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Biochemical and Trace Mineral Analysis of Silajit Samples From Pakistan

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Silajit **is a blackish brown pitch-like gummy substance. It is an important *Unani* and *Ayurvedic* medicine widely used in the treatment of many diseases and as nutritional supplement. It is found in certain mountainous regions of Pakistan, Afghanistan, India, Nepal, Australia and Russia. *Silajit* samples of Pakistani origin were obtained from different sources and analyzed for their physical characteristics and biochemical & elemental composition. X-ray diffraction studies on *Silajit* showed that it is an amorphous substance and has no crystalline structure. Also, pH studies of *Silajit* (2 g/L of water) indicated that it was slightly acidic in nature and ranged from 3.45 to 7.23. Conductivity ranged from 157.7 to 330. Amino acid analysis revealed that *Silajit* contained lysine and alanine in higher amounts than all other amino acids and ranged from 1456 to 2240 and 68 to 1615 ηmole/g, respectively. Mean concentration of arsenic, mercury, cadmium, lead, copper and zinc was 73.15, 104.92, 0.496, 3.89, 4.04 and 17.23 ppm, respectively. *Silajit* samples were also analyzed for calcium, potassium and sodium.**

Key words: *Silajit*, Trace minerals, Electrolytes, *Ayurvedic*

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INTRODUCTION

Silajit is a dark brownish pitch-like gummy substance. *Unani* and *Ayurvedic* systems of medicine use it as medicine for the treatment of many external and internal diseases and as nutritional supplement. In Urdu and Hindi, it is called *Silajit*, *Shilijat* or *Silaras*, in Arabic *Hajur-ul-Musa* and in Persian *Momai Faqir-ul-Yahud.*[1] There is no proper English name or term for it, perhaps because of the reason that it is an indigenous product/drug of the oriental origin. The terms asphalt[2] and asphaltum[3] have been used in the literature for *Silajit.* However, there are some serious concerns about this terminology.[4,5] Physical characteristics and microscopic examination of *Silajit* suggest that it is of vegetable origin. Its chemical composition, however, reveals the presence of hippuric acid and a high percentage of albuminoids, which make this supposition doubtful.[5] It was reported that *Euphorbia royleana* is related to the origin of *Silajit* and its formation.[6] It has been considered effective against phthisis, chronic bronchitis and asthma, digestive troubles, renal and bladder calculi, dropsy, nervous disorders, leprosy, diabetes and fracture of bones, etc.[7,8] Muslim physicians have been using it for many centuries as an antidote to poisons.[1] It is a mild laxative, diuretic and is postulated to improve digestion and appetite. *Silajit* also increases mental and physical stamina. It could also be useful for reproductive disorders in man.[5] Reports are also available for its use against tuberculosis and eczema, etc.[8]

Silajit is found in certain mountainous regions (altitude between 1,200 and 5,000 meters) of Pakistan, Afghanistan, India, Nepal and Russia.[9] Similar material is known to ooze out from the rock crevices in the Pollock Ranges, Western Australia.[10,11] In Pakistan, *Silajitis* available from mountains of Gilgit (Karrakuram), Baltistan and Azad Kashmir (Hamalya). It is always found at high altitudes on the steeps or overhanging rock cliffs. It slowly oozes out of the rock cracks in summer season and is successively deposited on the host rock surfaces. With the passage of time the size of the deposit increases.[5] The local inhabitants and the research workers have narrated many sensible and non sensible stories to the origin of Silajit.

Silajit is bitter in taste and soluble in water. According to some researchers, the odor of raw as well as purified *Silajit* is urinous.[3] Still another group claims that *Silajit*

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odor is typical of it and does not resemble any other natural or synthetic products[5]. No procedure to standardize *Silajit* exists in any country and adulteration with gall, gums and even cow's urine has been known in India.[9] The research work on *Silajit* is primarily limited to India and Pakistan.[1,12-14] A few studies outside of this region have also been reported.[15] Different research workers[1,9,16] have carried out the work on *Silajit* with respect to its physical, chemical and biochemical aspects by making use of samples collected from different mountainous regions. They have tried different approaches to explore the nature of *Silajit* However, beyond 1992, very scanty information is available in the literature about *Silajit*. The present study was an attempt to provide a fresh look of the physical, biochemical and trace mineral analysis of *Silajit* samples available in Pakistan.

METHODS & MATERIALS

1. Sample Collection

Thirteen samples of *Silajit* of the best available quality were obtained from different parts of Northern areas of Pakistan, i.e. Gilgit, Hunza, Sakardu and Muzaffarabad. Samples thus obtained were subjected to various qualitative and quantitative tests.

2. Determination of Solubility

Two gram of sample was mixed with 10 mL of different solvents, i.e. distilled water, acetic acid, methanol, ethanol, acetone, carbon tetrachloride, benzene and ammonia. All organic and inorganic solvents were of analytical grade obtained from Merck (Germany), Sigma (USA) or Fluka (Belgium). The samples in the solvents were shaken by mechanical shaker for 30 minutes and then left over for 12 hours. Solubility of the samples was also tried in the afore mentioned solvents by heating the solvents and samples together in a hot water bath (60℃) for 10 minutes.

3. Conductivity and pH Determination

For the conductometric studies, 2 g of *Silajit* was dissolved in one litre of the deionized water. Conductivity of the solution was measured with the help of conductometer WTW LF 521. The pH was measured by a digital pH meter.

4. X-Ray Diffraction Studies

Silajit samples were dried and then ground into the powdered form. Samples thus prepared were subjected to X-ray diffraction studies using an X-ray diffractometer (D-Max/II-A).

5. Amino Acid Analysis

Total amino acid analysis of *Silajit* was done by the method of Spackeman and Moore (17) on Beckman-Model 120C Amino Acid Analyzer.

6. Decomposition of Samples for Mineral Analysis

Samples were decomposed by wet digestion method.[18] When decomposition was complete, the samples were diluted to 20 mL with deionized water. Samples thus prepared were preserved in tubes and stored in a refrigerator to avoid volume loss by evaporation.

7. Mineral Analysis

Decomposed samples were analyzed for zinc, arsenic, mercury, lead and cadmium using Varian Atomic Absorption Spectrophotometer model 1275 AA equipped with lamps for different elements. Calcium, sodium and potassium were estimated by flame emission spectroscopy using Corning 410-flame photometer.

RESULTS AND DISCUSSION

1. Physical Properties of *Silajit*

All samples of *Silajit* were soluble in water and almost insoluble in all other solvents (acetic acid, ammonia, benzene, carbon tetrachloride, chloroform and ethanol). *Silajit* was sparingly soluble in hot methanol. This indicated the presence of water-soluble compounds in *Silajit*. Our results are in line with those of Chopra et al.,[8] and Ansari & Badrurad-Din,[1] who have reported the solubility of Silajit in water. The pH studies of *Silajit*

Table 1. pH & conductivity of different *Silajit* samples (2 g/L) at given temperature.

Sample No.	pH	Conductivity	Temperature (℃)
1	4.19	173.4	30.6
2	3.75	173.1	30.8
3	3.45	184.4	30.4
4	3.75	160.8	31.3
5	4.18	177.4	31.1
6	4.14	164.5	31.3
7	3.83	149.6	31.0
8	3.83	172.5	31.4
9	3.98	157.7	31.6
10	4.04	174.5	31.6
11	3.87	172.7	31.3
12	7.23	320.0	31.7
13	7.22	330.0	31.3

Table 2. Total amount of different amino acids in *Silajit* samples (ηmole/g)

Amino Acids \ SampleNo.	1	2	3	4	5	6	7	8	9	10	Mean
Asp	79.7	78.9	136.4	114.3	89.6	86.3	136.4	115.15	89.4	82.75	100.89
Thr	44.5	59.8	67	67.4	54.5	49.7	71.85	58.2	48.2	43.6	56.475
Ser	88	86.7	113.2	120.3	103.2	93.3	121.65	105.75	123.9	89.23	104.523
Glu	132.5	133.8	135.5	140.7	146	144	266.75	170.25	170.95	143	158.345
Ala	67.65	112	190	117.7	119.9	110.5	94.15	1615.3	1010.4	1124.35	456.195
Cys	59.2	58	59.6	57.7	63.6	49	72.45	57.7	56.45	49.45	58.315
Val	15.4	20.5	10.7	21.7	33	28	20.85	10.3	21.7	33	21.515
Met	36.25	39	37	45.6	62	65	63.15	44.85	38.55	43.25	47.465
Ile	54.35	52	53	55.8	93.7	65.6	105.2	72.7	66.15	65.2	68.37
Leu	14.4	10.5	11.9	9.8	15.2	23.4	123.6	8.5	13.5	9.05	23.985
Trp	4.35	12.9	14.7	14.9	12.7	40.3	53.85	41.15	44	39	27.785
Phe	13.35	13.7	11.3	12.5	11.4	11.9	-	11.95	-	-	8.61
Lys	1928	1478	1490	1660.6	1550	1455.5	1782.34	2239.65	1894.28	1455.85	1693.422
His	9.05	17	18	19.2	40.5	15.6	40.65	16	19.15	13.5	20.865
Arg	363.25	314.3	312	326	317	267	39.36	315.45	265.9	160.7	268.096
Pro	98.9	124.4	126.7	189.9	113.9	99.6	225.5	133.6	123.45	117.5	135.345

(2 g/L of water, Table 1) indicated that *Silajit* was slightly acidic in nature which may be due to the presence of benzoic acid, hippuric acid and other acids.[1)]

2. Amino Acid Analysis

The results of amino acid analysis (Table. 2) revealed that *Silajit* contained lysine in higher concentration than all other amino acids and ranged from 1456 to 2240 η mole/g. Alanine was also found in higher concentration ranging from 68 to 1615 ηmole/g of *Silajit*. Other amino acids found in *Silajit* were aspartate, threonine, serine, glutamate, cysteine, valine, methionine, leucine, isoleucine, tryptophan, phenylalanine, histidine, arginine, and proline. Phenylalanine was absent in some of the samples. This may be due to the difference in origin of Silajit or due to the different impurities contributed by different sources. The amino acid analyses obtained in the present study is not in line with that of Shakir *et al.*[2)] who reported higher concentrations of glycine, proline, threonine and hydroxyproline. Mahdihassan and Eradman[16)] also reported high concentration of glycine, i.e. about 8.25 percent, while other amino acids were reported in trace amounts. Glycine was not detected in the *Silajit* sample under study. The difference in the amino acid profile of the present and earlier studies may be attributed to sample collection from different locations having different geological origin. Another possibility of variant results may be the improvement in the amino acid analysis techniques and equipment.

3. Toxic Element Analysis:

The results of analysis of toxic elements (arsenic, cadmium, mercury and lead) are summarized in Table 3. Arsenic, a metalloid, was found to present in a range from 45 to 98 ppm. It is present in small quantities (< 0.5 mg/kg) in most human foods, and a value of 0.2 mg per day has been prescribed as a tolerance limit in the absence of any environmental contamination.[19)] Since a small dose of Silajit (ranging from 0.5 to 1 g) is used as a medicine, therefore, the amount of arsenic present in this dose (approx. 45-98 mg/g) may not pose any serious threat to health. The concentration of mercury in different samples ranged from 56 to 160 ppm. Mercury absorption in the human body depends on its form.

The absorption of methyl mercury is almost 100 percent and that of inorganic mercury compounds from food is about 7-8 percent. The major effects of its

Table 3. Concentrations of toxic elements (As, Cd, Hg, Pb) in different Silajit samples (ppm)

Sample No.	Arsenic	Mercury	Cadmium	Lead
1	49	56	0.290	2.70
2	70	100	0.522	4.66
3	82	88	0.522	4.30
4	86	56	0.444	4.80
5	83	100	0.452	3.90
6	88	116	0.522	4.60
7	98	108	0.522	4.36
8	98	108	0.518	4.20
9	50	100	0.330	2.38
10	45	160	0.640	4.00
11	64	116	0.522	3.96
12	72	140	0.640	4.30
13	66	116	0.530	2.38
Average	73.15	104.92	0.496	3.89
Range	45-98	56-160	0.29-0.64	2.38-4.80

poisoning are neurological and renal disturbances.[19] The WHO has set the provisional tolerable weekly intake of mercury at 1.5 μmole (300 μg), of which no more then 1.0 μmole should be methyl mercury.[20]. Since *Silajit* contained 56-160 μg (per gram of *Silajit*) of mercury, therefore, the high dose or long term use may cause health problems due to the toxic effects of mercury. Cadmium was found at relatively low concentration (0.29-0.64 ppm). It is a highly toxic metal and long-term low level exposure is known to result in renal dysfunction like tubular proteinuria, glucosuria, aminoaciduria, and increased excretion of β_2 microglubulin.[19,21] The FAO/WHO provisional tolerable weekly intake is 300-400 g. The weekly intake of cadmium from *Silajit* (1 g/daily) may range from 2.03 to 4.48 μg, which is quite small; therefore, it may not inflict toxic effects. We could not find any study for comparison of our results for the arsenic, mercury and cadmium contents of *Silajit*. Lead is a ubiquitous metal and its concentration in soil is about 2 to 200 mg/kg.[19] The lead content of *Silajit* ranged from 2.38 to 4.80 ppm, which is quite low when compared with soil content and results reported by Nizami et al.[22] It is therefore postulated that consumption of *Silajit* is not expected to pose the problem of lead toxicity.

4. Trace Element Analysis

The concentrations of copper and zinc are given in Table 4. The concentration of copper ranged from 1.6 to 7.32 ppm and zinc from 10 to 26.3 ppm. The results for zinc are partially in line with those of Peerzada *et al.*[9] They reported the zinc concentration of *Silajit* at 1.58 to 1.93 ppm. Both of these elements are required for normal physiological functions of the human body[19]. It may therefore be concluded that some of the remedial characteristics of Silajit are due the presence of these elements.

Table 4. Concentration of different electrolyte & trace elements in different Silajit sampl

Sample No.	Calcium (mg/gm)	Potassium (mg/gm)	Sodium (mg/gm)	Copper (ppm)	Zinc (ppm)
1	3.60	2.80	5.32	7.32	22
2	1.96	2.00	5.90	3.6	16
3	7.68	3.60	5.32	6.4	18.4
4	5.68	1.90	5.90	1.6	56
5	4.32	3.00	3.60	3.2	21
6	2.96	3.20	5.32	2.8	12.5
7	3.60	1.20	5.32	3.2	20
8	2.80	1.90	4.40	5.2	26.3
9	6.08	3.20	5.32	6	12.5
10	3.28	3.60	5.32	3.2	17.2
11	4.24	1.00	3.60	2.6	10
12	8.32	9.80	8.60	5	11.5
13	6.40	9.72	8.00	2.4	19
Average	4.69	3.60	5.49	4.04	17.23
Range	1.96-8.32	1.0-9.8	3.6-8.6	1.6-7.32	10-26.3

5. Electrolyte Analysis

Sodium, potassium, and calcium concentrations of *Silajit* are also summarized in Table 4. Peerazada *et al.*[9] reported the concentrations of sodium, potassium, and calcium as 1.87, 39.7 and 21.39 mg/g of *Silajit*, respectively. *Silajitis* used as an effective medicine in the treatment of bone fracture[1], and this therapeutic property of *Silajit* may be due to the presence of calcium in a readily absorbable form.

The important role minerals play in health and disease holds a fascination for researchers and health care providers. Some of the therapeutic properties of *Silajit* may be due to biological and protective roles of the minerals present in it, and perhaps *Silajit as* a medicine may be better interpreted with the present data.

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