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Comparative Pharmaceutico-analytical Study of *Shilajatu* Collected from Himachal Pradesh with that Samples Obtained from Standard Commercial Sources and their *In-vitro* Antioxidant Study- A Study Protocol

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Study Protocol

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ABSTRACT

Background: *Shilajatu* is one among the most used and important drugs in *Ayurveda*. However, a big percentage of *Shilajatu* samples available in market is not genuine. The analytical standards of *Shilajatu* are still not in *Ayurvedic* pharmacopoeia or any authoritative publication. So, the characterization and standardization of *Shilajatu* is required. Moreover, Shilajeet is vaguely being used by the layman and even professionals. It's therapeutic potential needs to be specifically evaluated.

Aims: To compare pharmaceutico-analytical characteristics of *Shilajatu* collected from Himachal Pradesh with that samples obtained from standard commercial sources and their in-vitro antioxidant study.

Methodology: The collection of samples from the site near *Rampur Bushahr, Himachal Pradesh* will be done in favourable season (in the month of April to June and September-October) by personal visits. The samples of *Shilajatu* from two sites shall be taken for this study. Two market samples shall also be bought from two standard commercial sources. All the samples shall be taken in triplicate to ensure data accuracy. Minimum 4 sources samples in triplicate, so total minimum 12 raw samples shall be taken. Further analysis of raw samples then pharmaceutical processing (Shodhana) and again analysis of processed samples shall be done. In analysis organoleptic, Physico-Chemical, elemental analysis and estimation of Humic acid and Fulvic acid shall be done of minimum 12 Raw samples and 12 processed samples (total minimum 24 samples). In-Vitro antioxidant study shall be done for both sources, processed samples.

Observations & Results: The observations noted while collection, analysis, processing of all samples shall be documented and presented in the form of data, photographs, tables, chart, etc. as applicable. Further the processed *Shuddha Shilajeet* shall be subjected to an In-Vitro antioxidant study. Findings will be analyzed for interpretation of results.

Conclusion: A comparative data of samples of *Shilajatu*, collected from site of natural occurrence and standard commercial sources, with respect to pharmaceutical work, analysis and In-Vitro antioxidant study will be generated.

Keywords: Shilajatu; himalayan shilajeet; fulvic acid; humic acid; rasayana; revitalizer.

1. INTRODUCTION

The description and therapeutic use of *Shilajatu* can be found from the oldest and commonest *Ayurveda* texts like *Charak Samhita* [1], *Sushruta Samhita* [2] and various texts of *Ras shastra*. However, even after such a long history of use; the availability, Identification and characterization of *Shilajatu* is a mystery till date. Even *Ayurveda* Pharmacopoeia of India do not have any details about Identification and standardization of *Shilajatu*. So, this work is focused on *Shilajatu* study.

Recently, availability of *Shilajatu* in *Himachal Pradesh* nearby *Rampur* region has been reported [3]. In this study, collection of raw *Shilajatu* sample from this site near *Rampur* area in *Himachal Pradesh* shall be done by personal visit. The location under study touches three districts; *Shimla, Kullu and Kinnaur of Himachal Pradesh* [4]. As per physiographic divisions [5] this area comes under lesser *Himalayas* or the central zone [5] and as per agroclimatic zones division [6], it is high hill temperate wet zone [6] respectively.

Maharishi Charaka discusses *Shilajatu* in a chapter on *Rasayana* [7] as rejuvenating or adaptogenic substance that confer long healthy life and so retard aging [7]. As per one another classical text, *Shilajatu* possess all the properties and actions of *Maharasa, Uparasa, Suta, Ratna and Lauha* [8]. *Shilajatu* has been used since long as a *Rasayana* (rejuvenator and anti-aging) and for treating diseases [9].

Raw *Shilajatu* that is found in high hill rocks, is somewhat brittle stony to crumbly material, without any specific shape and porosity and commonly have a good quantity (by weight), of stony, sandy and earthy impurities. It has not been categorized absolutely as mineral, plant or of animal origin. The composition of *Shilajatu* vary from source to source [10,11] and study to study, no work till date has reported a complete identification and standardization criteria; However, broadly the composition of *Shilajatu* has been reported to have inorganic to organic materials like more than 85 minerals, many elements and compounds- eighteen free amino acids, benzoic acid, m – hydroxybenzoic acid, sterols, tri-terpenes, ellagic acid, three

bencoumarins [11] and also Fulvic acid & Humic acid [12].

In the present study, collection of raw *Shilajatu* sample from the site near *Rampur area in Himachal Pradesh* has been planned by personal visit. A successful pilot study has already been done to ensure the availability of *Shilajeet* at planned sites. The analysis of the collected samples will be done as per general analysis protocol for *Ras Shastra* medicines, these analytical tests shall include organoleptic tests, physicochemical tests. Also, Elemental analysis and Humic acid and Fulvic acid estimation shall be done. Along with this, two different samples available in market shall be procured, identified and analyzed for comparison. Further, these collected samples and market samples shall be subjected to pharmaceutical processing that is extraction-shodhan as per *Ayurvedic Formulary of India (AFI)* [13]. The analysis of processed samples shall be repeated. All the samples shall be taken in triplicate to ensure reliable data generation. Further, In-Vitro antioxidant study shall be done on processed samples of both sources.

The observations of this study will provide qualitative and quantitative data for characterization and further studies on *Shilajatu.*

1.1 Research Gap

1. No Identification, Analysis & characterisation data about *Shilajeet* is available in *Ayurvedic pharmacopoeia of India* (API), AFI & any other official publication.
2. Although, tests in classical texts have been described to know the genuinity of *Shilajatu*, but, Modern day Analysis is not performed at that time. In today's context- Standard Analysis protocol is required.
3. No study is done and collected *Shilajatu* sample from the site specifically selected for this study (*Rampur Bushahr of Himachal Pradesh*). No Analysis and characterisation data is available for this *Shilajatu*.
4. Moreover, in case of *Shilajatu*, every new site is area for new study and a good possibility of new and unique research findings.
5. This is a comparative study; no previous study compares in-situ collected samples with the samples from commercial samples.
6. Various previous studies cannot be compared and compiled to form conclusive data as there is no in-situ sample collection, origin of samples is unknown, types of samples in various studies vary (*Shuddha/AShuddha*), only specific analysis has been done by various researchers previously and there is huge variation in analytical findings. Thus, none of the previous studies gave conclusive data of Characterisation.
7. Therefore, in this study it is proposed to work on characterization of *Shilajatu* collected from *Sub-Himalayan* region and compare it with market samples.

1.2 Aim of the Study

To compare pharmaceutico-analytical characteristics of *Shilajatu* collected from *Himachal Pradesh* with that obtained from standard commercial sources and their in-vitro antioxidant study.

1.3 Objectives

1. To collect samples of *Shilajatu* from *Himachal Pradesh* around *Rampur region.*
2. To study pharmaceutico-analytical characteristics of *Shilajatu* collected from *Himachal Pradesh* around *Rampur region.*
3. To study pharmaceutico-analytical characteristics of *Shilajatu* collected from standard commercial sources.
4. To compare pharmaceutico-analytical characteristics of samples from both sources.
5. In-vitro antioxidant study of *Shilajeet* from both sources.

1.4 Research Question

Whether there is variation in pharmaceutico-analytical characteristics of *Shilajatu* collected from *Himachal Pradesh* and procured from standard commercial sources?

2. MATERIALS AND METHODS

2.1 Materials

i. Field sample collection resources; like Mean of transport, sample collection tools and equipments.
ii. Well established Ras Sastra & Bhaishajya Kalpana laboratory for doing the pharmaceutical work.
iii. Well-equipped drug analysis laboratory or source for drug analysis work.

iv. Raw drugs and other reagents for processing (Shodhana) and analysis of various Shilajatu samples. Raw material is required as per AFI-I, Shodhan -47, are; Table 1; In AFI, for process of extraction and shodhana, water and decoction of Triphala has been advocated.

2.2 Methodology

The work will go through following sequence.

2.2.1 Collection of samples from the site at *Himachal Pradesh* and procurement from standard commercial sources

The collection of samples from the site near *Rampur Bushahr, Himachal Pradesh* will be done in favourable season (in the month of April to June and September-October) by personal visits. A short one-page SOP for collection of samples shall be prepared specifically applicable to the study and material, referring; Standard Operating Procedure for the Collection of Soil and Sediment Samples for the SCoRR Strategy Pilot Study, U.S. Geological Survey, Reston, Virginia: 2015 [14]. Three sites namely; *Vill. Bhatkiyol-Bajrah, Nirmand, district Kullu, Vill. Kunath-Bhadgaon, Distt. Shimla & Vill. Koyal-Nithar, Tehsil Nirmand, District Kullu,* have already been visited and identified in the pilot study.

The samples of Shilajatu from minimum two out of these three sites shall be taken for this study. Two market samples shall also be bought from two standard commercial sources. As the samples from all source be taken in triplicate to achieve reliable quality data during further part of study, so 6 samples from site of occurrence and 6 samples from standard commercial source shall be available, so, the magic number of statistics (i.e., N=6) shall be reached [15]. This value in statistics is very commonly used, where the N value supposed to be minimum due to various reasons like lesser number of animals for experimental work due to ethical issue or lesser availability of test samples as a whole. The proper documentation of work shall be done. Details of various samples taken for study has been described in Table 2.

2.2.2 Identification, pharmaceutico-analytical characterisation of samples

All raw samples; collected and commercial samples shall be subjected to:

2.2.2.1 Classical tests: - Shilajatu pariksha

Test on flame: *Vahano kshipatam – Bhaved Lingakaram* [16], *Adhoomakam* [16].

Test in water: *Ambhasi-Kshipatam addho-tantuvat* [17].

Organoleptic Tests: *Gomutra Gandhi* [17],*Maleenam* [17*],Gugulabh* [18], *Jatvabh* [18].

Further, standard analysis protocols of *API for Ras shastra* drugs has been planned, at some accredited drug testing laboratory/ies. those tests are detailed in Table 3.

Elemental Analysis: by Energy Dispersive X-Ray Analysis (EDX) as minerals being important constituents of *Shilajatu.*

Humic acid and Fulvic acid estimation: (as these constituents are postulated in some studies as important constituents responsible for it's pharmacological activities [10,19]).

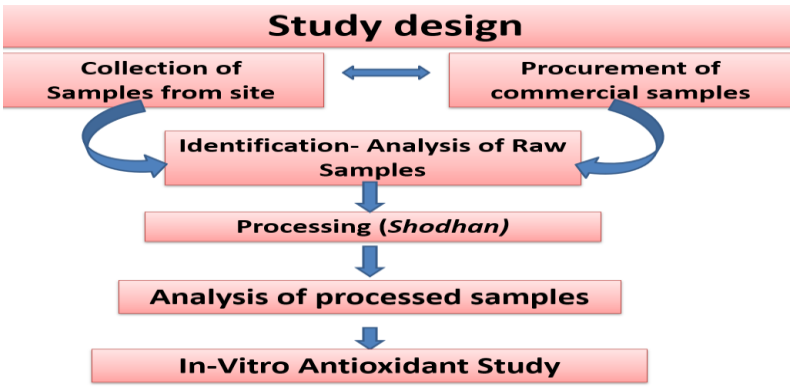
i. Humic acid estimation.
ii. Fulvic acid estimation

Table 1. Material for processing; *Shodhana process*

S.no.	Name	Botanical name	Part used	Proportion
1.	*Amalaki*	*Emblica officinalis* Linn.	Fr. P	1/3 parts
2.	*Haritaki*	*Terminalia chebula* Retz.	Fr. P	1/3 parts
3.	*Vibhitaka*	*Terminalia bellirica* Roxb.	Fr. P	1/3 parts
4.	*Shilajatu*	Raw form		2 parts

Table 2. Sample size for analysis and pharmaceutical study

Analysis sample Summary	No. of Samples
Collection of Field Samples (from *Rampur Bushahr, H.P.)*	Minimum 2 sites in Triplicate (6)
Procurement of Market Samples	Minimum 2 sources in Triplicate (6)
Processing (*Shodhan)* of raw Samples (Both Field & Market Samples)	Minimum 12 Samples
Analysis of Raw and Processed Samples (Both Field & Market Samples)	Minimum 24 Samples



Flow chart 1. Methodology; study design

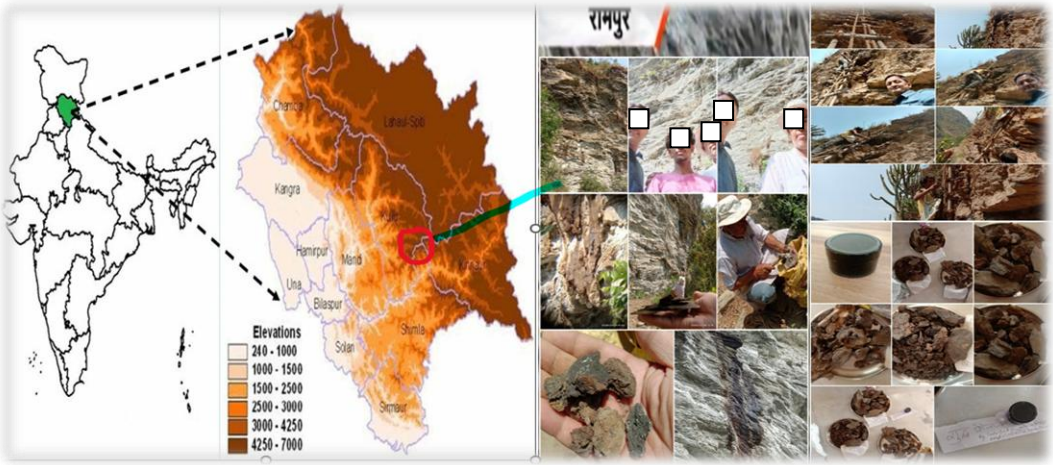


Image 1. Geographic location; Site of occurance of *Shilajeet* near *Rampur Bushehr region of Himachal Pradesh* also photographs during pilot study [3,5]

Table 3. Analysis plan

Organoleptic Parameters	Physico-chemical Parameters
Colour	*pH* (1% aqueous solution)
Odour	Loss on drying
Taste	Total ash
Touch	Acid-insoluble ash
Appearance	Alcohol-soluble extractive
	Water-soluble extractive

2.2.3 Pharmaceutical processing *(Shodhan);*

Pharmacognostic Evaluation of herbal contents to be used for processing *(Shodhan)* shall be done. *Shilajeet* samples shall be processed in triplicate as per classical method. The *Shodhana* method and material used shall be as per *AFI-I-Shilajatu Shodhan*-47 [13].

2.2.4 Analysis of processed *shuddha* samples

Processed *shuddha* samples, shall be subjected to analytical protocol as discussed for raw forms (2.2.2).

2.2.5 *In-Vitro* antioxidant study

Determination of 1,1, dipheny-2-picrylhydrazyl (DPPH) Radical Scavenging Activities. Shall be performed using 1,1 diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Brand-Williams et al. with some modifications as per the resources available at *Mahatma Gandhi Ayurved College, Hospital & Research Centre, Saloh(H), Wardha*. One processed *shuddha Shilajatu* sample from each source shall be taken for in vitro antioxidant study. The extraction of samples shall be done in best suitable solvent as per the findings of physicochemical study. 1 ml of each extract shall be transferred into a clean test tube along with 0.5 ml of 0.3mM DPPH in

suitable solvent. The mixture shall be shaken and left to stand in the dark at room temperature for 15 minutes. After incubation in the dark, the absorbance values shall be measured.

2.2.6 Analysis of data and conclusion

The collected data shall be analysed and discussed.

2.3 Study Type

Pharmaceutico-analytical & In-Vitro Antioxidant study.

3. OBSERVATION AND RESULTS

The observations noted while collection, analysis, processing and In-Vitro antioxidant study of all the applicable samples shall be documented and presented in the form of data, photographs, tables, chart, etc. The data will be analyzed for interpretation of results.

4. DISCUSSION

Although many research publications can be seen about various aspects of *Shilajeet*, and the reporting about *Shilajeet* composition vary from source to source [10,11], and study to study. Broadly, the composition of *Shilajatu* is reported to possess inorganic to organic materials, like more than 85 minerals, Ca, Fe, K, Li, Mg, Al, C, Mn, Na, Ni, P, Si, S etc. elements and compounds like eighteen free amino acids, benzoic acid, m – hydroxybenzoic acid, sterols, tri-terpenes, ellagic acid, three bencoumarins [11] and also Fulvic acid & Humic acid [19]. however, no work till date has reported a complete identification and analysis range that can be adopted as characterisation and standardisation protocol for *Shilajeet*. Limitation is that various previous studies cannot be compared and compiled to form conclusive data as in most of the studies [11,12,20-24], samples are from market; no in-situ sample collection has been done, so, origin of samples is unknown, types of samples in various studies vary *Shuddha/Ashuddha,* only specific analysis has been done by various researchers previously and there is huge variation in analytical findings for common criteria. Thus, none of the previous studies gave conclusive data about Characterisation.

As this study has been planned to collect the samples from site of occurrence and also samples from standard commercial sources shall also be studied. The analytical study of raw samples will be done, criteria being organoleptic, *pH*, Loss on drying, ash value, acid Insoluble ash, Water soluble Extractive and Alcohol soluble Extractive as per API protocol for *Ras Dravyas*. Also, as *Shilajeet* is rich in minerals, elements and organic constituents, the elemental analysis of the samples shall be done through Energy Dispersive X-Ray Analysis (EDX), to know the gross elemental composition [25-27]. In various previous studies [10,19] the Humic acid and Fulvic acid has been claimed as the organic constituents responsible for it's vast therapeutic properties, so in this study estimation of Humic acid and Fulvic acid will be an important part of this study. Further, an In-Vitro antioxidant study has been planned for the processed samples of *Shilajeet* of various origin, to initially assess the claimed *Rasayana,* rejuvenating and other vast therapeutic potential.

Observations of this study will provide qualitative and quantitative data for standardization of *Shilajatu* on the line of API protocol for *Ras Dravyas*. Also, the antioxidant study will provide an initial base and direction for working and evaluating the therapeutic potentials of *Shilajeet* of various origin.

5. CONCLUSION

A comparative data of different samples of *Shilajatu,* collected from site of natural and standard commercial sources, with respect to pharmaceutical work, analysis and In-Vitro antioxidant study will be generated.

6. SCOPE OF THE STUDY

i. A qualitative and quantitative data for characterization of Raw and Processed *Shuddha Shilajatu* shall be generated.
ii. Observations of this study will provide an Information and direction for further work on *Shilajatu.*

NOTE

The study highlights the efficacy of "ayurveda" which is an ancient tradition, used in some parts of india. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was received ethical approval from IEC; - Vide IEC letter of approval no.; - MGACHRC/IEC/June-2022/511(Dated; 18/06/2022).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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