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DETERMINATION OF HUMIC ACID AND FULVIC ACID IN SHILAJATU

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ABSTRACT

Shilajatu is a mineral explained in the treatises of Rasashastra. It is a pale brown to black coloured exudate available in mountainous regions. Based on the area it is collected from, the appearance and the composition varies. Research studies have put light that it is a combination of organic elements and inorganic substances. Shilajatu is said to comprise of humus; Humic substances are formed by the microbial degradation of dead plant matter, such as lignin. These are divided into three main fractions namely humic acids, fulvic acids, and humin.

High Performance Liquid Chromatography is a good method for quantification of fulvic acid and humic acid. The analysis proved that fulvic and humic acid content is more in the sample from Nepal.

Keywords: Shilajatu, Humic acid, Fulvic acid, HPLC

INTRODUCTION

Shilajatu, also known as salajit, shilajit, mumie or mummiyo, is a blackish-brown exudate coming out from layer of rocks

in many mountain ranges, especially in the Himalayas and Hindukush ranges of the Indian subcontinent.

In a research article it is said that Shilajatu comprises of humus (60–80 %) along with other components, such as benzoic acid, hippuric acid, fatty acids, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids and phenolic lipids. The major physiological action of Shilajit is said to be due to the presence of bioactive dibenzo- α -pyrones along with humic and fulvic acids, which acts as the carrier molecules for the active substances [1].

Humic substances are formed by the microbial degradation of dead plant matter, such as lignin. These can be divided into three main fractions: humic acids, fulvic acids, and humin (Humate fertilizer). Shilajatu has been found to consist of a complex mixture of organic humic substances and plant and microbial metabolites come about in the rock rhizospheres of its natural habitat. It is said that based on the ratio of fulvic acid (FA) with humic acid (HA), purity and action potential of shilajatu can be ascertained [2]. Humic substances are proved to be useful in musculoskeletal diseases, gynecological diseases and skin disease [3].

Fulvic acid is one of the major constituents of shilajatu. Fulvic acid is known to benefit digestion and helps the body absorb nutrients from food. Fulvic acid helps water

enter cells at a faster rate, leading to balanced hydration and mineral levels. Moreover, fulvic acid actively takes part in the transportation of nutrients into deep tissues and helps to overcome tiredness, lethargy, and chronic fatigue. It is also said that it works effectively as a tonic for the cardiac, gastric, and nervous system, adaptogen and anti-stress agent. Fulvic acid has been shown to discourage age-related cognitive impairment, Alzheimer's disease, jaundice, bronchitis and anemia as well. Thus, Shilajitu can be used as a source of fulvic acid and can be used as a material for prevention and treatment of a number of diseases [4].

The primary organic substance in aqueous extracts of Shilajit humus is fulvic acid (FA), and it has been suggested that FA may account for many biological and medicinal properties of Shilajit. FA has been used externally to treat hematoma, phlebitis, desmorrhesis, myogelosis, arthrosis, polyarthrititis, osteoarthritis, and osteochondrosis [5].

Shilajatu samples from different regions of the world vary in their physiological properties. Quantification of fulvic acid and humic acid in shilajitu is important to standardize and authenticate the samples.

MATERIALS AND METHODS

Shilajatu was collected from two sources one sample from India and another one sample from Nepal and authenticated by a geologist. High Performance Liquid Chromatography (HPLC) to determine Humic acid and Fulvic acid was carried out at BioChem Research & Testing Laboratory, Dharwad.

The samples were subjected to HPLC to quantify humic acid and fulvic acid.

Extraction of Fulvic and Humic acid [6, 7]

Sample (1.0 g) was extracted with methanol for removal of bioactive compounds. The residue was dissolved in 0.1N NaOH by vortexing. The suspension was filtered and the filtrate was acidified to a pH of less than 3 to precipitate the humic acid. The humic acid obtained was dissolved in 5 mL methanol and analyzed using HPLC. The filtrate obtained after removal of humic acid was shaken with macroporous ion-exchange resin in order to adsorb the FA, which was

then eluted using 0.1 N aqueous sodium hydroxide solution. The FA obtained in alkali was passed through hydrogen saturated cation exchange resin in order to exchange the sodium ions with hydrogen ions. The obtained fulvic acid solution is concentrated in vacuum evaporator, dissolved in methanol and analyzed using HPLC. The humic acid and fulvic acid extracts were pooled and quantified using HPLC.

HPLC conditions

Column – C18 (Waters, Spherisorb)

Mobile phase – A (water + 1%acetic acid), B (acetonitrile)

Gradient - Mobile phase B was maintained for 20 minutes, increased from 25% to 40% for 6 minutes, and maintained for 9 minutes.

Run time – 15 min

Flow rate – 0.7 mL/min

Column temperature – 28 °C

Wavelength – 230 nm

Detector – Photo diode array

RESULTS

Table 1: Quantitative parameter of Fulvic acid and Humic acid

Parameter	Shilajatu (Nepal)- Sample 1	Shilajatu (India)- Sample 2
Fulvic acid (%)	23.31	9.13
Humic acid (%)	15.25	3.53

Chromatogram- Images

1. Shilajatu (Sample from Nepal)

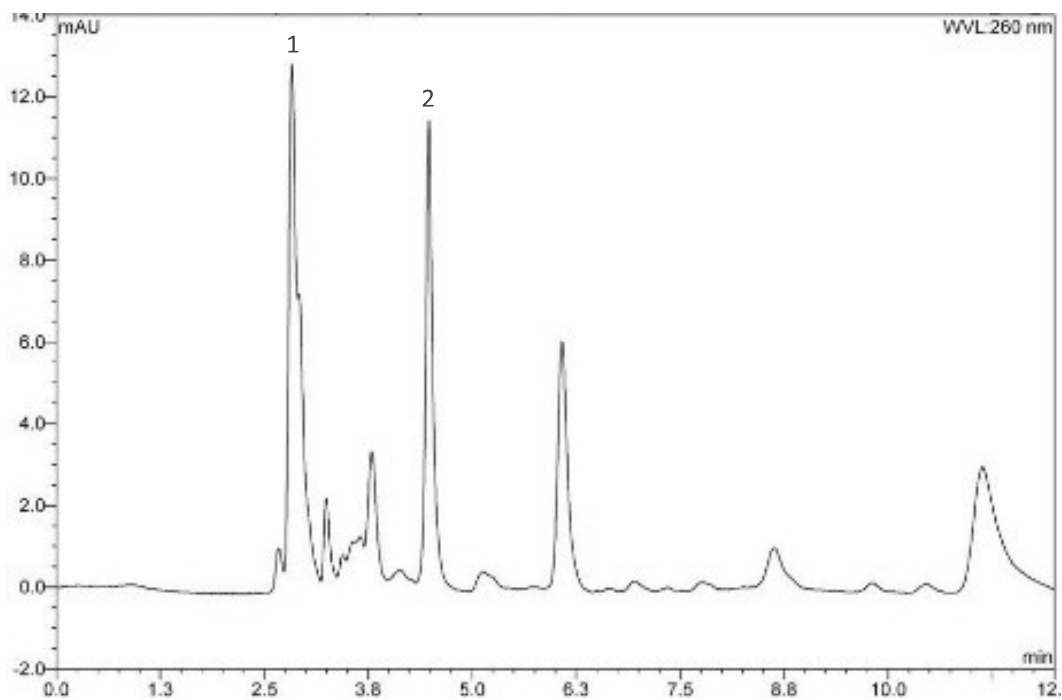


Figure 1

2. Shilajatu (Sample from Local market, India)

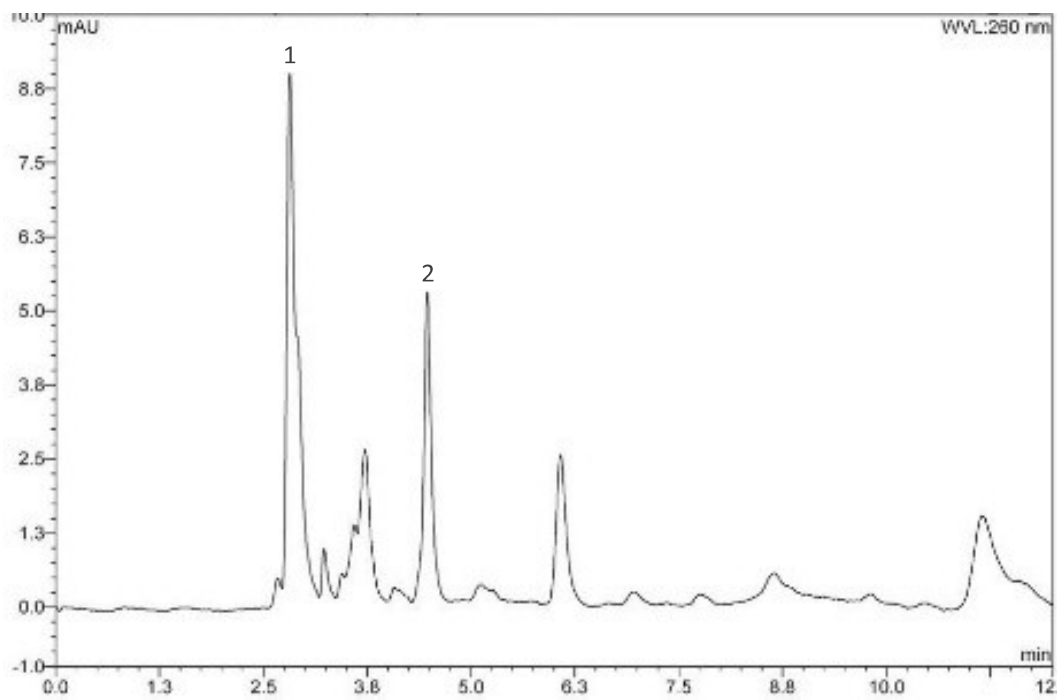


Figure 2

Note: 1 – Fulvic acid, 2 – Humic acid

DISCUSSION

The shilajatu collected from two different sources were black in colour with odour of cow's urine, which is due to the presence of hippuric acid. Standardization is defined as identification and quantification of one or more biochemical constituents using a marker compound [8].

The samples are collected from two different sources thereby their composition varies and hence and the quantity of humic acid and fulvic acid in the samples is also different. Humic substances are divided as humic acids, fulvic acids, and humin. Humic acid and fulvic acids are used therapeutically in different diseases.

Fulvic acid is said to have a unique capacity to dilate and permeate the thick cell walls to transmit the minerals into the cells, thereby overcome tiredness, lethargy, and chronic fatigue [9]. The HA application on human keratinocyte pre-treated with Urban dust revealed a general increase of viability suggesting a protective effect of humic matter due to the content of aromatic, phenolic and lignin components [10].

In sample 1 the FA is higher to HA. It is said that if FA is more than HA, then the sample is good; or vice versa. The reactive moiety is equal to FA. This was based on the principle that, in agricultural science

composting is believed to yield humus like substances through various biochemical process. This influences the quality of soil organic matter [11].

CONCLUSION

Shilajatu is said to consist of a complex mixture of organic humic substances and plant and microbial metabolites that come about in the rock rhizospheres of its natural habitat. Humic substances are formed by the microbial degradation of dead plant matter such as lignin; conversion of lignin and other components into humic substances like humic acids, fulvic acids, and humin. The ratio of Humic acid and Fulvic acid content of sample 1 was higher indicating that this sample is better than the sample 2.

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