



Pharmaceutical standardization of Indigenous traditional medicine: Dhatri lauh

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Abstract: The present study was carried out to set up quality control parameters for the Indigenous traditional preparation-Dhatri lauh by making the use of classical tests along with advanced analytical tools. Dhatri lauh prepared and has been characterized by determining various physicochemical parameters in comparison to marketed sample. X-ray diffraction techniques are proved to be useful in obtaining finger print profile of prepared sample. This is the first report of fingerprinting of Dhatri lauh using advanced analytical tool. This technique would be useful in studying qualitative and quantitative differences in inorganic as well as organic chemical constituents.

Keywords: Dhatri lauh, Quality control parameters, X-ray diffraction

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1. INTRODUCTION

The development of indigenous traditional systems of medicines with the perspectives of safety, efficacy, and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare. Classical Ayurveda prescribes metals and minerals or in combination with herbs as herbo-mineral formulations. Manufacturing procedures for these formulations are stringent and adverse reactions are possible if not standardized properly [1]. Traditional medicine as including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques

and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness [2]. There is a need to promote the traditional /herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them.

Standardization of drugs is the confirmation of its identity and determination of its quality and purity [3]. It is very important that a system of standardization is established for every plant medicine in the market because the scope for variation in different batches of medicine is enormous [4].

Dhatri lauh is an Ayurvedic formulation containing Dhatri (*Embelica officinalis*), Yastimandhu

(*Glycyrrhiza glabra*) and Lauh bhasma (*Iron preparation*) using Amratu kvatha (*Tinospora cordifolia*) for bhavana (lavigation), included in Ayurvedic formulary of India [2,5]. It is used in various therapeutic applications like Sularoga (Colic pain), Pandur (Anaemia), Amlapitta (Acidity) [6]. This formulation is described in Ayurvedic pharmacopeia of India and has been traditionally used since years but no work is done so far on its standardization. Hence, it is imperative to understand the scientific basis of various quality control tests for complete physicochemical characterization of *Dhatri lauh* to minimize the toxicity and improvement in therapeutic profile.

The objective of the present study is to elucidate this indigenous preparation and characterize *Dhatri lauh* in terms of its physico-chemical properties including crystalline phase analysis, and composition.

2. MATERIALS AND METHODS

All raw materials and marketed samples were collected from Chandigarh, India. All chemicals and solvents were of analytical grade.

2.1. Standardization of raw materials (*Embelica officinalis*, *Glycyrrhiza glabra*, *Tinospora cordifolia*)

The dried fruits of *Embelica officinalis*, roots of *Glycyrrhiza glabra* and stems of *Tinospora cordifolia* were powdered and was analysed for various physicochemical parameters like Water soluble extractive value, Alcohol soluble extractive value, Moisture content and Total ash value [6,7].

2.1.1. Determination of Alcohol Soluble Extractive

5g powder of plant was macerated with 100ml of alcohol in a closed flask for 24 hours, shaken frequently during six hours and allowed to stand for eighteen hours. Filtered rapidly, 25ml of the

filtrate evaporated to dryness in a tared flat bottomed shallow dish, and dried at 105 °C, to constant weight and weighed. The percentage of alcohol soluble extractive value was calculated with respect to air dried drug.

2.1.2. Determination of Water Soluble Extractive

Same procedure followed for determination of water soluble extractive value as followed for determination of alcohol soluble extractive value but chloroform-water I.P. (2.5ml CHCl₃ make up the volume 1000ml with water) was used instead of alcohol. The percentage of water soluble extractive value was calculated with respect to air dried drug.

2.1.3. Determination of Moisture Content (Loss on Drying)

10g powder of plant was placed in silica dish and dried at 105 °C for 5 hours, and weighed. The percentage of moisture content was calculated.

2.1.4. Determination of Total Ash

2g powder of plant was incinerated in silica crucible at temperature not exceeding 450 °C until free from carbon. It was cooled and weighed. Total ash value was determined in percentage.

2.2. Preparation of Dhatri lauh

All the ingredients were made into very fine powder, taken into a mortar pestle and mixed with Lauha bhasma. The mixture was triturated for one week for half an hour in sun light and dried.

2.3. Standardization of marketed Lauh Bhasma, marketed Dhatri lauh and prepared Dhatri lauh

Comparative standardization of marketed sample of Lauh bhasma, marketed sample of Dhatri lauh and prepared sample of Dhatri lauh was done using various physicochemical parameters like Loss on drying, Total ash, Acid insoluble ash, Fines of particle, Floating test, loss of metallic state and

identification test for iron as per Ayurvedic Pharmacopoeia of India [6].

2.3.1. Loss on drying

500 mg of sample (without preliminary drying) was placed in Petridis dish and was dried at 105 °C for 7 hours and weighed. The loss on drying percentage was then calculated.

2.3.2. Total ash

1 gram of sample was weighed and incinerated in the muffle furnace at 600 °C for 7 hour. The total ash percentage was calculated.

2.3.3. Acid insoluble ash

50 ml of dilute hydrochloric acid was added to crucible containing 500 mg of sample. The insoluble ash was collected on an ash less filter paper. The residue washed with hot water until the filtrate became neutral. The filter paper containing the insoluble ash was transferred into the original crucibles. It was further dried in hot plate and ignited to constant weight in incinerator. The content of acid insoluble ash was calculated in percentage.

2.3.4. Fineness of particle (Rekhapurna)

5 mg of samples were taken between the fingers and rubbed to feel fineness of particle.

2.3.5. Floating test (Varitaratavam)

10 mg of samples were taken and sprinkled on water surface in a petri dish.

2.3.6. Loss of metallic state (Nishchandrika)

10 mg of sample was taken and heated to red hot for 5 minute along with very thin silver sheet and cool to room temperature.

2.3.7. Identification test for iron [8,9]

Chemical test were done in order to identify the presence of iron in sample for qualitative estimation.

a. Test for ferric iron

Sample preparation:

30 mg of sample to be tested was dissolved in 10 ml of 2% HCl. Different chemical test to know the presence of ferric iron in the sample to be tested was done according to table 1.

Table 1: Qualitative test for presence of ferric iron

S. No.	Chemical test	Observations
1.	Addition ammonium thiocyanate solution (10% w/v 1 g of sample in 100 ml of water) and extracted with 2% amyl alcohol	Red blood color formed
2.	Acidified sample was treated with Potassium ferricyanide solution	Reddish brown color formed
3.	Acidified sample was treated with potassium ferrocyanide solution	Intense blue precipitate observed

b. Test for ferrous iron

Sample preparation:

30 mg of sample to be tested was dissolved in 10 ml of 2% HCl. Different chemical test to know the presence of ferrous iron in the sample to be tested was done according to table 2.

Table 2: Qualitative test for presence of ferrous iron

S. No.	Chemical test	Observations
1.	Acidified sample was treated with 6% potassium permanganate solution. On addition of ammonium thiocyanate solution	Faint pink colour observed. Blood red color formed
2.	Acidified sample was treated with 2% Potassium ferricyanide solution	Dark blue colour formed
3.	Acidified sample was treated with potassium ferrocyanide solution	A white precipitate formed

2.3.8. Test for quantitative estimation of Iron [8,9]

a. Assay for total iron

10 ml of hydrochloric acid was added to an aliquot of the sample (50 mg) and boiled for 2 minutes. It was evaporated to dryness on a steam bath to render SiO₃ insoluble. The residue was moistened with 5 ml of hydrochloric acid and

boiled for 6 minutes. Add 30 ml of water, heat on the water bath for few minutes, filtered and washed thoroughly, and made the volume.

Determination

To an aliquot of the sample, 2 ml of hydrogen peroxide solution (6%) was added to oxidize the iron and boiled. Sufficient hydrochloric acid was added to adjust the concentration of hydrochloric acid to approximately 3 N. After that 2 g of potassium iodide was added, set aside for 3 minutes and titrated with 10 ml of 0.1 N sodium thiosulphate. Total iron content in the sample to be estimated was calculated using following relation.

Each ml of 0.1N Sodium thiosulphate = 5.585mg of Fe

b. Assay for ferrous iron

20 ml of dilute hydrochloric acid was added to accurately weighed quantity of the sample (50 mg). It was then filtered, washed with water and acidified with sulphuric acid. The sample was then titrated with N/10 $KMnO_4$. Iron content as ferrous iron in the estimated sample was calculated by following relation.

Each ml of N/10 $KMnO_4$ = 0.005585 g of Fe (as Ferrous iron)

c. Assay for ferric iron

20 ml of dilute hydrochloric acid was added to accurately weighed quantity of the sample (50 mg). The sample was heated to dissolve the iron and filter. The filtrate was washed with water and acidified with hydrochloric acid. The sufficient hydrochloric acid was added to adjust the concentration to approximately 3N. 2g of potassium iodide was added and set aside for 3 min and filtered. The filtrate was titrated with N/10 sodium thiosulphate. Iron content as ferric iron in the estimated sample was calculated using following relation.

Each ml of N/10 sodium thiosulphate = 0.005585 g of Fe (as Ferric iron)

2.3.9. X-Ray Diffraction Analysis (X-RD)

It was used to determine the nature (crystalline or amorphous) of different sample of marketed Lauh Bhasma (LB), marketed Dhatri lauh (DH1) and prepared Dhatri lauh (DH2). The sample was weighed and taken into the chamber. The XRD was consist of a copper X-ray source (wavelength=1.54 Å), a solid state detector and analysed the diffraction data [10].

3. RESULTS AND DISCUSSION

3.1. Standardization of raw materials (*Embelica officinalis*, *Glycyrrhiza glabra*, *Tinospora cordifolia*)

Water soluble extractive value, Alcohol soluble extractive value, Moisture content and Total ash value were determined for *Embelica officinalis*, *Glycyrrhiza glabra*, *Tinospora cordifolia*. The results of these physicochemical parameters are shown in table 3 and results were within the limit of standard.

Table 3: Physicochemical parameters of raw materials

S. No.	Parameters	<i>Embelica officinalis</i>	<i>Glycyrrhiza glabra</i>	<i>Tinospora cordifolia</i>
1.	Water soluble extractive	46%	63%	26%
2.	Alcohol soluble extractive	35%	46%	23%
3.	Moisture content	89%	76%	67%
4.	Total ash	6.6%	7.2%	14.6%

3.2. Preparation of Dhatri lauh

Weigh quantity of all the standardized raw materials were made into very fine powder and mixed with lauha bhasma as shown in figure 1.

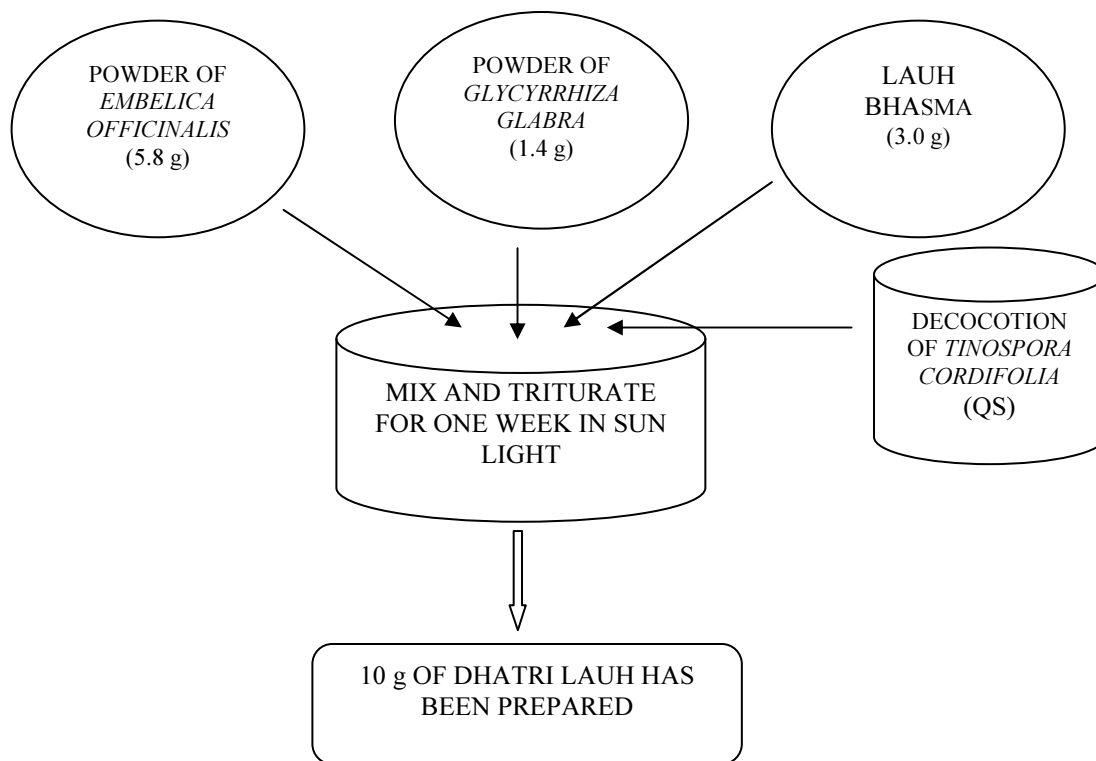


Fig. 1: Preparation of Dhatri lauh

3.3. Standardization of marketed *Lauh Bhasma*, marketed *Dhatri lauh* and prepared *Dhatri lauh*

Various physicochemical parameters were determined to standardize marketed sample of

lauh bhasma (LB), Marketed sample of *Dhatri lauh* (DH1) and prepared *Dhatri lauh* (DH2). The results of these samples were shown in table 4.

Table 4: Physicochemical parameters of *Lauh Bhasma*, *Dhatri lauh* (DH1), *Dhatri lauh* (DH2)

S. No.	Parameters	<i>Lauh Bhasma</i> (LB)	<i>Dhatri Lauh</i> (DH1)	<i>Dhatri Lauh</i> (DH2)
1.	Loss on drying (%)	8.0	18	14
2.	Total ash (%)	93	81	89
3.	Acid insoluble ash (%)	33.8	25	27
4.	Fineness of particle	Fine particle	Particles are not fine	Particles are not fine
5.	Floating test	Floated on water surface	Not Floated on water surface	Not Floated on water surface
6.	Loss of metallic state	Loss of metallic state	No loss of metallic state	No loss of metallic state
7.	Identification test for iron <ul style="list-style-type: none"> • Ferric iron • Ferrous iron 	present present	present present	present present
8.	Quantitative estimation of Iron <ul style="list-style-type: none"> • Total iron content • Ferrous content • Ferric content 	32.39 mg 6.14 mg 25.13 mg	13.96 mg 1.11 mg 10.61 mg	15.63 mg 2.23 mg 12.28 mg

▪ X-RD analysis of *Lauh Bhasma* (LB) and *Dhatri lauh* (DH1, DH2)

X-RD study of marketed sample of *Lauh bhasma* (LB), Marketed *Dhatri lauh* (DH1) and prepared *Dhatri lauh* (DH2) was done. X-RD scan of different sample was found to be with high intensity peak as shown in

figure 2. X-RD spectrum suggests the crystalline nature of sample. All the samples have a sharp peak at 45° showed the presence of iron oxide in the sample.

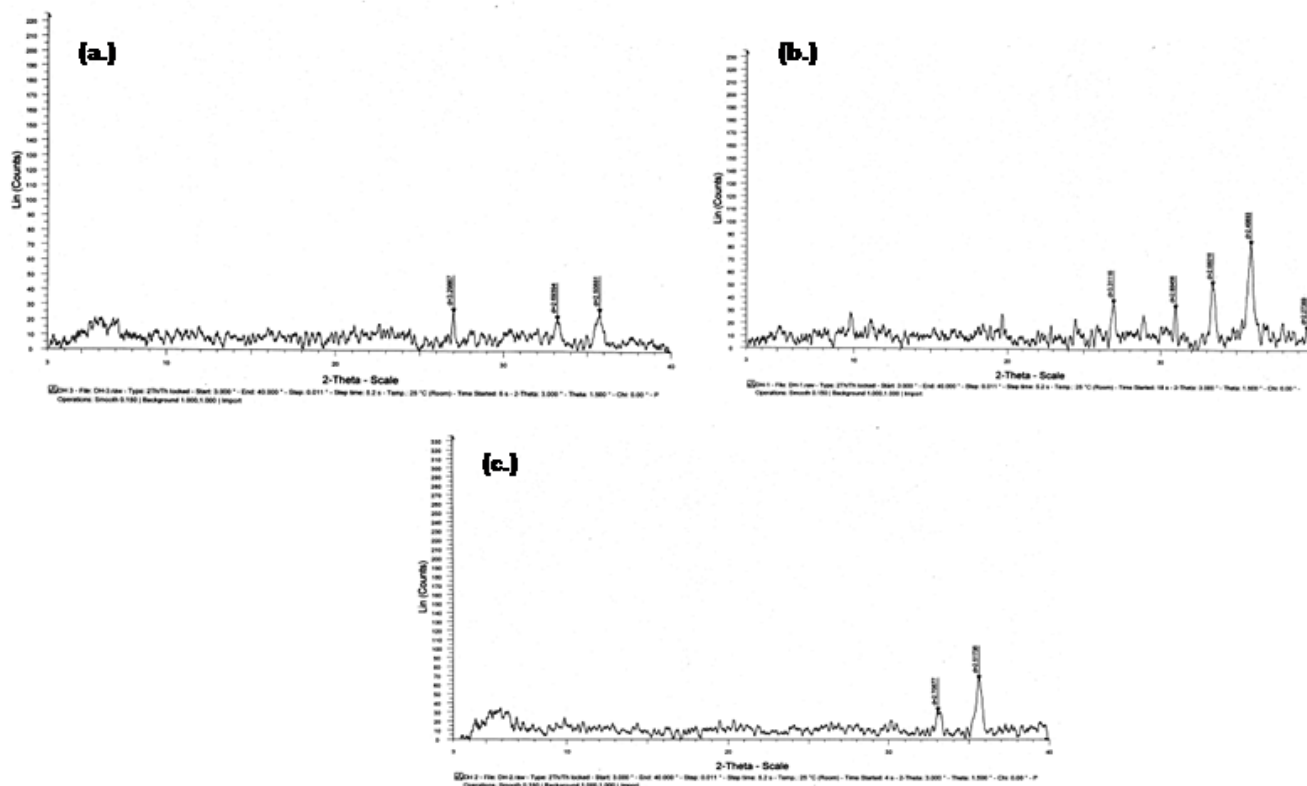


Fig. 2: X-RD spectrum of (a.) Lauh bhasma [LB], (b.) Dhatri lauh [DH1] and (c.) Dhatri lauh [DH2]

4. CONCLUSION

Various physicochemical parameters of raw material used in *Dhatri lauh* preparation, marketed sample of *Dhatri lauh* and prepared *Dhatri lauh* was determined. Results of physicochemical evaluation of raw materials were found to comply with limits as given in the Ayurvedic Pharmacopoeia of India. Dhatri lauh have been prepared and various physicochemical parameters are compared with marketed formulation of Dhatri lauh. Results of prepared sample of *Dhatri lauh* and marketed sample of *Dhatri lauh* for various standardization parameters were not shown any significant difference. Marketed formulation and prepared formulation was further standardized using the modern analytical technique X-RD. It was

observed that there is a difference in results as they were observed for the physical properties and the composition. The herbs mixed with the bhasma, changed the iron content of the formulation and make the iron more bioavailable or increase the absorption of iron in the body. Therefore, standardization of traditional system of medicine by modern analytical technique had revealed its importance to assess the quality and characteristic of traditional drug like Dhatri lauh, so that its quality will be further increased to improve its efficacy.

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