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Plant-derived phenolic acids in Shilajit: a comparative HPLC–MS/MS analysis across five regions

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Shilajit, a resinous exudate traditionally used in Asian medicine, is thought to originate from the decomposition of plant material, yet direct chemical evidence for this hypothesis has remained limited. Here, we report the first comprehensive quantification of phenolic acids, as the key plant-derived antioxidants, in eleven Shilajit samples from Iran, India, Nepal, Russia, and Kyrgyzstan using HPLC–MS/MS. Nine phenolic acids (gallic, vanillic, syringic, caffeic, *p*-coumaric, ferulic, sinapic, chlorogenic, and rosmarinic acids) were identified and quantified. Most samples had more hydroxybenzoic acids (gallic, vanillic, syringic) than hydroxycinnamic acids. Gallic acid was the most abundant compound, reaching up to 2839.28 μg g^{-1} in Iranian Shilajit, whereas rosmarinic acid displayed exceptionally high levels (216.26 μg g^{-1}) in one Iranian sample. The pronounced geographical variation in phenolic acid composition suggests a strong environmental and botanical influence on Shilajit formation. These findings provide molecular evidence supporting its plant-based origin and highlight phenolic acids as contributors to Shilajit's well-documented antioxidant and therapeutic effects. The study also establishes a robust analytical platform for future standardization and quality assessment of Shilajit as a natural medicinal product.

Keywords Shilajit, Phenolic acids, Natural product analysis, Antioxidant compounds, Natural medicine standardization

Shilajit, also known as Mumijo, is a dark-brown resin that has been used in traditional medicine for thousands of years. It is usually found in high mountain areas, where it leaks from rocks during warmer months[1]. In Central Asia, including India, China, Iran, Pakistan, Afghanistan, Nepal, Bhutan, Mongolia, Kyrgyzstan, Kazakhstan, and Russia, Shilajit has been used for over 3,000 years. One of its earliest applications was the treatment of bone fractures. Over time, its therapeutic scope has expanded, and modern research has demonstrated a wide range of biological functions. Antiallergic, analgesic, anti-inflammatory, antifungal, anti-diabetic, antioxidant, anticancer, anti-anxiety, antiviral, hepatoprotective (fatty liver), and neuroprotective functions like preventing Alzheimer's disease are among the effects that have been reported[2]. Today, Shilajit is being sold worldwide in the unprocessed form, as well as a dietary supplement, demonstrating its recognized beneficial effects on human health[3].

Chemically, Shilajit is composed of 18–20% w/w minerals,13–17% w/w proteins, 18–20% w/w nitrogen-free compounds, 4.0–4.5.0.5% w/w lipids, 3.3–6.5% w/w steroids, 5% w/w trace elements, 1.5–2.5% w/w carbohydrates, and 0.05–0.08% w/w alkaloids, amino acids and other nitrogen containing compounds[4]. The chemical composition and therapeutic potential of Shilajit depends on many factors such as, the geological composing of the rock and soil, temperature, humidity, altitude and the types of plants growing in the region. These variables can alter the ratio and presence of bioactive components, even though Shilajit exhibits similar physical characteristics across regions[5]. Despite being widely used, the composition and origin of Shilajit are not fully known yet. According to the biological hypothesis (one of the most widely accepted ones) Shilajit is produced when plant residues and animal excreta are gradually decomposed under specific physicochemical conditions[6].

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In most countries, Shilajit is recognized and marketed as an herbal medicine, emphasizing its botanical origin. Consequently, the contribution of plants to its formation is undeniable. One of the most distinctive chemical signatures of this plant-derived contribution is the presence of phenolic acids (PAs, aromatic carboxylic acids) widely distributed throughout the plant kingdom. PAs are important secondary metabolites that play key roles in plant growth, reproduction, and defense against environmental stress. They are commonly found in many edible plants, including black currants, walnuts, cranberries, strawberries, raspberries, and grapes. In humans, dietary intake of PAs has been associated with a reduced risk of chronic diseases due to their strong antioxidant and bioactive properties[7].

Structurally, PAs are classified into two main groups: hydroxybenzoic acids (derived from benzoic acid, such as salicylic, 4-hydroxybenzoic, protocatechuic, gentisic, ellagic, hexahydroxydiphenic, gallic, vanillic, and syringic acids) and hydroxycinnamic acids (derived from cinnamic acid, such as caffeic, *p*-coumaric, ferulic, sinapic, chlorogenic, and rosmarinic acids). The most common PAs are gallic acid (GA), caffeic acid (CA), vanillic acid (VA), *p*-coumaric acid (*p*-COA), ferulic acid (FA), sinapic acid (SIA), chlorogenic acid (CGA), rosmarinic acid (RA) and syringic acid (SA); their characteristics are listed in Table SI1. These compounds not only protect plants from external stress through complex biochemical signaling networks but also contribute significantly to human health as natural antioxidants and as precursors for bioactive molecules used in food, cosmetic, and pharmaceutical products. Their structural diversity strongly influences their biological activities[8–10]. For example, caffeic acid (CA) with two hydroxyl groups on its aromatic ring generally shows stronger antioxidant effects than *p*-COA, which has only one[11]. CA's biological activities are largely attributed to its antioxidant properties, which arise from the presence of two hydroxyl groups on its aromatic ring[12]. Beyond its antioxidant effects, CA has drawn attention to numerous potential therapeutic applications. CA is also being explored in cosmetics for its skin-protective and anti-aging benefits[13]. Previous study by Mishra et al.[14]reported the presence of CA in Shilajit from India at a concentration of 35.54 μg g^{-1}. Another PA is ferulic acid (FA), (E)−3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid). It is a derivative of caffeic acid and is one of the most widely distributed hydroxycinnamic acids in plants. FA exhibits strong antioxidant activity and multiple pharmacological effects[15]. Previous research on Shilajit reported FA concentrations as 751 μg g^{-1} in a sample from India[14]. Compared to other PAs, FA shows higher bioavailability due to faster absorption and prolonged circulation in the bloodstream, making it valuable in food, pharmaceutical, nutraceutical, and cosmetic industries[15].

p-Coumaric acid (*p*-COA) is another PA which is a hydroxy derivative of cinnamic acid and exists in three isomeric forms: *o*-coumaric, *m*-coumaric, and *p*-coumaric, with the *p*-isomer (4-hydroxycinnamic acid) being the most abundant in nature. *p*-COA is widely distributed across the plant kingdom and commonly found in fruits, vegetables, and cereals[16].

Sinapic acid (SIA), another PA, is one of the most common hydroxycinnamic acid and is widespread in plant kingdom. SIA has been reported to be used in Chinese traditional remedies as a major active component[17]. Chlorogenic acids (CGAs) are a group of phenolic compounds widely found in plants[18]. In the food industry, CGAs act as natural preservatives due to their antimicrobial and antioxidant effects, while also offering prebiotic benefits[19,20]. Rosmarinic acid (RA) is the first polyphenolic ester isolated from rosemary (*Rosmarinus officinalis* L, Lamiaceae Martinov) and is also found in many herbs of the Lamiaceae family[21]. Chemically, RA is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid ($C_{18}H_{16}O_8$). RA exhibits a wide spectrum of biological and pharmacological activities[22]. Gallic acid (GA) is a widely distributed phenolic compound present in many plants and commonly consumed foods. Its accumulation in plants can be influenced by both biotic and abiotic factors, including exposure to ultraviolet radiation, microbial attacks, and environmental stresses. Due to these broad biological activities, GA is of interest in the food, cosmetic, and pharmaceutical industries[23]. Previous research using HPLC-DAD reported GA levels in Indian Shilajit samples ranging from 18 to 415.20 μg g^{-1}[14]. Vanillic acid (VA) is a naturally occurring derivative of benzoic acid and an oxidized form of vanillin. It is commonly used in the food industry as a flavoring agent, preservative, and additive. VA exhibits a broad spectrum of biological activities, it possesses anticoagulant and antiulcer properties, emphasizing its multifaceted health benefits[24,25]. Syringic acid (SA), or 4-hydroxy-3,5-dimethoxybenzoic acid, is a naturally occurring O-methylated trihydroxy benzoic acid widely distributed in plants. It has been associated with a range of pharmacological effects, including antimicrobial, anti-inflammatory[26]. More detailed information about the main source of phenolic acid and the role of plants in the formation of Shilajit is mentioned in one of the formation theories, and the presence of PAs provides strong evidence for it. This research fills the gap regarding the presence and role of plants in Shilajit formation and also proves that the antioxidant and health benefits of Shilajit can be attributed to the presence of these PAs. For this reason, the present study focuses on quantifying the most abundant PAs in Shilajit samples from different regions using HPLC–MS/MS. The compounds analyzed include gallic acid (GA), caffeic acid (CA), vanillic acid (VA), *p*-coumaric acid (*p*-COA), syringic acid (SA), ferulic acid (FA), sinapic acid (SIA), chlorogenic acid (CGA), and rosmarinic acid (RA).

Experimental

Chemicals, reagents, and materials

Analytical standards of 9 PAs (Fig. 1), caffeic acid (CA)(≥98%), *p*-coumaric acid (*p*-COA) (≥99.6%), vanillic (VA) (97%), syringic (SA) (98%), gallic acid (GA) (97.5–102.5.5.5%), *trans*- sinapic acid (SIA) (≥98%), ferulic acid (FA) (99%), chlorogenic acid (CGA) (≥95%) and rosmarinic acid (RA) (≥98%) were obtained from Sigma-Aldrich (Missouri, USA). HPLC-grade methanol, acetonitrile and ammonium acetate (>98% purity) were purchased from the same company. Membrane filters (0.2 μm pore size) were supplied by Labindex. LC–MS grade water was produced using a Milli-Q® Eq. 7000 ultrapure water system (Merck KGaA, Germany). The filtration system with 18.2 MΩ·cm^{-1} at 25 °C and a total organic carbon ≤ 5 μg L^{-1} was used for all experiments. For extraction steps (Cimarec i Poly 15 Multipoint Stirrer, Thermo Fisher Scientific, USA) was used.

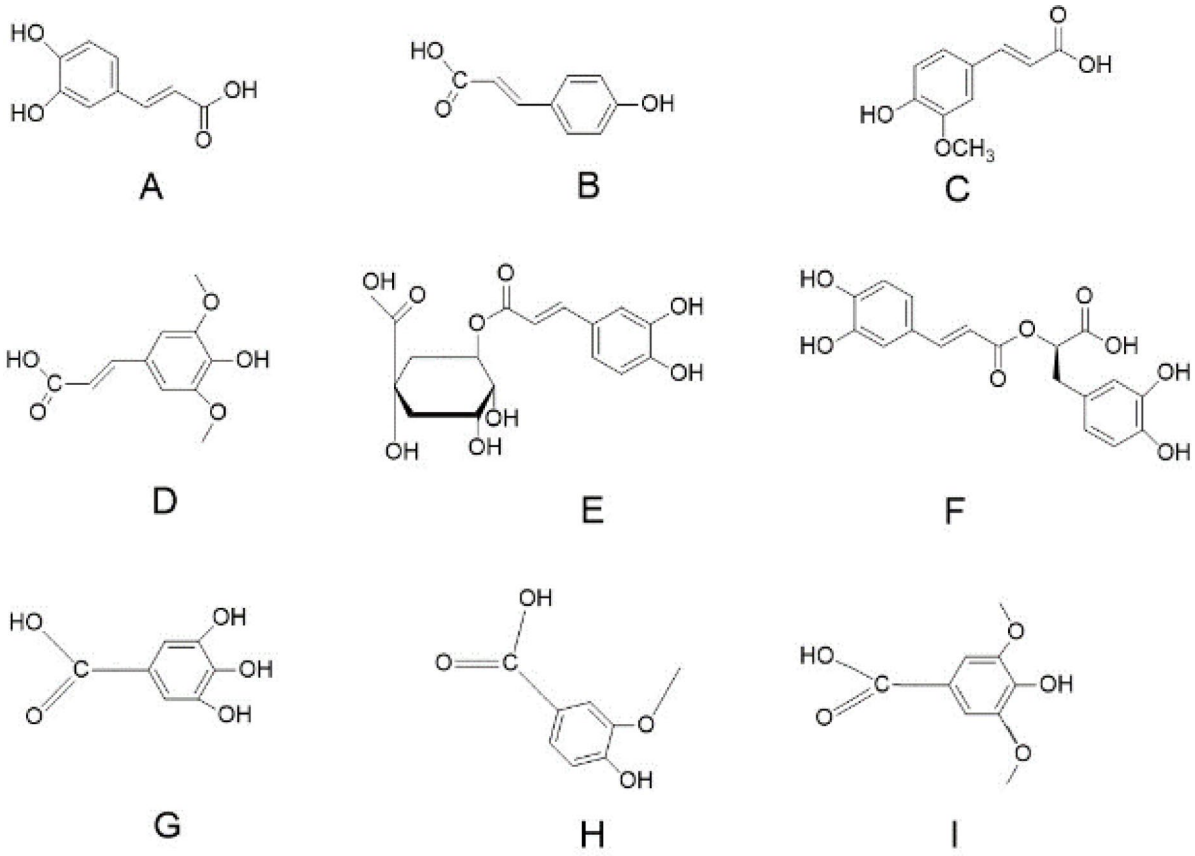

Fig. 1. Chemical structure for the nine PAs analyzed. Hydroxycinnamic-acid derivatives: (**A**) CA, (**B**) p-COA, (**C**) FA, (**D**) SIA, (**E**) CGA, and (**F**) RA. Hydroxybenzoic-acid derivatives: (**G**) GA, (**H**) VA, and (**I**) SA.

Instrumentation

PA analysis was performed using an HPLC–MS/MS system consisting of an UltiMate 3000 RSLC instrument (Dionex, Thermo Scientific, Waltham, MA, USA) coupled to an API 4000 QTRAP triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with an electrospray ionization source operating in negative ionization mode.

Chromatographic separation was achieved on a Luna C_{18} analytical column (150 mm × 2.0 mm, 3 µm particle size; Phenomenex, Torrance, CA, USA). The column temperature was maintained at 35 °C, and the injection volume was 5.0 µL. The mobile phase consisted of (A) water containing 5 mmol L^{-1} ammonium acetate and (B) acetonitrile. The gradient program started at 90% B (0.0–2.5 min), increased to 100% B at 3.5 min, and was held for 0.5 min. The flow rate was 0.20 mL min^{-1}. A post-run equilibration time of 4.0 min was applied before the next injection.

The operating conditions for mass spectrometry are listed in Table SI2. Quantitative analysis was conducted in multiple reaction monitoring (MRM) mode. For each analyte, one transition of the deprotonated molecular ion and its corresponding product ion was monitored. These transitions (m/z) along with the associated declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) are summarized in Table 1.

PAs in methanolic extracts of Shilajit were quantified using a four-point standard addition method. The spiking concentrations were adjusted according to the expected levels of each phenolic acid in the samples. Quantification was based on the peak areas of the respective analytes.

Sample Preparation and extraction

A total of eleven raw Shilajit samples collected from different geographical regions were analyzed in this study. The origins of these samples are listed in Table 2. The samples analyzed in this study were obtained as raw Shilajit materials with supplier-declared geographic origin and were selected to represent multiple Shilajit-producing regions (Iran, India, Nepal, Russia, and Kyrgyzstan). Because the samples were not collected as part of a geo-referenced field campaign, exact coordinates and site-level ecological descriptors (including vegetation inventories around exudation zones) were not available. Therefore, their origin is reported at the mountain/region level based on the information provided by suppliers.

Due to the inherently sticky and viscous nature of Shilajit, all samples were first lyophilized and then ground into a fine powder using a laboratory mill to ensure homogeneous extraction. The solubility of phenolic compounds strongly depends on both the polarity of the extraction solvent and the matrix composition. Pure organic solvents are generally ineffective for extracting highly polar phenolic compounds; thus, solvent mixtures

Compound	[M-H]⁻	DP [V]	MRM 1	CE [V]	CXP [V]	MRM 2	CE [V]	CXP [V]
Caffeic acid	179	−51	179→ 135	−22	−7	179→106.3	−32	−7
Chlorogenic acid	353	−65	353→191	−24	−9	353→85	−64	−5
Ferulic acid	193	−55	193→134	−20	−7	193→178	−18	−9
Gallic acid	169.1	−55	169.1→125	−22	−9	169.1→79	−32	−5
Vanillic acid	167	−50	167→107.9	−26	−7	167→122.9	−18	−21
P-coumaric acid	163	−55	163→119	−24	−9	163→92.9	−44	−3
Syringic acid	197	−65	197→120.9	−24	−9	197→153	−18	−9
Sinapic acid	223	−60	223→148.7	−28	−25	223→163.8	−20	−7
Rosmarinic acid	359	−60	359→160.8	−24	−9	359→196.6	−26	−9

Table 1. Optimal operating parameters of the tandem mass spectrometer for the tested compounds.

Shilajit sample	Country/region
S1	Iran/Unknown
S2	Nepal/Unknown
S3	Iran/Kurdistan
S4	Iran/Ravar
S5	India/Unknown
S6	India/Unknown
S7	Russia/Unknown
S8	Iran/Kahnuj
S9	Kyrgyzstan/Unknown
S10	Iran/Saravan
S11	India/Himalaya

Table 2. Geographical origin of the analyzed Shilajit samples.

with increased polarity are preferred. Water–organic solvent mixtures such as 75% acetone, 80% ethanol, or 80% methanol are commonly employed for phenolic acid extraction[27]. To determine the optimal extraction conditions for Shilajit, various methanol–water ratios (100%, 90%, 70%, and 50%) and extraction durations (4, 8, and 24 h) were tested. The most efficient extraction was obtained using 70% methanol with a 4 h extraction period. The efficiency was calculated based on the area under the peak for each PA. Accordingly, approximately 20 mg of powdered Shilajit was mixed with 3 mL of 70% methanol and agitated for 4 h. The mixture was then centrifuged at 320 rpm, and the supernatant was carefully collected and filtered through a 0.22 µm PTFE membrane. Each sample was extracted in triplicate, yielding a total of 33 extracts, which were immediately subjected to HPLC–MS/MS analysis.

Method validation

The HPLC–MS/MS method used in this study was validated by assessing key analytical parameters, including the limit of detection (LOD), limit of quantification (LOQ), and linearity range for each PA. The LOD was defined as the lowest concentration that produced a signal-to-noise (S/N) ratio of at least 5, whereas the LOQ corresponded to the lowest concentration yielding an S/N ratio of 10 or higher. Linearity was evaluated by preparing a series of standard solutions for each analyte, and calibration curves were constructed to determine the relationship between analyte concentration and detector response. The calculated LOD, LOQ, and linearity ranges for all PAs analyzed in this study are summarized in Table 3. These validation results confirm the method's suitability for accurate and sensitive quantification of PAs in complex Shilajit samples.

Results and discussion

The HPLC–MS/MS analysis enabled the identification and quantification of nine PAs across eleven Shilajit samples collected from different geographical regions (Table 4).

Each sample was extracted three times ($n = 3$). Results are reported as average values, and the variation between repeats is given as standard deviation (SD). Because the number of samples per region is small and unequal, we did not use formal statistical tests to compare regions and instead describe regional differences qualitatively. The resulting concentration profiles show distinct compound-specific patterns as well as distinct regional variability. As can be seen, in all Shilajit sample, hydroxybenzoic acid PAs (GA, VA, SA) were found at a higher amount than hydroxycinnamic acids (CA, *p*-COA, FA, SIA, CGA, RA) (Fig. 2). The only exceptions are sample S3 (Iran) and S11 (India), where the concentration of hydroxycinnamic acid is higher. In the current study, GA was the most abundant PA across the Shilajit samples, ranging from 22.19 to 2839.28 µg g^{-1}. The highest concentration was observed in Iranian and Indian samples, with 2839.28 and 2649.74 µg g^{-1}, respectively. The lowest GA content was observed in sample S3 from Iran. For comparison, the concentration of GA in S2 is ten times higher than S4,

Phenolic acid	Linear equation	R^2	Range of linearity [µg/mL]	LOD [µg mL^{-1}] (S/*N*)	LOQ [µg mL^{-1}] (S/*N*)
Caffeic acid	*y = 4E+06x − 3280.7 y = 1E+07x − 419,920	0.9810 0.9913	5×10^{-4} – 2.5×10^{-2} 5×10^{-2} – 1	2.5×10^{-4}	5×10^{-4}
Chlorogenic acid	y = 1E+06x + 23,083	0.9981	1×10^{-2} – 5×10^{-1}	2.5×10^{-5}	5×10^{-4}
Ferulic acid	y = 3E+06x + 2443.4	0.9998	5×10^{-4} – 5×10^{-1}	2.5×10^{-4}	5×10^{-4}
Gallic acid	y = 5E+06x − 32,484	0.9955	5×10^{-2} – 1	5×10^{-4}	1×10^{-3}
Vanillic aicd	y = 1E+06x + 1562.5	0.9995	2.5×10^{-3} – 5×10^{-1}	1×10^{-3}	2.5×10^{-3}
P-coumaric acid	y = 9E+06x + 37,354	0.9986	1×10^{-3} – 5×10^{-1}	2.5×10^{-4}	1×10^{-3}
Syringic acid	y = 806545x + 7135.4	0.9986	5×10^{-3} – 1	1×10^{-3}	5×10^{-3}
Sinapic acid	y = 2E+06x + 78.569	0.9998	1×10^{-3} – 5×10^{-1}	5×10^{-4}	1×10^{-3}
Rosmarinic acid	y= 5E+06x − 236,720	0.9939	1×10^{-1} – 1	5×10^{-4}	1×10^{-3}

Table 3. Calibration parameters and sensitivity data (linearity, LOD, and LOQ) for phenolic acids analysed by HPLC–MS/MS (LOD and LOQ were calculated from S/N in the chromatograms (LOD: S/$N \geq 5$; LOQ: S/$N \geq 10$)). *Two equations present the dual range of linearity for caffeic acid.

Sample	*p*-COA ± SD	VA ± SD	GA ± SD*	CA ± SD	SA ± SD	FA ± SD	CGA ± SD	RA ± SD	SIA ± SD
S1	59.71 ± 4.13	97.99 ± 3.81	73.85 ± 4.94	112.33 ± 5.42	365.28 ± 21.49	< LOD**	34.25 ± 3.87	< LOD	< LOD
S2	67.94 ± 3.95	178.85 ± 7.23	618.60 ± 86.96	121.10 + 0.38	75.53 ± 2.38	< LOD	< LOD	< LOD	< LOD
S3	7.28 ± 0.11	15.32 ± 2.11	22.19 ± 1.63	4.89 ± 0.10	92.67 ± 2.62	< LOD	14.09 ± 0.003	216.26 ± 2.36	< LOD
S4	15.17 ± 0.80	96.53 ± 0.49	60.62 ± 0.54	11.77 ± 0.45	110.64 ± 2.19	3.46 ± 0.15	8.21 ± 0.54	8.19 ± 1.28	< LOD
S5	202.04 ± 2.13	1424.16 ± 13.76	2649.74 ± 267.59	271.17 ± 9.43	160.50 ± 5.46	59.15 ± 2.96	27.10 ± 3.37	< LOD	< LOD
S6	72.74 ± 0.53	223.18 ± 1.88	772.49 ± 1.40	524.40 ± 12.64	< LOD	< LOD	< LOD	< LOD	< LOD
S7	111.71 ± 4.57	1099.28 ± 11.11	2383.14 ± 25.59	479.55 ± 11.05	292.43 ± 5.60	50.46 ± 6.72	35.35 ± 0.93	< LOD	44.22 ± 4.76
S8	87.55 ± 6.40	344.53 ± 14.86	2839.28 ± 30.53	163.94 ± 8.17	211.59 ± 11.26	< LOD	< LOD	< LOD	< LOD
S9	145.79 ± 1.62	350.15 ± 6.80	41.08 ± 6.66	239.04 ± 14.85	107.74 ± 2.65	38.96 ± 0.01	10.73 ± 1.08	5.40 ± 0.23	< LOD
S10	61.56 ± 4.57	114.99 ± 6.54	76.56 ± 2.20	47.02 ± 2.76	78.08 ± 1.21	< LOD	45.26 ± 2.29	3.74 ± 0.40	< LOD
S11	153.40 ± 4.54	408.71 ± 36.86	769.39 ± 19.98	1698.23 ± 92.42	< LOD	< LOD	< LOD	< LOD	< LOD

Table 4. Concentrations of PAs (µg g^{-1}) in Shilajit samples determined by HPLC–MS/MS ($n = 3$). *SD: standard deviation; **< LOD: lower than limit of detection.

while S7 contained almost 107 times more GA than S3. The level of GA is reported in Shilajit in the range18 to 415.20 µg g^{-1}[14]. The wide concentration range observed for GA suggests substantial regional differences, which may be attributed to variations in local vegetation, microbial activity, and climatic conditions affecting organic matter decomposition. Following GA, VA and CA were the next most abundant PAs. VA concentrations ranged from 15.32 µg g^{-1} (Iran) to 1424.16 µg g^{-1} (India). CA levels ranged from 4.89 µg g^{-1} (Iran, S3) to 1698.23 µg g^{-1} (India, S11). Notably, S3 exhibited the lowest concentrations of CA, GA, and VA. In S11, CA levels were four times higher than VA, while in S7, VA levels were almost 2.3 times higher than CA. Samples from Iran (S1, S8, S3, S4 and S10) generally contained low CA level (112.33, 163.94, 4.89, 11.77 and 47.02 µg g^{-1}, respectively). In contrast, Indian samples contained significantly higher concentrations, particularly S5 (271.17 µg g^{-1}), S6 (524.40 µg g^{-1}), and S11 (1698.23 µg g^{-1}). The only reported concentration of CA in Shilajit is 35.54 µg g^{-1} in sample from India[14]. In this study, *p*-COA concentrations ranged from7.28 to 202.04 µg g^{-1}. The lowest levels were observed in samples S3 and S4 (Iran), while the highest concentrations were found in Indian samples S5 (202.04 µg g^{-1}) and S11 (153.40 µg g^{-1}). Additionally, samples S1 and S10 exhibited nearly identical *p*-COA concentrations. The highest SA levels were detected in sample S1 (Iran, 365.28 µg g^{-1}) and sample S7 (Russia, 292.43 µg g^{-1}). By contrast, sample S2 and S10 have almost the same and the lowest concentration of SA among Shilajit samples. The SA level in S8 was about twice that of S4. SA was below the limit of detection (LOD) in samples S6 and S11. These findings indicate that although SA and *p*-COA are not as abundant as GA, VA, or CA, they are present in substantial amounts in certain Shilajit samples and may meaningfully contribute to its antioxidant and therapeutic properties. The concentrations of FA, SIA, CGA, and RA in Shilajit were generally below 60 µg g^{-1}, categorizing them as low-abundance PAs. Exceptionally, sample S3 exhibited a comparatively elevated rosmarinic acid concentration (216.26 µg g^{-1}). Although rosmarinic acid is commonly associated with certain plant taxa (e.g., Lamiaceae), the present study does not include geo-referenced metadata or site-level botanical surveys; therefore, the observed enrichment cannot be attributed to specific local flora. Other Iranian samples (S4, S9, and S10) contained < 10 µg g^{-1} of RA, while in samples S2, S6 and S11, the concentration of RA, FA, CGA, and SIA were below LOD. Previous research reported FA concentrations as high as 751 µg g^{-1} in an Indian sample[14]. In the present study, however, FA was much lower, with the highest level in S5 (India, 59.15 µg g^{-1}). Considerably smaller amounts were detected in S4 (3.46 µg g^{-1}), while samples S1, S2, S3, S6, S8, S10, and

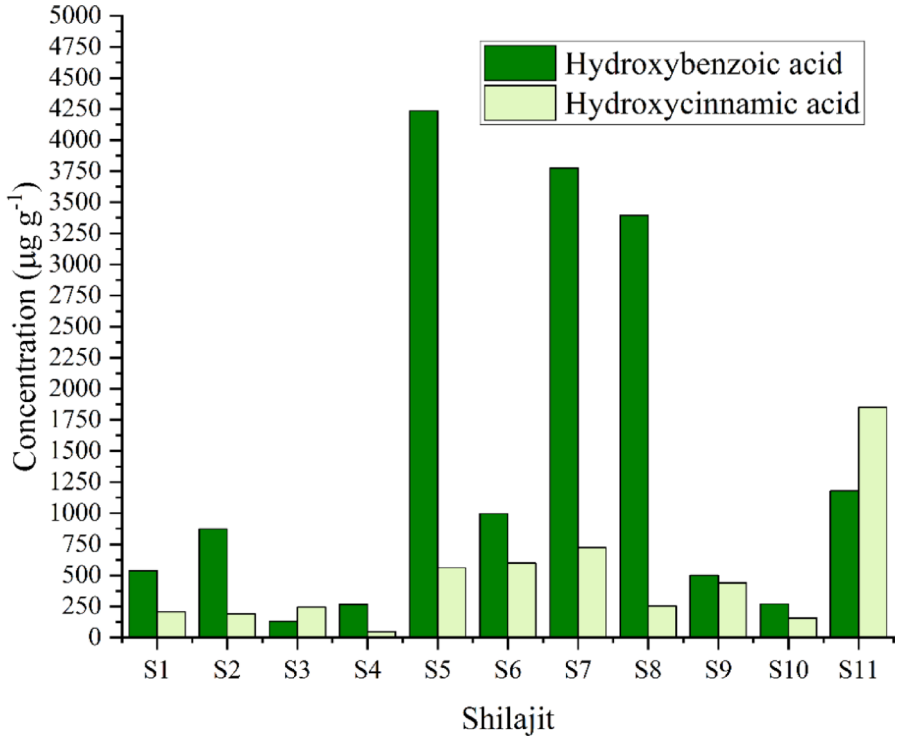

Fig. 2. Hydroxybenzoic acid and hydroxycinnamic acid concentration in Shilajit.

S11 contained FA below LOD. CGA was detected in several samples, with concentrations varying by origin. The highest level occurred in S10 (Iran, 45.26 µg g^{-1}). Similar levels were observed in S1 and S7, while the lowest concentration was recorded in S4 (Iran, 8.21 µg g^{-1}). SIA was only found in S7 (Russia, 44.22 µg g^{-1}). In all other samples, SIA was below LOD.

Beyond reporting concentrations, the phenolic-acid fingerprints obtained here provide a practical compositional basis for comparing Shilajit materials from different declared origins. Because Shilajit is commonly described as the product of long-term transformation of organic matter under microbial and geological processes, differences in phenolic-acid distributions across samples are plausibly influenced by multiple factors, including historical botanical inputs, local geochemical environment, and transformation history. Therefore, the profiles observed in this study are best interpreted as integrative chemical signatures of origin and processing history rather than direct reflections of any single present-day environmental parameter. A notable aspect of the dataset is the predominance of hydroxybenzoic acids in many samples, while hydroxycinnamic acids show more variable presence and, in several cases, fall below detection. This pattern may reflect differences in the stability and long-term transformation behavior of phenolic structures during extended maturation, although the present dataset was not designed to isolate mechanistic pathways. Importantly, the measured profiles provide analytically useful markers that can support product characterization and comparability across sources when applied with appropriate provenance documentation. Comparison with previously published data shows both agreement and notable discrepancies, particularly in the reported levels of GA, CA, and FA. These differences may due to the variation in sample origin, extraction procedure and analytical method. The present study expands the available data by providing a broader comparative overview of several PAs analyzed under the same analytical conditions, therefore offering improved reliability for inter-sample comparison.

From an applied perspective, the proposed LC–MS/MS approach can support raw-material screening and quality control in Shilajit-based products by enabling marker-based comparison across batches and suppliers. In particular, a defined phenolic-acid panel can be used as part of a broader authenticity and standardization strategy, alongside other compositional parameters typically considered for Shilajit quality assessment.

Conclusion

This study presents the first comprehensive quantification and comparison of PAs in Shilajit samples from diverse geographical regions using HPLC–MS/MS for profiling and quantifying selected plant-derived phenolic acids in Shilajit samples from multiple declared origins. The proposed workflow provides a practical analytical basis for compositional characterization and comparison of Shilajit materials, supporting efforts toward improved quality assessment, authenticity screening, and standardization of Shilajit-based products. From an applied viewpoint, such marker-based profiling can assist industry in raw-material qualification and batch-to-batch consistency, and it can also support more reproducible, evidence-informed use of Shilajit in phytotherapy.

This work is limited by the number of available samples per region and by the lack of site-level geo-referenced provenance and ecological metadata for all materials. In addition, the study focuses on a defined phenolic-

acid panel and does not capture the full chemical complexity of Shilajit. Future studies should expand geo-referenced sampling, evaluate additional marker classes and potential contaminants, assess stability during storage/processing, and link compositional fingerprints to biological performance and product-relevant quality attributes.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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EK: investigation, writing- original draft, data curation; AS: investigation; JZ: supervision, review and editing; PłK: writing and editing; MK: supervision, writing, review and editing. All the authors read and approved the final manuscript.

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