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Humic acid from Shilajit – a physico-chemical and spectroscopic characterization

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Abstract: Shilajit is a blackish–brown exudation, consisting of organic substances, metal ions and minerals, from different formations, commonly found in the Himalayan region (1000–3000 m) from Nepal to Kashmir. Shilajit can also be collected throughout the mountain regions in Afghanistan, Bhutan, China, Bajkal, throughout Ural, Caucasus and Altai mountains also, at altitudes between 1000 to 5000 m. The major physiological action of shilajit has been attributed to the presence of bioactive dibenzo- α -pyrones together with humic and fulvic acids, which act as carrier molecules for the active ingredients. In this work, the aim was to extract humic acid from Shilajit from various sources and characterised these humic acids based on their physicochemical properties, elemental analysis, UV/Vis and FTIR spectra, X-ray diffraction pattern and DSC thermograms. The spectral features obtained from UV/Vis, FTIR, XRD and DSC studies for samples of different origins showed a distinct similarity amongst themselves and in comparison to soil humic acids. The surfactant properties of the extracted fulvic acids were investigated by determining the effect of increasing concentration on the surface tension of water. The study demonstrated that humic acids extracted from shilajit indeed possessed surfactant properties.

Keywords: Shilajit; humic acid; FTIR spectra; DSC; XRD; surfactant properties.

INTRODUCTION

Shilajit, also known as salajit, shilajatu, 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.¹ It is also found in Russia, Tibet, Norway and other countries, where it is collected in small quantities from steep rock faces at altitudes between 1000 and 5000 m. Shi-

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lajit samples from different regions of the world, however, vary in their physiological properties.²

It mainly consists of palaeohumus (around 80–85 %) and organic compounds derived from vegetation fossils that were compressed under layers of rocks for hundreds of years and underwent significant metamorphosis due to the prevalent high temperature and pressure conditions.⁵

Extensive research has been performed to determine the exact chemical nature of Shilajit. Earlier work on shilajit showed that its major organic constituents included benzoic acid, hippuric acid, fatty acids, resin and waxy materials, gums, albuminoids and vegetable matter with benzoic acid being the active substance.^{6,7} Extensive research in the eighties showed that the major organic mass of Shilajit was comprised 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.⁸ The major physiological action of Shilajit was found to be due to the presence of bioactive dibenzo- α -pyrones along with humic and fulvic acids, which acted as the carrier molecules for the active substances.^{9–11} Recently, the physico-chemical, spectral and thermal properties of shilajit and its humic substances were reported, which further confirmed its humic nature.^{12–15} Elemental analysis and spectroscopic techniques, such as UV/Vis, FTIR and X-ray diffraction and DSC analyses have been widely used for the characterization of humic acids obtained from lignite, charcoal, soil, sewage sludge and compost.^{16,17} In this study, these methods were applied for the first time to humic acids extracted from Shilajit from different sources.

EXPERIMENTAL

Materials and methods

An authentic sample of rock Shilajit (RS) 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. The humic acid was extracted from all the samples of Shilajit and characterised based on their physico-chemical properties and their elemental analysis. Scanning electron microscopy and spectral analysis, such as UV/Vis, FTIR, DSC and X-ray diffraction, were performed. The E_4/E_6 ratio was also determined. The spectral properties were compared with a humic acid standard from Sigma Aldrich.

Extraction of humic acid from Shilajit

Finely powdered shilajit was successively extracted¹⁸ with 500 ml each of hot organic solvents of increasing polarity, *i.e.*, chloroform, ethyl acetate and methanol, to remove the bioactive components, specifically oxygenated dibenzo- α -pyrones. The so-obtained extracted Shilajit was taken and dispersed in 0.10 M aqueous sodium hydroxide with intermittent shaking under nitrogen at room temperature for 24 h. The suspension was filtered to remove humin (insoluble in water at all pH values) and the filtrate was acidified with dilute HCl to a pH of less than three. The solution was allowed to stand at room temperature (25 °C) overnight. The humic acid, which separated out as a coagulate, was filtered, dried and pulverized.

Elemental analysis

The C, H, N and S contents were determined by packing the fulvic acid powder in tin boats after careful weighing (Balance – Mettler Toledo, MX5) with the aid of a CHNS analyzer (Vario EL-III). The obtained values are expressed as dry weight of powder, in mass %.

UV/Vis Spectroscopy

The UV/Vis spectra of various HA extracted from shilajit of different origins were obtained on a Shimadzu, 1601 UV/Vis spectrophotometer by dissolving the various HA samples in water and recording the spectra in a 1 cm quartz cuvette in the wavelength range 200–800 nm. Since humic substances usually yield uncharacteristic spectra in the UV and visible, the E_4/E_6 ratio (ratio of the absorbance of the solution at 465 and 665 nm)¹⁹ was determined for the various samples.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of HA samples were recorded on a Win-IRrez (Bio-Rad, Hercules, CA, USA) using the potassium bromide (KBr) disc technique. The samples (2 mg) were mixed with potassium bromide (about 100 mg) in a clean glass pestle and mortar and compressed to obtain a pellet. The base line was corrected and scanning was performed from 4000–400 cm^{-1} .

Powder X-ray diffraction

Powder X-ray diffraction patterns of powdered samples of HA 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 $^\circ/\text{min}$.

Differential scanning calorimetry (DSC)

A Perkin–Elmer Pyris 6 instrument was used for recording DSC thermograms of the HA samples obtained from different shilajit sources. Samples (2–8 mg) were accurately weighed and heated in closed aluminium crimp cells at a rate of 10 $^\circ\text{C}/\text{min}$ under a dynamic nitrogen atmosphere (flow rate 20 ml/min) over the 50–300 $^\circ\text{C}$ temperature range.

Scanning electron microscopy

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

Surfactant properties

The surfactant properties of the humic acids were investigated by determining the effect of increasing the concentration of humic acid on the surface tension of water. The surface tension of the solutions was determined by the drop-weight method using a stalagmometer. Solutions of fulvic acids in the concentration range 0–1.4 % w/v were prepared. Each solution was separately sucked into the stalagmometer and allowed to drop slowly from it. The drop rate was adjusted to approximately 2–3 drops/min. and the weight of 10 drops was measured.

RESULTS AND DISCUSSION

Extraction of humic acid from shilajit

The yields obtained at the different stages of the earlier reported method and the improved method for the extraction of humic acid from shilajit are compared in Table I. The yields of HA extracted from shilajit from Dabur, Gurukul Kangri,

Natural Remedies and Pioneer Enterprises were 2.5, 9.2, 8.7 and 8.7 %, respectively. These are high proportion compared with those reported in the literature.¹⁸ The maximum yield of HA was obtained from the Gurukul Kangri shilajit.

TABLE I. Comparison of the yields of humic acid from Shilajit obtained from different sources

Shilajit	Yield of fulvic acid, %							
	Reported method				Improved method			
	I	II	III	Mean \pm SD	I	II	III	Mean \pm SD
RS	1.2	1.0	1.4	1.2 \pm 0.2	2.2	2.8	2.6	2.5 \pm 0.3
GK	7.5	7.8	7.3	7.5 \pm 0.3	9.2	8.9	9.5	9.2 \pm 0.3
NR	5.8	6.2	6.9	6.3 \pm 0.6	8.1	8.9	9.1	8.7 \pm 0.5
PE	6.9	6.4	7.1	6.8 \pm 0.4	9.3	8.8	8.5	8.9 \pm 0.4

Physical characteristics

The physical characteristics of the humic acids extracted from Shilajit of different origin are listed in Table II. The extracted HA from shilajit of different origins exhibit very similar physico-chemical characteristics, indicating no qualitative variation in the Shilajit samples and in their extraction procedure.¹⁸ Slight variations are to be expected and were seen in these HA samples. All the HA samples were brownish black in colour and had a typical characteristic odour and taste. The pH of 2 % aqueous solutions ranged from 3.46 to 3.86. The ratio of the absorbance at 465 and 665 nm (E_4/E_6) has been widely used by soil scientist for characterization purposes. The E_4/E_6 ratio for all the examined HA samples ranged from about 3.0 to 4.0, which are consistent with those reported in the literature.¹⁷

Elemental analysis

Elemental analysis of humic substances is generally used to establish their nature and origin.²⁰ As shown in Table III, a comparison of carbon, hydrogen, ni-

TABLE II. Comparison of the physical characteristics of humic acid from Shilajit of different origins

Characteristic	Humic acid (RS)	Humic acid (GK)	Humic acid (NR)	Humic acid (PE)
Nature	Dark brown powder	Dark brown powder	Dark brown powder	Dark brown powder
Colour	Dark Brown	Dark Brown	Dark Brown	Dark Brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Characteristic	Characteristic	Characteristic	Characteristic
pH of 2 % aq. solution	3.86	3.77	3.46	3.68
Absorbance at 465 nm (E_4)	0.513	0.542	0.284	0.222
Absorbance at 665 nm (E_6)	0.144	0.180	0.072	0.072
E_4/E_6 ratio	3.56	3.01	3.94	3.08

trogen and sulphur contents of the humic acids extracted from Shilajit of different origins with those of soil humic acids²¹ and the Sigma Aldrich standard humic acid revealed that the content of C, H, N and S were very low in the case of the humic acids extracted from the pioneer shilajit. The carbon, hydrogen, nitrogen and sulphur contents also varied significantly among the samples of humic acids. These differences may be due to differences in the origin, different isolation techniques and error in sampling and analysis. The C/N ratio also varied among the samples of humic acids.

TABLE III. Elemental analysis of humic acids extracted from shilajit of different origins

Source of humic acid	% C	% H	% N	% S	C/N ratio
Rock Shilajit (Dabur)	36.46	5.15	3.03	0.70	12.0
Shudh Shilajit (Gurukul Kangri)	45.36	5.92	2.31	0.39	19.63
Shilajit extract (Natural Remedies)	51.48	5.89	3.27	0.81	15.73
Shilajit extract (Pioneer Enterprises)	27.44	2.90	1.24	0.26	22.10
Sigma Aldrich (Std. HA)	42.28	4.25	0.57	0.81	73.09

UV/Vis Spectra

The UV/Vis spectra of the various samples of humic acids extracted from Shilajit of different origin were recorded in water from 200 nm to 800 nm are shown in Fig. 1. The samples did not exhibit any sharp maxima but exhibited a slight hump near 260–280 nm, which is characteristic of humic substances.¹⁹ As discussed previously, this hump is attributed to the absorption of radiation by the double bonds C=C, C=O and N=N of the aromatic or unsaturated components of humic

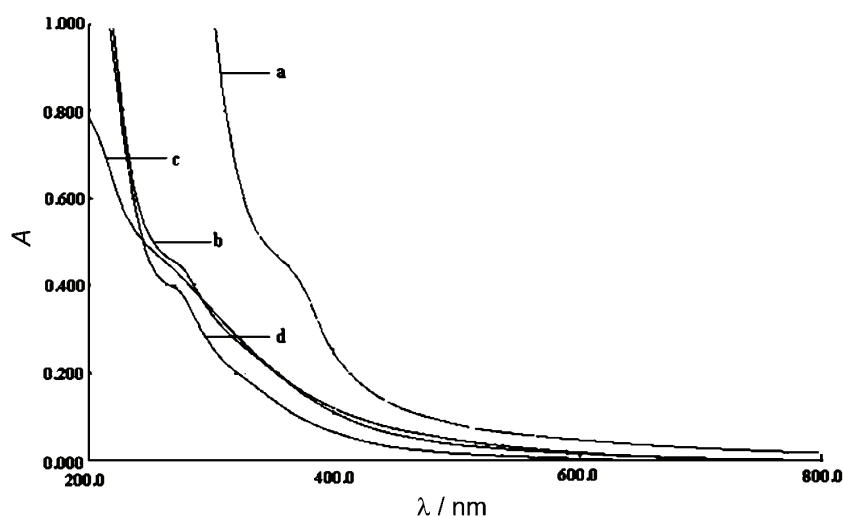


Fig. 1. UV/Vis Spectra of humic acid extracted from Shilajit of different origins: a) RS, b) GK, c) NR and d) PE.

substances.²² The variation in the hump observed with the different samples of Shilajit could be attributed to variations in the concentrations of aromatic compounds, which in turn is characteristic of the difference in the humification process.

FTIR Spectra

The FTIR spectra (Fig. 2) of the extracted humic acids were characterised by relatively few broad bands. All the humic acid samples exhibited broad bands at about 3400, 1725 and 1630 cm^{-1} , which can be attributed to hydrogen bonded OH groups, C=O stretching of COOH groups and C=C double bonds, respectively. Sharp bands were observed in the region of 2925, 1400 and 1050 cm^{-1} , which can be attributed to the bending vibration of aliphatic C-H groups, the O-H bending vibrations of alcohols or carboxylic acids and the OH bending deformation of carboxyl groups, respectively.¹⁹

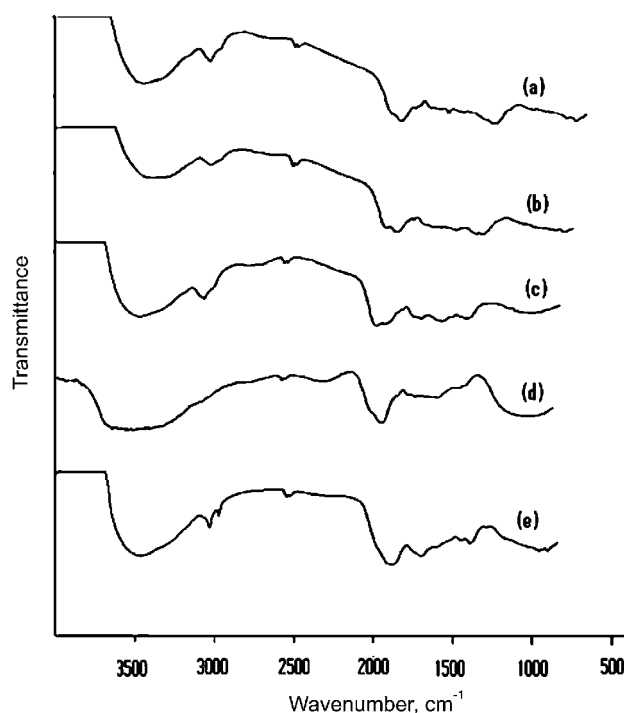


Fig. 2. FTIR Spectra of humic acid extracted from Shilajit of different origins: a) RS, b) GK, c) NR, d) PE and e) Laurentian humic acid.

X-Ray diffraction pattern

The X-ray diffraction pattern in the 2θ range from 10 to 70° of humic acid extracted from rock a shilajit sample (Fig. 3) exhibited very small diffuse peaks with a few intense peaks, implying its non-crystalline nature. This behaviour is

consistent with the behaviour observed in the case of humic substances from other sources.^{23,24}

Differential scanning calorimetry (DSC)

The humic acid of pioneer Shilajit exhibited no sharp endothermic peak, indicating that it does not have any defined melting point (Fig. 4). A shallow endotherm could be observed near 100 °C, which could be attributed to dehydration of the sample. On the other hand, it showed an exothermic peak near 331 °C, which could be attributed to the thermal degradation of carbohydrates, dehydration of aliphatic structures and decarboxylation of carboxylic groups.²⁴

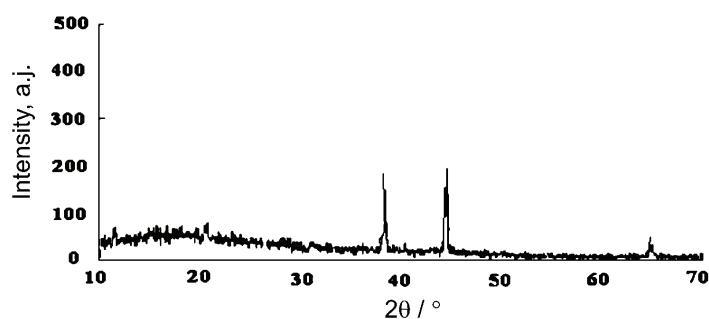


Fig. 3. XRD Pattern of humic acid extracted from the rock Shilajit.

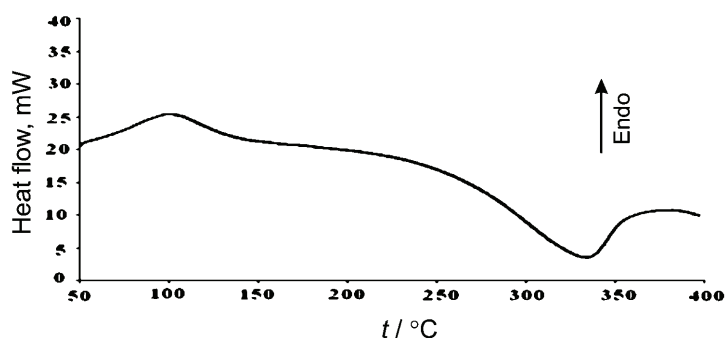


Fig. 4. DSC Spectra of humic acid extracted from the Pioneer Shilajit.

Scanning electron microscopy

The scanning electron micrographs (Fig. 5) of humic acid extracted from rock Shilajit of Dabur showed a loose spongy structure of humic acids with the particles tending to aggregate to each other.

Surfactant properties

As can be seen in Fig. 6, increasing the concentration of extracted humic acids in water clearly led to a decrease the surface tension. The decrease was ini-

tially gradual until a concentration of about 0.8 %, w/v, after which it rose slightly and then became almost constant. This could be due to the formation of micelle at this concentration. This demonstrates that humic acids extracted from Shilajit indeed possess surfactant properties. The value of 0.8 %, w/v, for the critical micelle concentration (CMC) is in agreement with the reported value of 0.7 %, w/v, for humic acids extracted from soil.²⁶

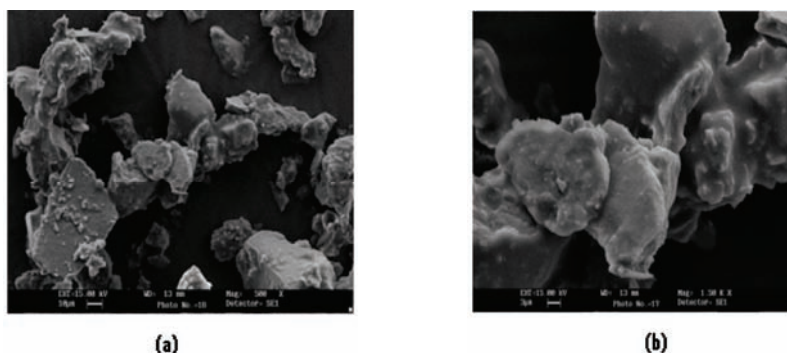


Fig. 5. Scanning electron micrographs of humic acid from rock shilajit; a) 500 \times ; b) 1500 \times .

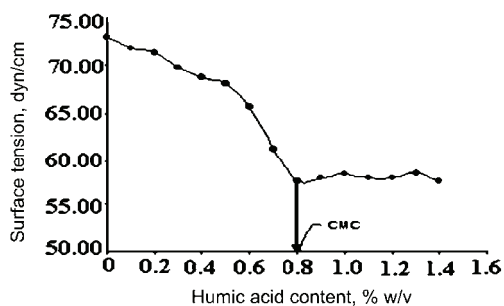


Fig. 6. Effect of humic acid concentration on the surface tension of water.

CONCLUSIONS

Humic acids from the various samples of shilajit were characterised and their physico-chemical and spectral properties compared. Such results are presented for the first time herein. The spectral features obtained from UV/Vis, FTIR, XRD and DSC studies for samples of different origins showed a distinct similarity amongst themselves and in comparison to soil humic acids. The surfactant properties of humic acids were investigated by determining the effect of increasing concentration of humic acids on the surface tension of water. The study demonstrated that the humic acids extracted from shilajit indeed possessed surfactant properties.

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ИЗВОД

ХУМИНСКА КИСЕЛИНА ИЗ ШИЛАЦИТА – ФИЗИЧКО–ХЕМИЈСКА И СПЕКТРОСКОПСКА КАРАКТЕРИЗАЦИЈА

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Шилацит је црно-мрк ексудат, који се састоји од органских супстанци, металних јона и минерала, различитог састава. Широко је распрострањен у хималајској регији (на висинама 1000–3000 m) од Непала до Кашмира. Шилацит се такође може наћи у планинским регијама Авганистана, Бутана, Кине, Бајкала, као и на Уралу, Кавказу и Алтају, на висинама између 1000 и 5000 m. Основна физиолошка активност шилацита приписана је присуству биоактивних дибензо- α -пирона поред хуминске и фулвинске киселине, које делују као носећи молекули активних састојака. У овом раду, циљ је био да се екстрахују хуминске киселине из шилацита различитог порекла и да се оне окарактеришу на основу физичко–хемијских својстава, елементалне анализе, UV/Vis и FTIR спектра, дифрактограма X-зрака и DSC термограма. Спектралне карактеристике узорака различитог порекла добијене UV/Vis, FTIR и XRD методама, као и информације добијене DSC техником, показале су очигледну међусобну сличност узорака и сличност са хуминским киселинама из земљишта. Испитивана је и површинска активност екстрахованих фулвинских киселина одређивањем утицаја њихове концентрације на површински напон воде. Испитивања су показала да су екстраховане хуминске киселине заиста површински активне.

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