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Physico-chemical, Spectral and Thermal Characterization of Shilajit, A Humic Substance with Medicinal Properties†

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In the present study, shilajit samples from various sources were characterized based on their physico-chemical properties, UV, FTIR and ^{1}H NMR spectra, X-ray diffraction pattern and DSC thermograms. All the samples showed a distinct similarity with respect to their physico-chemical as well as spectral and thermal characteristics. Being natural in origin, some variation can be expected and was seen in these samples too. The studies confirmed the complex multicomponent and humic nature of shilajit.

Key Words: Shilajit, ^{1}H NMR, FTIR spectra, DSC, XRD, Scanning electron microscopy.

INTRODUCTION

Shilajit, also known as salajit, shilajatu, mumie or mummiyo is a pale-brown to blackish-brown exudation, of variable consistency, coming out from layer of rocks in many mountain ranges of the world, especially the Himalayan ranges of the Indian subcontinent[1,2] (Fig. 1). It is also found in many other mountain ranges of the world, *e.g.*, Afghanistan (Hindukush, Badakh-Shan), Australia (Northern Pollock Ranges) and in the former USSR (Tien-Shan, Pamir, Caucasus, Ural), where it is collected[3] in small quantities from steep rock faces at altitudes between 1000 and 5000 m. Shilajit samples from different region of the world however vary in their physiological properties. Although the name shilajit means Winner of Rock, shilajit itself is not a rock comprising inorganic material but is a complex mixture of organic humic substances and plant and microbial metabolites occurring in the rock rhizospheres of its natural habitat[4].

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Fig. 1. Rock Shilajit in its raw form

Shilajit has been used for thousands of years in one form or the other as food, tonic or medicine as part of folk medicines in a number of countries[5-7]. Shilajit has been used traditionally under the indigenous systems of medicine like ayurveda and unani as a rasayana or health tonic[8]. Many therapeutic properties have been ascribed to it, a number of which have been verified by modern scientific evaluation[5,9]. It has been said that there is hardly any curable disease which cannot be controlled or cured with the aid of shilajit. Although this is a tall order, scientific studies over the last 20-25 years have shown that it is indeed a panacea of traditional medicine, effective in a number of ailments.

Extensive research has been carried out to know the exact chemical nature of shilajit. It has been found to be mainly comprising of humus (60-80 %) along with other components such as benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin and waxy materials, gums, albuminoids, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids and phenolic lipids[10-12]. Humus or humic substances are organic materials that are formed by the profound alteration of organic matter in natural environments. The biological effects of shilajit have been ascribed to two distinct classes of compounds[13]: (1) The low molecular weight bioactive organic compounds such as oxygenated dibenzo-α-pyrones and (2) The medium molecular weight fulvic acids and humic acids. While the benzopyrones act as the active principles, fulvic and humic acids act as carrier molecules for *in vivo* transportation of these bioactive substances[13].

While a lot of work has been reported on the nature and chemical constituents of shilajit, not much work has been reported on its spectral and thermal characterization. The present study deals with the physico-chemical, spectral and thermal characterization of shilajit from different sources in India. Shilajit, being a scanty and costly material, is often adulterated with non-humic materials such as rock soil, plant debris and quercus gums[11]. The data obtained in this study will not only be useful in documenting the variability observed in the samples from different sources but will also be useful for its authentication and differentiation from non-humic adulteration.

EXPERIMENTAL

An authentic sample of rock shilajit was obtained from Dabur Research Foundation, Ghaziabad, India. Dried shilajit extracts were also obtained from three different commercial sources in India, *viz.*, Pioneer Enterprises (PE), Mumbai, Natural Remedies (NR), Bangalore and Gurukul Kangri (GK), Haridwar. All the samples were characterized based on their physico-chemical properties such as colour, odour, taste, solubility in water and other organic solvents, *etc*. Scanning electron microscopy, elemental and spectral analysis such as UV, FTIR, ^{1}H NMR and XRD diffraction were performed. Thermal properties of the samples were analyzed by means of DSC thermograms.

UV-Vis spectroscopy: UV-Vis spectra of various shilajit samples were obtained on a Shimadzu, 1601 UV/VS spectrophotometer by dissolving the various shilajit samples in water and recording the spectra in a 1 cm quartz cuvette by scanning from 200 to 800 nm. Since humic substances usually yield uncharacteristic spectra in the UV and visible, E4/E6 ratio (ratio of the absorbance of the solution at 465 and 665 nm)[14] was determined for the various samples.

Fourier transforms infrared spectroscopy: The infrared spectra of Shilajit samples were recorded on a FTS 40 (BioRad, USA) FTIR instrument and WIN IR software by the KBr pellet technique. 2 mg of previously dried sample was mixed with 100 mg KBr and compressed into a pellet on an IR hydraulic press. These pellets were made immediately prior to the recording of the spectrum. Scanning was done from 4000 to 450 cm^{-1}.

^{1}H Nuclear magnetic resonance spectroscopy: ^{1}H NMR spectra of shilajit samples were recorded using a Bruker DRX-300 NMR spectrometer. All the spectra were recorded in deuterated DMSO (DMSO-d_6) solvent. DMSO-d_6 was preferred to other NMR organic solvents since it is a good solvent for humic substances[15].

Differential scanning calorimetry: A perkin elmer pyris 6 instrument was used for recording DSC thermogram of the shilajit samples obtained from different sources. Samples (2-3 mg) were accurately weighed and heated in closed aluminium crimp cells at a rate of 10 ºC/min under nitrogen purge with a flow rate of 20 mL/min over the temperature range of 50-300 ºC.

Powder X-ray diffraction: Powder X-ray diffraction patterns of powdered samples of shilajit were obtained using a Panalytical X-ray diffractometer PW3719. All the samples were treated according to the following specifications:

Target/filter (monochromator) : Cu; Voltage/current : 40 KV/50 mA; Scan speed : 4°/min; Smoothing : 0

Scanning electron microscopy: Scanning electron micrographs of powdered samples were obtained using a Joel JSM-840 scanning microscope with a 10 KV accelerating voltage. The surfaces of samples for SEM were made electrically conductive in a sputtering apparatus (Fine Coat Ion Sputter JFC-1100) by evaporation of gold.

RESULTS AND DISCUSSION

Shilajit samples obtained from different sources showed a distinct similarity with respect to their physico-chemical characteristics. Being natural in origin, some variation can be expected and was seen in these samples also. All the samples were brownish black in colour and had a typical characteristic odour and taste. The characteristic odour of shilajit which resembles that of cow's urine has been attributed to the presence of a number of compounds including 2-ethylhexanoic acid, *p*-cresol, 3-4-ethylphenol, naphthalene, benzothiazole, 2,4-dimethylquinoline and 2,4-*bis*(1,1-dimethylethyl) phenol[16]. The pH of a 2 % aqueous solution ranged from about 5.0 to 7.0 while the E4/E6 ratio for all the samples ranged from about 2.5 to 3.5. The results are shown in Table-1 and are consistent with those reported in the literature[13,16].

The UV-Visible spectra of the various samples of shilajit are shown in Fig. 2. The samples did not exhibit any sharp maxima and the absorbance value decreased with increasing wavelength. A lack of maxima can be expected considering the complicated, multicomponent nature of shilajit with the variety of chromophores of its constituents contributing variable absorptivities. The results are consistent with those obtained for humic rich soils and humic substances[14]. A shoulder was observed at the region around 270 nm which is generally attributed to the absorbance of the aromatic groupings which are characteristic of humification[17].

FTIR Spectra of the samples were characterized by a relatively few bands that were broad. This is expected since shilajit consists of a complex mixture of diverse materials and extensive overlapping of individual absorption peaks due to various functional groups present is likely to

TABLE-1

COMPARISON OF THE PHYSICAL CHARACTERISTICS OF SHILAJIT FROM DIFFERENT SOURCES

Characteristic	Shilajit (Dabur)	Shilajit (GK)	Shilajit (NR)	Shilajit (PE)
Nature	Brownish black rock	Brownish Black semisolid mass	Brownish black powder	Brownish black solid mass
Colour	Brownish black	Brownish black	Brownish black	Brownish black
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Characteristic pungent and astringent	Characteristic pungent and astringent	Characteristic pungent and astringent	Characteristic pungent and astringent
Solubility	Partially soluble in water and alkali. Slightly soluble in ethanol and methanol. Almost insoluble in chloroform and ethyl acetate.	Partially soluble in water and alkali. Slightly soluble in ethanol and methanol. Almost insoluble in chloroform and ethyl acetate.	Partially soluble in water and alkali. Slightly soluble in ethanol and methanol. Almost insoluble in chloroform and ethyl acetate.	Partially soluble in water and alkali. Slightly soluble in ethanol and methanol. Almost insoluble in chloroform and ethyl acetate.
pH of 2% aq. solution	5.52	6.63	6.70	5.06
Absorbance at 465 nm (E_4)	0.455	0.655	0.532	0.492
Absorbance at 665 nm (E_6)	0.145	0.245	0.159	0.157
E_4/E_6 ratio	3.14	2.67	3.33	3.13

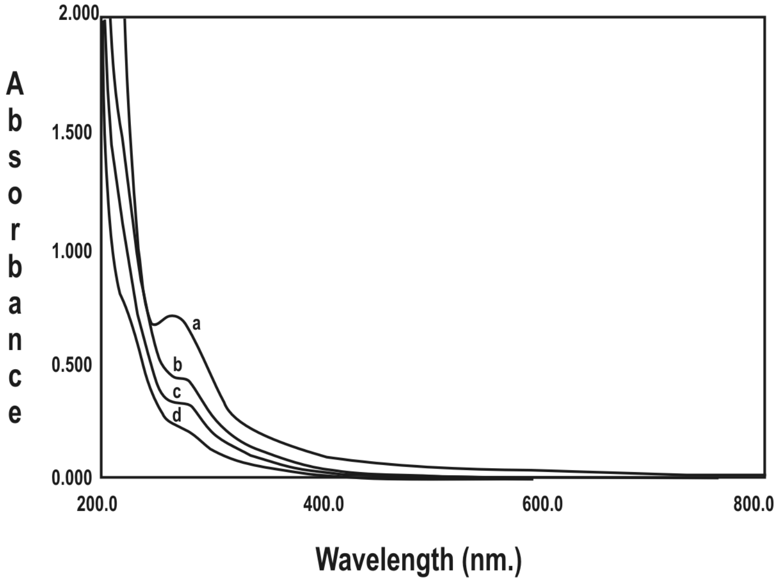

Fig. 2. UV-Visible spectra of Shilajit from different sources: (a) PE (b) NR (c) Dabur (d) GK

occur. The results are consistent with those reported in the literature for humus rich soil and humic and fulvic acids[14,18].

Taking a clue from the extensive work done on the humic substances derived from soil, peat, *etc.*, the broad bands observed in the spectrum of shilajit at 3390, 1725 and 1638 cm^{-1} can be attributed to hydrogen bonded OH group, C=O stretching of COOH and C=C double bond, respectively[19-21]. The sharp bands observed at 2923, 1386 and 1036 cm^{-1} can be attributed to the bending vibration of aliphatic C-H group, the O-H bending vibrations of alcohols or carboxylic acids and OH bending deformation of carboxyl groups, respectively. The spectrum reflects the preponderance of oxygen-containing functional groups like COOH, OH and C=O in shilajit.

A typical ^{1}H NMR spectra obtained with shilajit sample and spectra from other samples exhibited a combination of broad and sharp absorption signals which suggest the presence of high molecular weight, complex, heterogenic, multi-component systems, as would be expected. The spectra may be divided into three main regions: (1) 0.5-2 ppm, signals due to methyl and methylene protons; (2) 2-5 ppm, signal due to H in structures containing methyl or methylene groups linked to heteroatoms, such as O and N, as well as H of OH groups in alcohols, phenols and carboxyls and (3) 6-8 ppm, signals due to aromatic protons. The results are similar to those observed in case of humic substances from other sources[15, 20,21].

Shilajit samples did not exhibit any sharp endotherm indicating that it does not have any defined melting point. A shallow endotherm could be observed near 90 °C which could be attributed to dehydration of the samples. An exothermic event could be observed at a temperature above 250 °C which could be attributed to the thermal degradation of carbohydrates, dehydration of aliphatic structures and decarboxylation of carboxylic groups[22]. A few more thermal events seen in some of the samples could be attributed to the presence of small amounts of lipid crystallites and other components of natural organic matter[23].

A typical X-ray diffractogram of the rock shilajit sample from Dabur, India is shown in Fig. 3. The sample largely exhibited a non-crystalline nature as evident from the absence of sharp diffraction peaks. A few peaks were however observed which could be attributed to the presence of lipid crystallites, clay particles and other crystalline and micro-crystalline materials in the shilajit sample. The behaviour is consistent with the multi-component nature of shilajit and with the behaviour observed in case of humic substances from other sources[23,24].

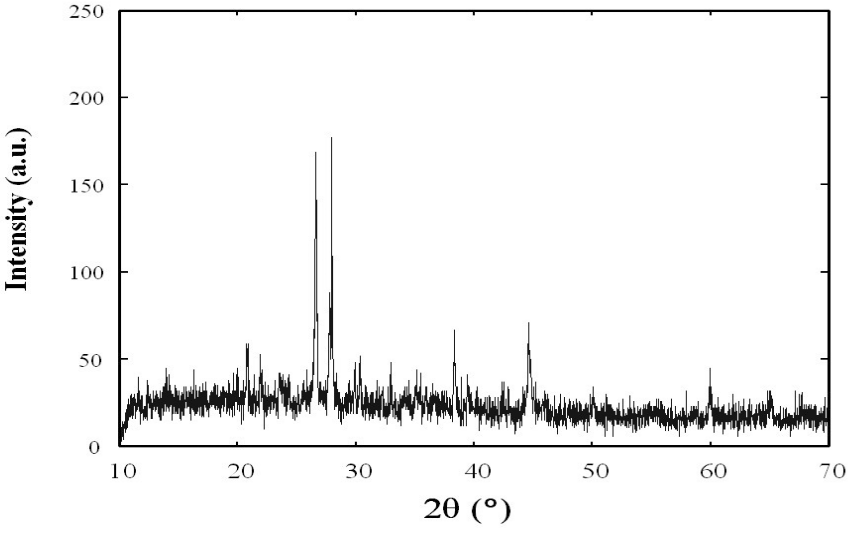

Fig. 3. X-ray diffraction pattern of Rock Shilajit (Dabur)

Fig. 4 shows a scanning electron micrograph of the powdered shilajit sample from Dabur at magnification of 1500x. The shilajit particles exhibited a spongy structure with presence of internal spaces. The particles also showed a tendency to aggregate.

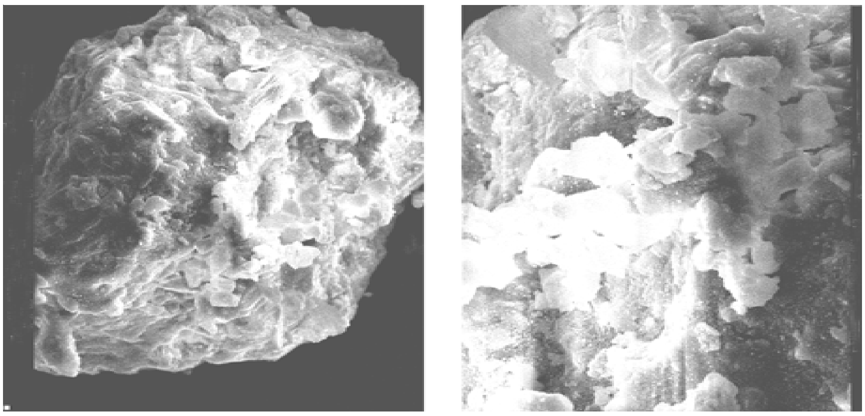

Fig. 4. Scanning electron micrographs of Rock Shilajit (Dabur)

Conclusion

Shilajit samples from various sources were characterised and compared for their physico-chemical, spectral and thermal properties. All the samples showed a distinct similarity with respect to their physico-chemical as well as spectral and thermal characteristics. Being natural in origin, some variation can be expected and was seen in these samples also. The spectral properties confirmed the complex multicomponent and humic nature of shilajit.

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