectively). The

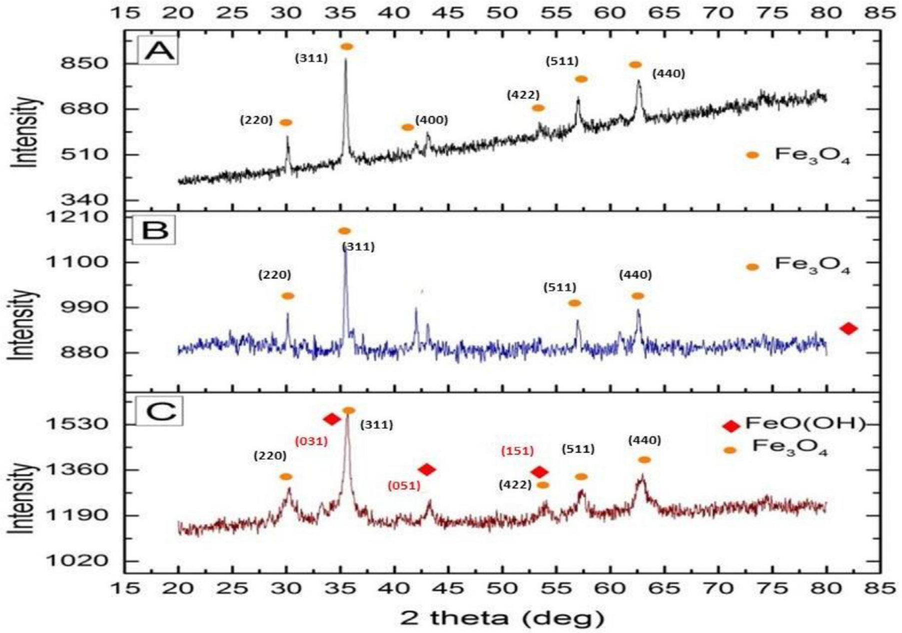

Fig. 2. PXRD spectra of solid intermediate product formed at the *Vishesha Shodhana* and *Bhanupaka Sthalipaka* steps (A) Solid iron sample after been dipped in *Triphala Kwatha* (Second step)(B) Solid iron sample after exposed to the sunlight (*Bhanupaka*) (C) Solid iron sample after exposed to the Furnace heat (*Sthalipaka*).

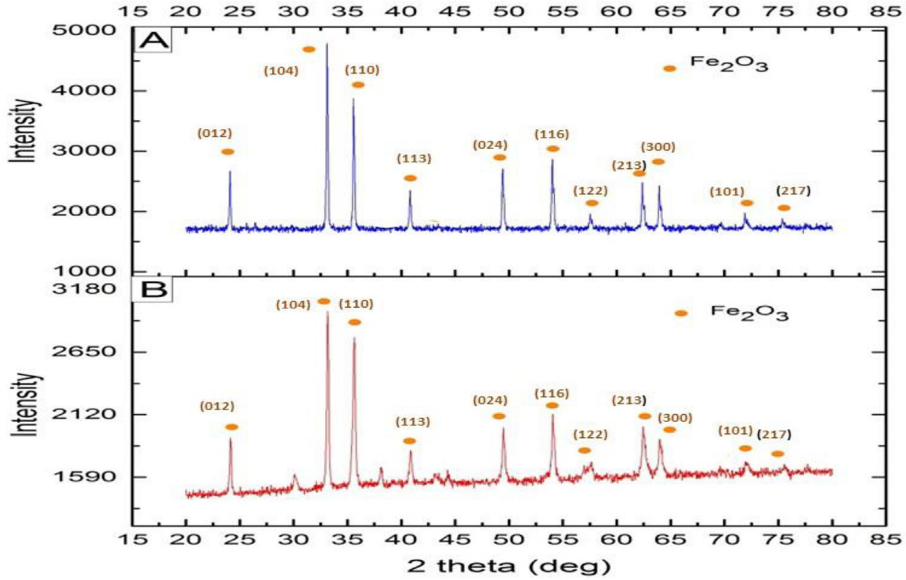

Fig. 3. PXRD spectra at the *Putapaka* step of LB preparation process (A) Iron sample after the *Putapaka* process (Synthesized LB) (B) Commercially available LB.

spectrum of the solid intermediate product obtained after exposure to the furnace heat at the *Sthalipaka* step was confirmed to be a mixture of magnetite and iron (III) oxide hydroxide (Compounds ICDD card numbers were 01-076-7165 and 01-074-3080 respectively). Several chemicals can label as iron (III) oxide hydroxide. These chemicals are oxide-hydroxide of iron and it may be available in anhydrous (FeO(OH)) or hydrated (FeO(OH)·H2O) forms [38,39]. Qualitative analysis of PXRD has confirmed that the intermediate sample contains 85% of Iron(III) oxide-hydroxide or ferric oxyhydroxide and 15% of magnetite. The chemical composition of the final product after the *Putapaka* step was confirmed to be Hematite by PXRD. Afterward, the chemical composition of the synthesized LB powder and commercially available LB powder were compared to ensure the accuracy of the process. PXRD spectra have confirmed that both samples contained similar peak positions and it is hematite by chemical nature (Compounds ICDD card numbers were 01-071-5088 and 01-080-2377 respectively). The physical nature of the synthesized LB powder and commercially available LB powder were observed using a scanning electron microscope (SEM). SEM images of both samples depicted the presence of irregularly shaped aggregated nanoparticles (Fig. 4). Furthermore, the surface energy of nanoparticles is increased when the size of the particles is reduced. Agglomeration may have taken place because organic stabilizing agents cannot survive at high temperatures (600 °C). The electron micrograph showed the presence of small cavities which may have appeared due to agglomeration. Small cavity

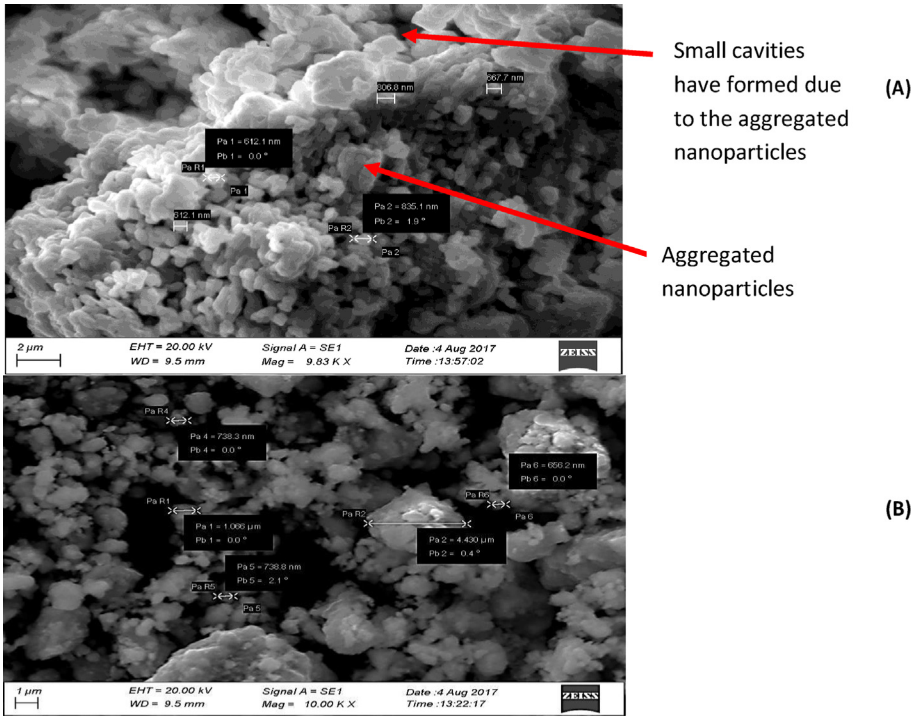

Fig. 4. Magnified SEM images (A) Magnified SEM of synthesized LB powder (B) Magnified SEM of the commercially available LB powder.

Table 4
Variation of Fe^{2+} content in each step.

Solid intermediate iron samples	Fe^{2+} percentage	Degree of Fe^{2+} reduction in successive steps
Initial Red hot iron	86.27%	–
After quenched in *Tila Taila* (first step)	79.60%	6.67%
After quenched in *Thakra* (first step)	77.50%	2.1%
After quenched in *Gomuthra* (first step)	74.12%	3.38%
After quenched in *Kanji* (first step)	66.25%	7.87%
After quenched in *Kulattha Kwatha* (first step)	59.17%	7.08%
After the second step	51.70%	7.47%
After exposed to the sunlight (3rd step)	33.53%	18.17%
After exposed to the furnace heat (3rd step)	19.45%	14.08%

formation can decrease the powder density. Consequently, solid powder can float on the water surface [40].

Primary physical tests which were conducted after completing the *Putapaka* step have confirmed the formation of *Lauha Bhasma* and the Final product (synthesized LB powder) was found positive in 95% *Rekhapurna* and 75% *Varitaratvam* test. These two tests are carried out by weighing the materials. This floating test (*Varitaratvam*) reveals that the powder became light-weighted due to the reduction of particle density. The formation of agglomerated nanoparticles may have caused the reduction of density during this preparation process [40,41]. The PXRD spectra in the *Shodhana* steps and *Bhanupaka Sthalipaka* step have confirmed the existence of different type of magnetite in the intermediate iron samples (different compound ICDD numbers). Magnetite has a broad range of chemical formulas depending upon the amount of structural Fe^{2+}. Therefore, a single chemical formula cannot be given for the magnetite formation [42,43]. Consequently, the variation of Fe^{2+} was determined using titrimetric analysis. Titrimetric results imply that Fe^{2+} content in the intermediate solid samples has gradually decreased during the LB preparation process. However, the *Bhanupaka Sthalipaka* step has shown a large depletion of Fe^{2+}. *Bhanupaka Sthalipaka* step contains organic matter from the *Triphala Kwatha* and the pH of the *Triphala Kwatha* solution was found to be 2.98, indicateing that the *Triphala Kwatha* is acidic. Moreover, the Contact time between the *Lauha Churna* and the *Triphala Kwatha* is high at the *Bhanupaka Sthalipaka* step. All these factors are favorable for iron oxidation [44]. Thus, the highest oxidation has taken place at the *Bhanupaka Sthalipaka* step.

5. Conclusion

Minor metals present in the starting iron sample (Cd, Cr, Zn, Cu, Pb, and Mn) have significantly decreased during the LB preparation process. Among these minor metals, Cd has shown the highest elimination during the LB preparation process. The reduction percentages of Cu and Pb were similar. However, the reduction percentages of Mn and Cr were reasonably low compared to that of Cu and Pb. PXRD spectra have qualitatively confirmed the oxidation of *Teekshana lauha* to Hematite during the LB preparation process. Titrimetric analysis has quantitatively determined the oxidation of *Teekshana lauha* and it has confirmed that the highest iron oxidation has taken place at the *Bhanupaka Sthalipaka* step of the LB preparation process. The SEM images have confirmed that the formation of irregular shaped aggregated nanoparticles at the end of *Putapaka* process. Small cavities that were generated due to the aggregated iron nanoparticles decreased the powder density of LB medicine.

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Author contributions

B.T. Punchihewa: Data curation, Writing- Original draft preparation, Investigation. **M.A.B. Prashantha:** Conceptualization, Supervision, Validation, Writing - Review & Editing, Resources. **P.I. Godakumbura:** Validation, Supervision, Writing - Review & Editing. **S.K.M.K. Herapathdeniya:** Supervision, Validation, Resources, Methodology.

Declaration of competing interest

None.

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