Research Article

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CHARACTERIZATION OF AN AYURVEDIC DRUG (SHILAJATWADI LAUHA): AN APPROACH TO STANDARDIZATION

Singh T.R[1]*, Gupta L.N[2], Kumar V[3], Kumar N[4]

[1]PG Scholar, Department of Rasa Shastra, Faculty of Ayurveda, IMS, Banaras Hindu University, Varanasi, India

[2]Assistant Professor, Department of Rasa Shastra, Faculty of Ayurveda, IMS, Banaras Hindu University, Varanasi, India

[3]Associate Professor, Neuropharmacology Research Laboratory, Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi, India

[4]Professor, Department of Rasa Shastra, Faculty of Ayurveda, IMS, Banaras Hindu University, Varanasi, India

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***Corresponding author**

Dr. Thakur Rakesh Singh, PG Scholar, Department of Rasa Shastra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, UP, India E-mail: rakeshayu1984@gmail.com

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ABSTRACT

Shilajatwadi Lauha (SL) is an Ayurvedic Herbo mineral compound formulation used in the treatment of Kshaya and Madhumeha from ancient times. The present study was selected to standardize SL which can be adopted by different Ayurvedic pharmacies for its standardization. For preparation of SL pure Shilajit, Swarna Makshik Bhasma, Shunthi-Marich-Pippali Churna (Trikatu) were taken in equal quantity in mortar and pestle and six times of Lauha Bhasma were added in it and triturated until it becomes fine powder and homogenously mixed. Each of the ingredients was prepared according to the protocols of Ayurvedic texts and by applying Electric muffle furnace as heating device for incineration. To ensure the proper preparation of Bhasmas and other ingredients bhasma parikshas and other tests were employed. After completion of all tests, SL was prepared and subjected for XRD and SEM analysis. XRD study reveals that strongest peak obtained was Fe_2O_3 and other identified compound was CuS and FeS_2 and SEM study shows that the particle size of SL was between 2 to 20 µm. This is the first study in establishing the characterization of SL.

Keywords: Characterization, Shilajatwadi Lauha, X-ray diffraction, Scanning electron microscopy.

INTRODUCTION

The basic purpose of the science Ayurveda is to keep the human beings free from disease, old age and death. Rasashastra and Bhaisajya kalpana is one of the branches of Ayurveda which has a rich knowledge of Herbo mineral formulations. In earlier days, the quality of a medicine was not subjected for critique, but it was based on the sacred trust which existed between the physician and the patient. The technological development and apprehensions of modern science obligated the patients and physicians to be watchful about the quality assurance, safety and efficacy of the medicine[1]. Ayurvedic medicines have no exception in this regard. Hence it is the need of the hour to produce fingerprints for quality medicines. SL is one of the herbo mineral formulations which have been used for treatment of Kshaya, Madhumeha, Mutraroga, Pandu etc from ancient times[2-4]. Hence this formulation was selected for study. Till date no scientific work has been carried out on this formulation with respect to physicochemical characterization, which is essential for drug standardization.

MATERIALS AND METHODS

Preparation of Shilajatvadi Lauha (SL)

Pure Shilajit, Swarna Makshik Bhasma, Shunthi-Marich-Pippali Churna (Trikatu) were taken in equal quantity in mortar and pestle and added six times of Lauha Bhasma in it and triturated until it becomes fine powder and homogenously mixed[5]. This drug was kept in air tight container. For preparing individual ingredients, the guidelines were adopted from various classical texts of Rasa Shastra and expert opinions (Table 1).

Characterization of ingredients of Shilajatvadi Lauha by classical parameters

The final bhasmas, Shilajit and other ingredients were analyzed for quality control as per the Ayurvedic texts and then subjected for the preparation of SL.

Physical characterization

Varna (Color)

Well prepared bhasmas and other ingredients possess a specific color as per the classics. In the present study the following were the colors of the ingredients, Lauha Bhasma- ripened Jamoon fruit (purple), Swarna Makshika Bhasma - Raktotpaldal (Reddish brown), Pure Shilajit- black, Trikatu churna- light brown, SL- dark brown.

Nischandratvam (lusterless)

Metal loses its metallic luster after proper incineration. This was examined under sunlight. No luster was found in any of the bhasmas.

Rekhapurnata (finger lines test)

The particles were so fine that when a pinch of bhasmas was taken between index finger and thumb, all the particles entered the furrows of the fingers.

Varitara (floating on water)

Small quantity of each bhasma was sprinkled on still water and it floated for some time on water.

Unama
On each varitara bhasma rice grain was kept, it floated on the water.

Reaction on fire
When pure Shilajit reacts on fire, it burns without fumes and gives lingakar akriti.

Avami test
Ingestion of 5-10 mg of Swarna Makshik bhasma did not produce nausea/vomiting. Thus, these bhasmas and other ingredients passed all the tests for physical characterization which were quoted in different classical texts of Rasa Shastra.

Chemical characterization
Apunarbhava
It is the inability of bhasmas to revert to its original form. Bhasmas were mixed with mitra panchaka triturated, and subjected for puta. After cooling no metallic particles were detected.

Niruttha
It is the inability to regain its alloy forming metallic property. If the bhasma is subjected to puta and kept along with silver foil, the weight of silver foil did not increase. Both the bhasmas passed these two tests.

Characterization of Shilajatwadi Lauha by using modern parameters
Loss on drying
This test was performed to find out the moisture content of the sample. 1 g of exactly weighed SL was taken in a previously weighed petridish and dried in an oven at 110°C till constant weight. Then the petridish was taken out, weighed after self cooling and from the weight loss the percentage of loss on drying was calculated and expressed as %w/w.

Ash value
This test was conducted to assess the total ash content of the sample. It was determined by incinerating about 2 g of accurately weighed sample in tarred silica crucible in an electric muffle furnace at 700°C, then allowed for self cooling and weighed. The percentage of ash was calculated and expressed as % w/w.

Acid insoluble ash
This test was carried out to assess the percentage of acid insoluble inorganic content of the sample. The ash obtained in process of determination of ash value was boiled for 5 minutes with 50 ml of dilute hydrochloric acid; the insoluble matter was collected on ash less filter paper, washed with hot water and ignited to get constant weight. The percentage of acid insoluble ash was calculated and expressed as % w/w.

X-ray diffraction
XRD Study was carried out in the Department of Physics, Faculty of Science, BHU, Varanasi, India. The sample of SL was grinded well in agate mortar and desiccated well prior to the experiment, as any sort of moisture will give improper results. The samples of the study were mounted on the sample holder of a commercial high resolution X-ray power Diffractometer fitted with a curved crystal monochromator. This diffractometer operates on "Bragg-Brentano geometry". An 18 Kw rotating anode generator was used as a source of X-ray. This machine was of Rigaku make with model No. Rint 2000/PC series. The XRD data were collected in the fully automatic mode and stored in the personal computer[6] (Figure 1, Table 2).

Scanning electron microscope
SEM was performed in the Department of Physics, Faculty of Science, BHU, Varanasi, India. For the present study SEM 840 A (JEOL - Japan electronics optical limited) was used for taking the micrographs. It is a state of the art high resolution SEM. Its maximum limit of magnification is 3 lakh[7].

RESULTS
In physio-chemical analysis of SL, percentage of loss on drying was 4.12, ash value was 65.66 and acid insoluble ash was 8.66. In XRD study data were recorded from $2\theta = 10^0$-80^0 at a scanning rate of 4^0/mm of 6 kw energy. XRD pattern is shown in the Figure 1 of SL. The strongest peak identified was Fe_2O_3 and other identified compound was CuS and FeS_2. Six Fe_2O_3 peaks. Two CuS peaks and one FeS_2 peak were also observed (Table 2). Particle size analysis of SL sample had been done with SEM and the results are shown in (Figure 2 and 3). The photomicrograph of bulk particle shows a wide distribution of size in the sample. We also observed that the particles were irregular in shape and were present in aggregated form. In SEM pictures (Figure 2 and 3) of SL, individual particles were visualized clearly. The horizontal line in the right corner of the micrograph corresponds to 10 and 5-μ length. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the Bhasma particle was calculated. The SEM study pictures shown that the particles were homogenously mixed. The particle size ranges from < 2 to 20 μ and the bigger particles were agglomeration of smaller particles itself.

DISCUSSION
Preparation procedure of bhasma is very elaborative. However, this process has been followed strictly till today for maintaining the quality and efficacy of the product. Previously, the Herbo mineral formulations were being prepared on small scale by the Ayurvedic physicians themselves, but now they are manufactured on large scale in pharmaceutical houses. This new approach has created several troubles, as the uses of new appliances have not been standardized with respect to the quality of these preparations. For standardized formulations, there is a need for scientific approach which includes physical and chemical standardization of drug. In Herbo mineral formulations, bhasmas are one of the important ingredients. For quality assurance of bhasmas process standardization is also essential. Ancient scholars of Rasa Shastra have explained some tests (Bhasma parikshas) to identify the bhasmas.

Table 1: Pharmaceutical preparation of Shilajatwadi Lauha

Ingredients	Quantity	Shodhana	Pre incineration process	Bhavna dravya	Putapaka method		
					Peak T	Duration of T	No. of incineration cycles
Teekshna Lauha (Iron turning)	6 part	Quenching each 7 times in sesame oil, butter milk, cows urine, sour gruel decoction of horse gram and triphla	1. Bhanupaka (sun drying) with triphla kwatha 2. Sthalipaka (boiling) with triphla kwatha	Triphla kwatha	650^0C	1 h	20
Swarna makshika (Chalcopyrite)	1 part	Roasting in Nimbu swarasa for 3 days	-	Nimbu swarasa	650^0C	1 h	10
Shilajit	1 part	Dissolution and filtration in water	-	-	-	-	-
Pippali powder(*Piper longum*)	1 part	-	-	-	-	-	-
Shunthi powder(*Zingiber officinale*)	1 part	-	-	-	-	-	-
Marich powder (*Piper nigrum*)	6 part	-	-	-	-	-	-

T: Temperature

Table 2: X-Ray Diffraction of the Shilajatvadi Lauha

S. No.	Compounds	Position (2θ)	Heights (cts)	FWMH (2θ)	d space (Å)	Relative intensity (%)
1.	Fe_2O_3	33.32	8.83	0.11	2.68	8.35
		35.77	105.74	0.27	2.50	100.00
		43.44	16.95	0.31	2.08	16.03
		53.86	12.02	0.23	1.70	11.37
		57.39	27.16	0.31	1.60	25.68
		63.05	33.09	0.31	1.47	31.29
2.	CuS	30.40	40.55	0.23	2.93	38.35
		33.32	8.83	0.11	2.68	8.35
3.	FeS_2	37.28	4.25	0.47	2.41	4.02

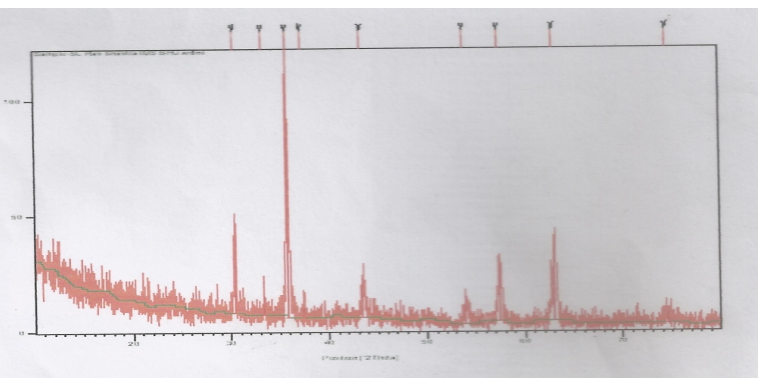

Figure 1: X- ray diffraction of Shilajatwadi Lauha

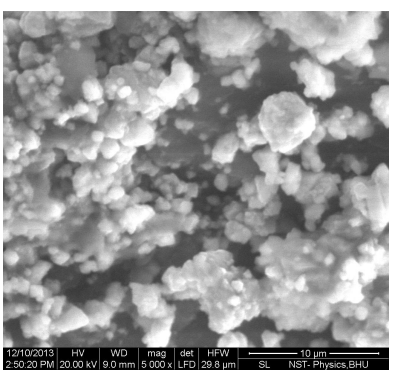

Figure 2: SEM image of SL (up to 10µ m)

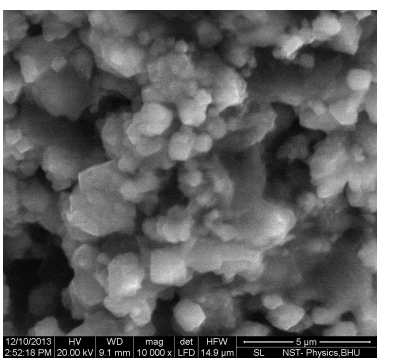

Figure 3: SEM image of SL (up to 5µ m)

They may be classified into nondestructive (Nischandratvam, Rekhapurnata, Varitara, and Unama) and destructive (Apunarbhava and Niruttha)[1]. The prepared bhasma and purified Shilajit passed all the physio-chemical parameters of ancient classics. Physio-chemical analysis of SL was done on parameters of loss on drying, ash value and acid insoluble ash. The loss on drying (% w/w) value of SL indicates the moisture content in the drug. The total ash content, which usually consists of carbonate, silicates, silica, physiological and non physiological ash, was found to be high in SL which is due to the presence of mineral and metal derived substances in the formulation[8]. The acid insoluble ash value indicates the quantity of acid non digestible mass in the drug. Hence lower acid insoluble drug indicates higher bioavailability of drug. XRD pilot study confirms the presence of ingredients of SL. Six Fe_2O_3 (maghamite) peaks, two CuS peaks and two FeS_2 peak peaks were observed in the study (Figure 1). These peaks indicate the presence of respective bhasmas. The XRD pattern of SL shows the presence of Fe_2O_3 (maghamite) as the major phase, which is marked to the corresponding peak of each phase and remaining peaks of CuS, FeS_2 were minor phases. The presence of Fe_2O_3 (maghamite) in Lauha bhasma, CuS peaks along with FeS_2 peaks in Swarna Makshika bhasma were reported by the earlier scholars[9]. XRD study also confirmed that absence of any free metal in SL. The SEM study pictures (Figure 2 and 3) show that the reduction in particle size and the particles were homogenously mixed. Reduction in particle size indicates the proper preparation of bhasma and is responsible for high biological activities of these metals and minerals derived substances. It also facilitates absorption and assimilation of the bhasma in the system[9]. The particle size ranges from < 2 to 20 µ and the bigger particles were agglomeration of smaller particles itself. More interestingly various crystallites in different shapes namely rod shaped, cubical, square and rectangular shaped is embedded in the lumps so bigger particles has been obtained. Smaller particles may be because of presence of large quantity of Lauha bhasma and also due to presence of Swarna Makshika bhasma. The earlier SEM studies on Swarna Makshika bhasma reported that 1-2 µm and the particle size distribution of Lauha bhasma in a range of 2.5-40.9 µm[1]. Particle size depends on the nature of drug and calcination cycles.

CONCLUSION

Shilajatvadi Lauha (SL) was standardized on classical and modern analytical parameters. The data of physio-chemical analysis of SL may be useful for evaluation and standardization. In this study authors reported first time that characterization of SL which will be useful in standardization of Shilajatvadi Lauha.

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