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Screening and quantification of inorganic anions in Shilajit and its supplements

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Abstract

Shilajit, a natural substance with ancient medicinal roots, is increasingly used in modern supplements for its purported health benefits. However, there is a lack of comprehensive chemical characterization, particularly regarding inorganic anions. This study addresses this gap by quantifying common inorganic anions in 14 raw Shilajit samples sourced from Iran, India, Nepal, Kyrgyzstan, and Russia, as well as in 6 commercially available supplements from Poland, Russia, and Kyrgyzstan. Using ion chromatography, key anions including chloride, sulphate, nitrate, hydrogen phosphate, and fluoride were analyzed. Results revealed that chloride was the most prevalent anion, with concentrations ranging from 0.102 to 9.496 $mg.g^{-1}$ in raw Shilajit samples and up to 0.931 $mg.g^{-1}$ in supplements. Sulphate levels were significant, with concentrations up to 12.412 $mg.g^{-1}$ in raw Shilajit and 0.854 $mg.g^{-1}$ in supplements. Nitrate was detected in lower concentrations, peaking at 9.504 $mg.g^{-1}$ in raw Shilajit. Fluoride was quantifiable in only one sample at 0.064 $mg.g^{-1}$. The study concludes that Shilajit's geographical origin significantly influences its anion composition, leading to variability in its potential health effects. These findings highlight the necessity for standardized formulations and stringent quality control measures in Shilajit supplement production to ensure consumer safety and product efficacy.

Keywords Shilajit, Ion chromatography, Inorganic anions, Natural product, Health supplements

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Introduction

Shilajit is a naturally occurring substance with a historical presence spanning millennia that is predominantly found in high-altitude mountain regions, particularly in Central Asia. Rooted in biological transformation processes under specific physicochemical conditions, Shilajit is a complex blend of organic humic substances, plant and animal fossils, mineral resins, inorganic materials, and microbial metabolites present in rock rhizospheres. Recognized for its therapeutic properties, Shilajit has played a pivotal role in traditional and contemporary medicine for internal and external applications, including the treatment of bone fractures, skin diseases, peripheral nervous system disorders, treating male sexual dysfunction, anti-anxiety, antiviral activity, fatty liver diseases, anti-cancer [1–4]. Nowadays, many companies and dietary supplement manufacturers sell thousands of Shilajit-based supplements and recommend them for a wide variety of treatments [5, 6]. These supplements mainly consist of more than 90% refined Shilajit, as one of our unpublished works revealed, recently. On the other hand, there are a lot of trace elements in Shilajit, such as Pb, Hg, As, and Tl. High concentrations of these trace elements can be dangerous for human body.

While Shilajit has gained widespread popularity as a drug and supplement, not enough information about its chemical formulation, or even qualitative data is available yet [6]. This information is vital since consumption of the precise quantity and specific types of Shilajit and its supplements is dependent on such data. Unfortunately, none of the pharmaceutical companies reported its contents or ingredients. So far, we know that Shilajit is composed primarily of humic substances, proteins, nitrogen-free compounds, lipids, steroids, trace elements, carbohydrates, alkaloids, amino acids, and other nitrogenous compounds. Shilajit's chemical composition varies with its geographical origin [4, 6]. To date, the only available data in the literature regarding anions of Shilajit are chloride, sulphur, and phosphorus which are collected in Table 1 [7, 8].

The presence of inorganic anions plays a vital role in human health and functionality [8]. Additionally, there is a growing interest in identifying inorganic ions responsible for potential toxicological effects on human beings. Understanding the precise quantity of inorganic anions in Shilajit and supplements holds paramount importance for several key reasons. Firstly, the inorganic anion composition directly influences the overall chemical profile of these natural substances, and variations in their concentrations can significantly impact their therapeutic or nutritional value. As Shilajit and its supplements are consumed widely for their purported health benefits, having a detailed understanding of the inorganic anion content is crucial for ensuring consistency and efficacy in their usage. Secondly, certain inorganic anions, such as chloride, sulphate, nitrate, and hydrogen phosphate, play essential roles in various physiological processes within the human body. Deviations in their concentrations may have implications for health, potentially influencing metabolic pathways, enzymatic activities, and cellular functions [9, 10]. By quantifying these anions, our research provides valuable insights into the potential health effects and benefits associated with the consumption of Shilajit and supplements. As can be seen in Table 1, the data are scattered and inconsistent. To address this gap, our study offers a groundbreaking quantitative analysis of common inorganic anions in both Shilajit and Shilajit-based supplements. The attention garnered by our research stems from the imperative need for clinical chemists to comprehend the specific inorganic ions required, at optimal concentration levels, for the proper functioning of living organisms. This research, for the first time, provides a quantitative examination of the amount of the most common inorganic anions present in Shilajit. Given the widespread use of Shilajit as a supplement and its oral use as traditional medicine, particularly in Asia, our findings underscore the crucial importance of quantifying inorganic anions in Shilajit for a more comprehensive understanding of its health implications. Here, we quantified a broader spectrum of anions beyond sulphur, phosphorus, and chloride. Specifically, anionic species Cl^-, F^-, NO_2^-, NO_3^-, SO_4^{2-}, HPO_4^{2-}, and Br^- were analysed in Shilajit samples sourced from Iran, India, Nepal, Kyrgyzstan, and Russia, alongside Shilajit supplements from Poland, Russia, and Kyrgyzstan.

Table 1 Identified anions of Shilajit reported in the literature

Anion	Amount found (mg.kg^{-1})	Origin of the sample	Ref.
Chloride	84,050	Afghanistan	[8]
	186,140	Pakistan	
Sulphur	109,550	India	[7]
	133,150	Pakistan	
	212,990	Afghanistan	[8]
	91,200	Pakistan	
Phosphorus	357	India	[7]
	320	Pakistan	
	204.27	Afghanistan	[8]

Experimental

Chemicals

All reagents used in this study were of analytical grade were purchased from Merck KGaA (Darmstadt, Germany). 1000 mg.L^{-1} standard solutions of anions and sodium bicarbonate and sodium carbonate (used as mobile phase components) were acquired from the same company. Deionized water with a resistivity of 18.2 MΩ·cm^{-1} at 25 °C and a total organic carbon ≤ 5 µg.L^{-1} was used in all experiments. The water was produced by a Milli-Q® Eq. 7000 ultrapure water system (Merck KGaA, Germany).

Instrumentation

Samples were analysed using an ion chromatographic (IC) 930 Compact Flex system from Metrohm Poland. The IC was equipped with a conductivity detector, peristaltic pump, oven, sequential suppression, degasser and 919 IC Autosampler Plus (Metrohm, Switzerland). Data acquisition and analysis were performed using the IC Net 3.1 chromatographic workstation with MagIC Net Basic software. Anion analysis was carried out with sequential background suppression (chemical and CO_2) on a Metrohm Metrosep A Supp 19, 150 × 4.0 mm anion exchange column packed with hydrophilized polystyrene-divinylbenzene copolymer with quaternary

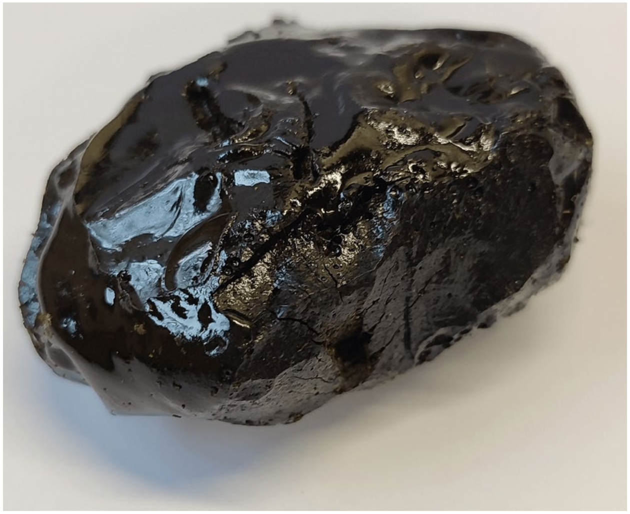

Fig. 1 Black Shilajit, collected from the south-east part of Iran by one of the authors

ammonium groups. The mobile phase consisted of a standard eluent (8.0 mmol.L^{-1} sodium carbonate, 0.25 mmol.L^{-1} sodium hydrogen carbonate) at a flow rate of 0.7 mL.min^{-1}. Prior to analysis, a blank solution (ultrapure water) was injected as a system check.

Methods

Sample Preparation

This study utilized 14 raw Shilajit samples labelled S1 to S14. Sample origins were as follows: Iran (S1, S2, S4, S5, S6, S7, S12, S14); India (S8, S9, S10); Russia (S11); Kyrgyzstan (S13); and Nepal (S3). Figure 1 shows a Shilajit sample from south-eastern Iran. Additionally, six supplements were analyzed: Sp1 (capsule) from Poland; Sp2, Sp4, and Sp5 (tablets) from Russia; and Sp3 and Sp6 (tablets) from Kyrgyzstan.

For IC analyses, 50 mg of raw Shilajit or one tablet of supplement was dissolved in 10.0 mL of deionized water (resistivity: 18.2 MΩ·cm^{-1}). The mixture was stirred at ambient temperature (23–25 °C) for 4 h using an electrical stirrer at 500 rpm, then centrifuged at 2000 rpm for 10 min, and finally filtered through a 0.22 µm nylon syringe filter to remove suspended particles. For each sample, extraction was done three times, and the analysis was conducted in triplicate. To confirm complete dissolution of anions, a separate set of samples was treated with deionized water for 10 h. Analysis of both sets of samples revealed that anion concentrations remained constant after 4 h, indicating full dissolution within this timeframe. As is shown in Fig. 2 at 4 h, the nitrate peak is well-defined and exhibits higher intensity, confirming its clear presence and quantification while at 10 h, the nitrate peak is reduced in intensity, likely due to the matrix effects or minor sample degradation over extended timeframes.

Method validation

Analyses of all the anions were performed using six points calibration curves, and each sample was analysed in at least three replicates. The analytical features of the method are summarized in Table 2. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the $3(S_d)/m$ and $10(S_d)/m$ criteria, respectively. Here, "S_d" represents the standard deviation which is based on the calibration curve and the standard deviation of y-intercepts of regression lines. The value "m" refers to the slope of the calibration curve within the linear range [11]. To validate the analytical method,

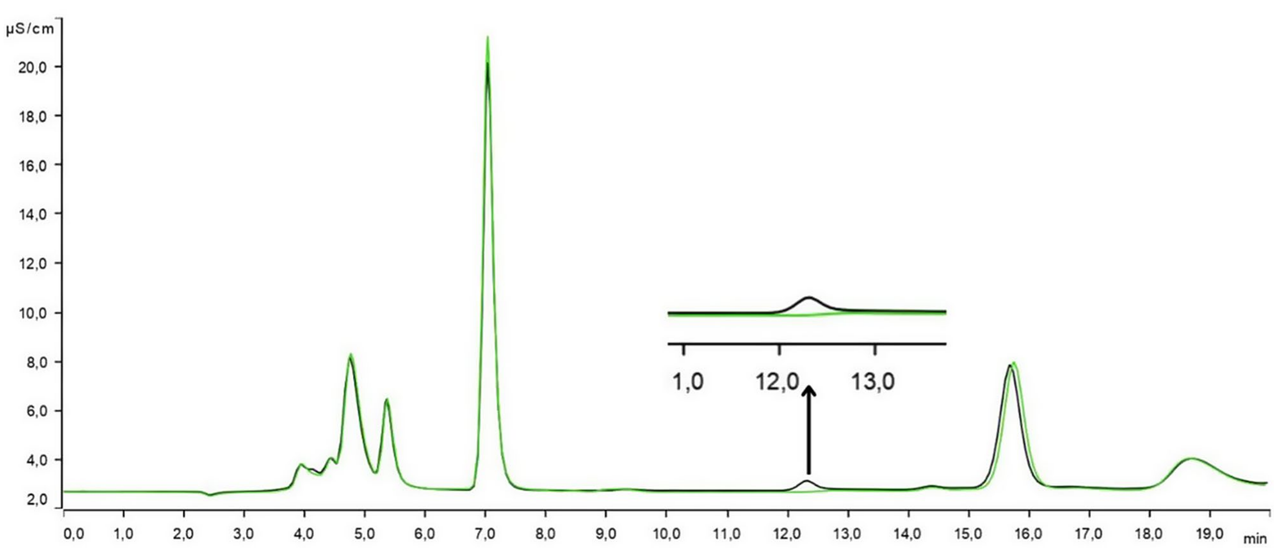

Fig. 2 Chromatogram of water extract Shilajt 9, nitrate retention time (12.28 min), time extraction 10 h (green line), 4 h (black line)

Table 2 Analytical parameters applied for the quantification of anions

Anion	Linear range (μg.mL^{-1})	Equation	Correlation coefficient (R^2)	LOD (μg.mL^{-1})	LOQ (μg.mL^{-1})
F^-	30–100	$y=2.0\times10^{-4}x-9.0\times10^{-4}$	0.9978	5.66	17.17
Cl^-	15–100	$y=1.0\times10^{-4}x+1.0\times10^{-4}$	0.9999	1.23	3.71
Br^-	60–260	$y=6.0\times10^{-5}x+2.0\times10^{-4}$	0.9925	23.32	70.68
NO_3^-	60–200	$y=6.0\times10^{-5}x-2.1\times10^{-3}$	0.9911	23.85	72.28
NO_2^-	100–500	$y=1.0\times10^{-4}x-2.3\times10^{-3}$	0.9941	46.43	140.69
HPO_4^{2-}	150–600	$y=4.0\times10^{-5}x-3.5\times10^{-3}$	0.9996	17.16	52.00
SO_4^{2-}	100–350	$y=1.0\times10^{-4}x-1.4\times10^{-3}$	0.9992	10.95	33.20

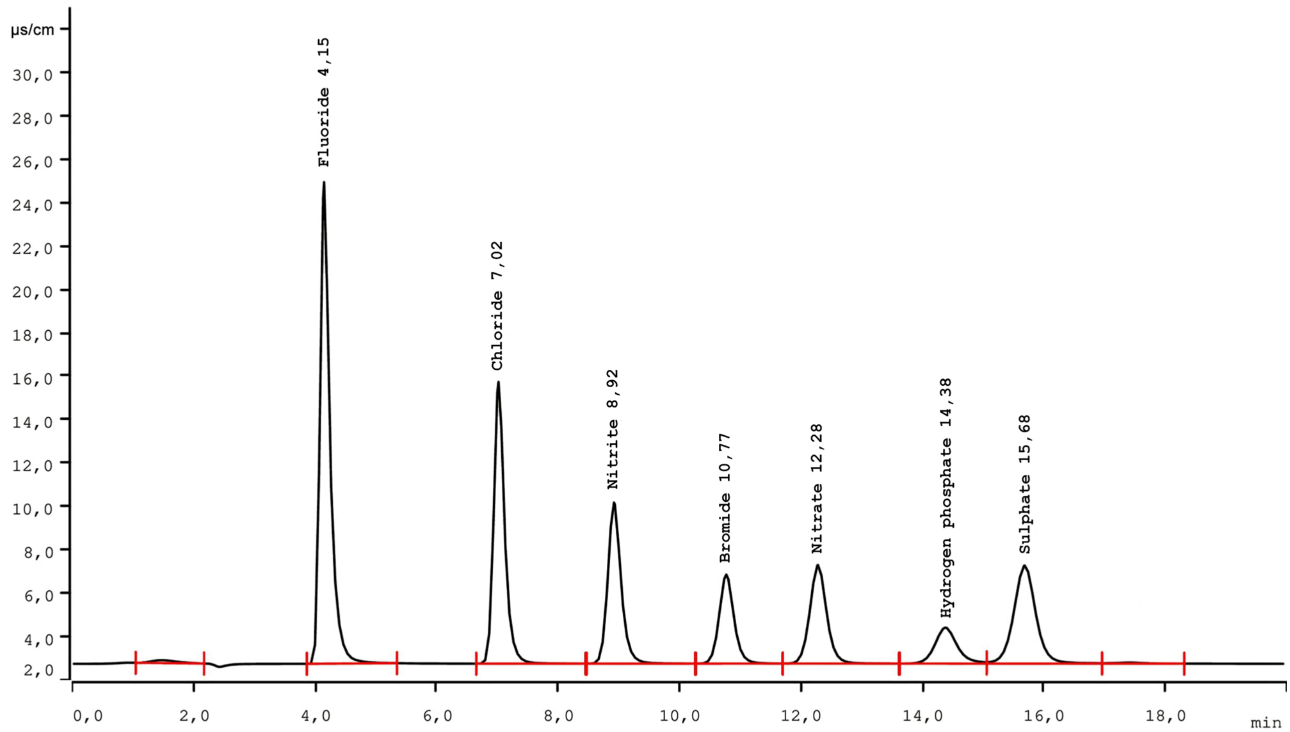

Fig. 3 Standard anions at a concentration of 10 mg.L^{-1}

the quality control (QC) was performed by using standard addition technique. Figure 3 illustrates the standard anions while Fig. 4 shows the standard addition of nitrate to Shilajit sample 7 at different concentrations. The retention time for nitrate is 12.28 min. Following the standard addition, an increase in peak height was observed, directly proportional to the concentration of the standard nitrate added. Importantly, the alignment of retention times before and after standard addition confirms the validity of the method.

For quality assurance (QA), since there is no certified reference material (CRM) for unique Shilajit matrix. To ensure the accuracy of the analyses, a certified standard solution was used for calibration purposes and to confirm the retention times of all target anions.

Results

A specimen chromatogram for Sh1 is depicted in Fig. 5. As can be seen, the concentration (mg.g^{-1}) range of the anions is: sulphate (2.921); hydrogen phosphate (2.451); chloride (1.451); and nitrate (0.041). Figures 6, 7, 8, 9 and 10 show the results obtained for the quantitative analysis of anion concentrations in the analysed samples. Chloride is the sole anion present in all raw Shilajits and supplements (Fig. 6). The highest amount of chloride was found in S12 with 9.496 mg.g^{-1}, while the lowest was in S2 with 0.102 mg.g^{-1}. Among supplements, Sp2 had the lowest chloride content (0.040 mg.g^{-1}), and Sp5 the highest (0.931 mg.g^{-1}).

Following chloride, sulphate (SO_4^{2-}) was found as the second most common anion after chloride (Fig. 7). The highest sulphate concentrations were observed in S10 (12.412 mg.g^{-1}), S12 (11.772 mg.g^{-1}), and S14 (9.328 mg.g^{-1}). In comparison, Sp5 has the highest sulphate concentration among the supplements (0.854 mg.g^{-1}),

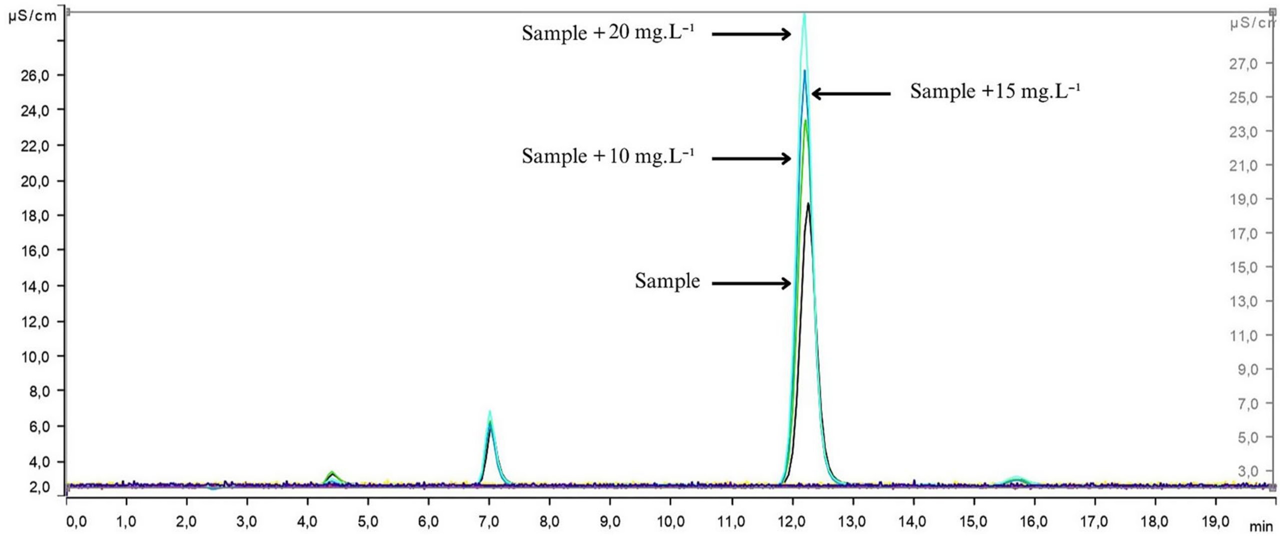

Fig. 4 Standard addition of nitrate (Rt, 12.28 min) at concentrations of 10, 15, and 20 mg.L^{-1} to Shilajit sample 7

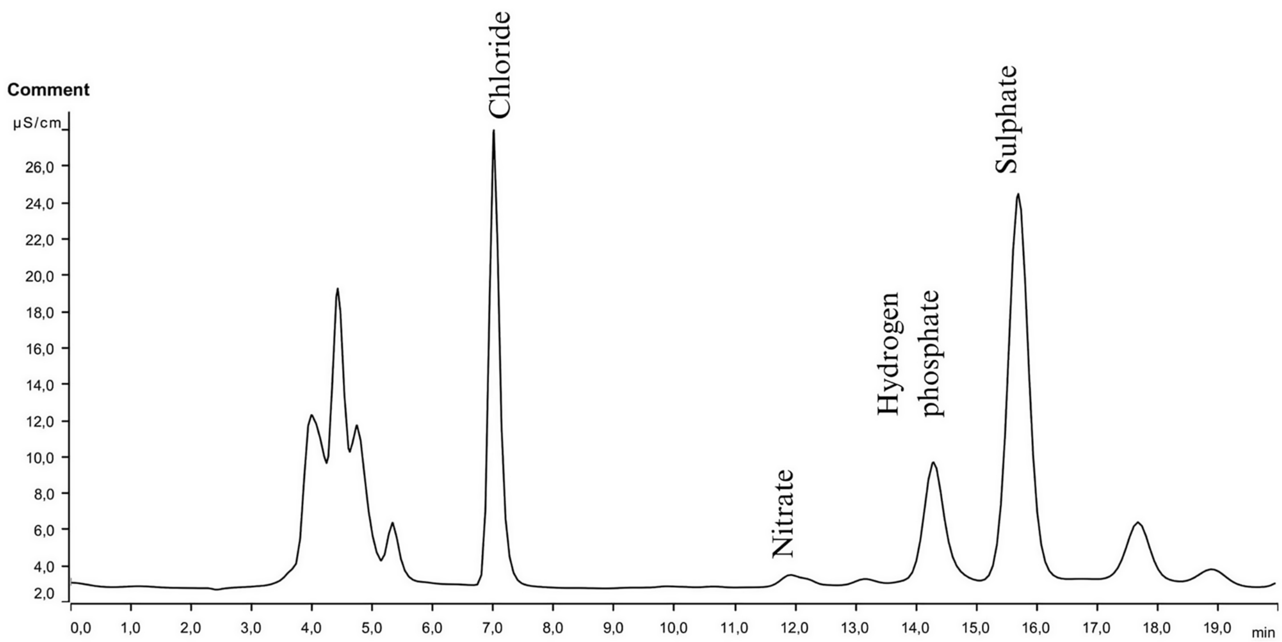

Fig. 5 A sample chromatogram of the anions of a Shilajit (S1)

while Sp2 had the lowest (0.024 mg.g^{-1}). Nitrate concentrations were lower than the limit of detection (LOD) in samples S2, S4, S11, and Sp3 (Fig. 8). The highest nitrate concentration is discerned in S7 at 9.504 mg.g^{-1}, while the lowest concentration among raw Shilajits is quantified in S1 at 0.041 mg.g^{-1}. Supplements reveal nitrate concentrations in the range 0.002 to 0.443 mg.g^{-1}, with S6 shows the highest concentration, exceeding even S1, S5, S8, S9, S13, and S14. Nitrite was quantified in S9, S11, and Sp6, with concentrations of 0.106, 0.137, and 0.056 mg.g^{-1}, respectively (Fig. 9). Hydrogen phosphate was not detected in S2, S7, and some supplements (Sp3, Sp4, Sp5, and Sp6) (Fig. 10). The highest concentrations were found in S6, S10, S1, and S13. Fluoride was quantified only in S2 (0.064 mg.g^{-1}), while no other halogens were found in supplements or raw Shilajits.

Discussion

Chloride's presence in Shilajit samples and supplements is likely due to the high content of NaCl salt. The chloride content in S1 surpasses that of several raw Shilajit samples, including S2, S8, S7, S5, and S6. In one report [8], the average amount of chloride in Shilajits obtained from Afghanistan and Pakistan was reported to be 84.050

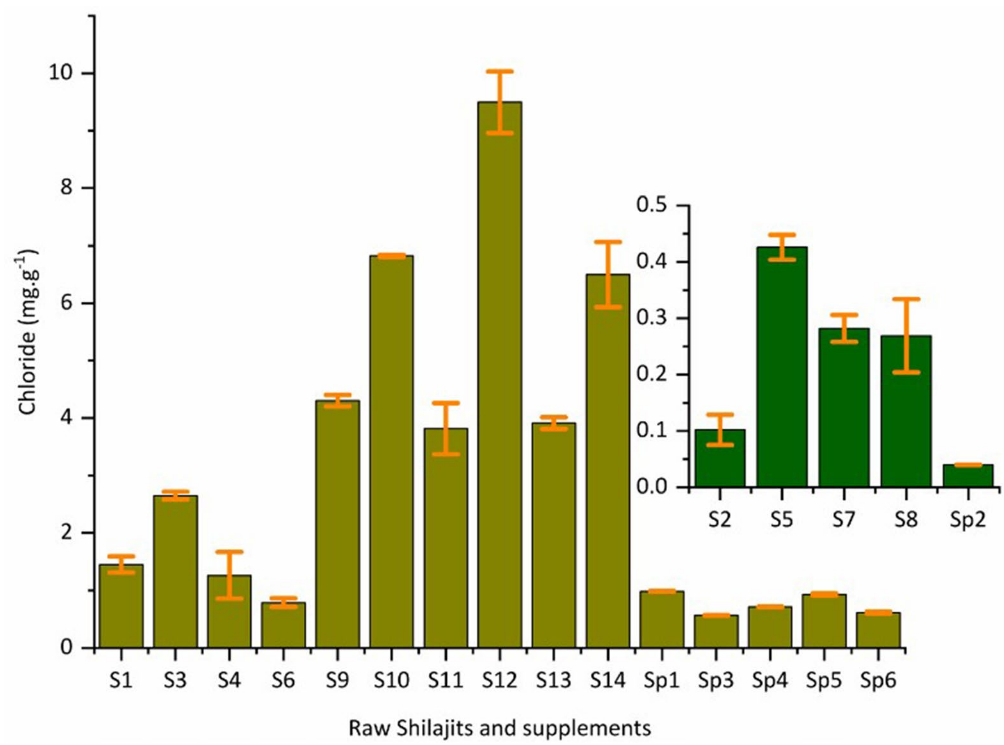

Fig. 6 Chloride content of raw Shilajits and supplements

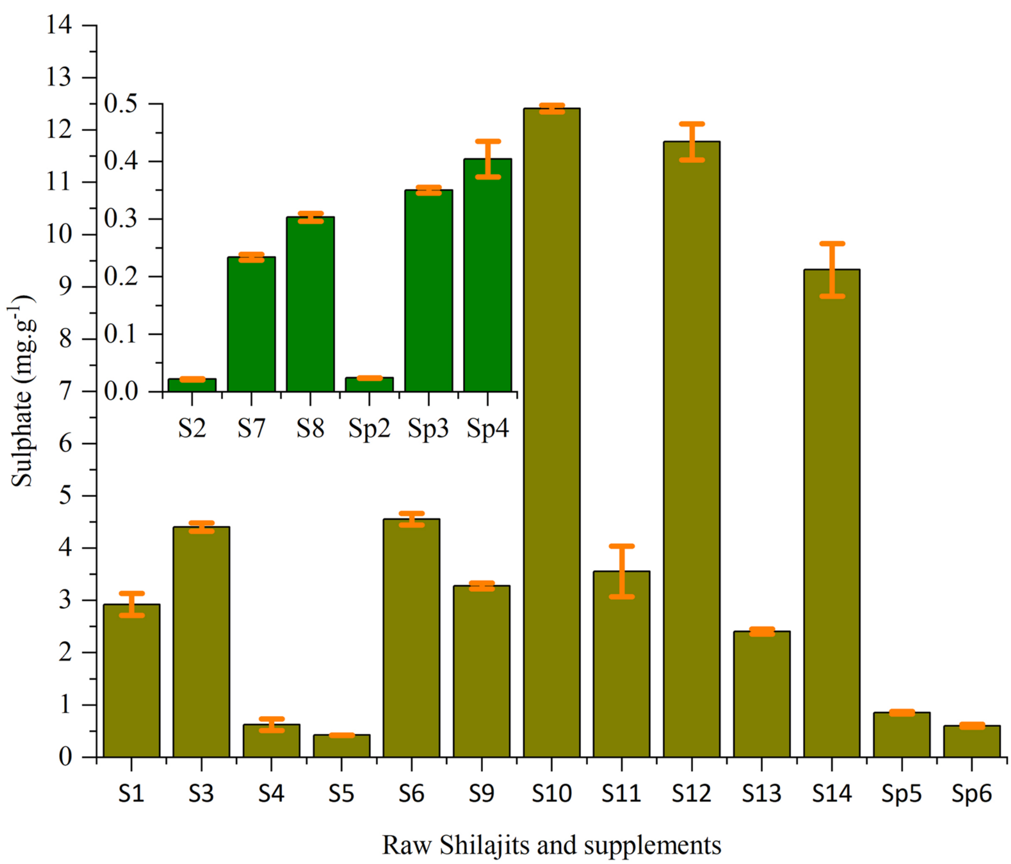

Fig. 7 Sulphate content of raw Shilajits and supplements

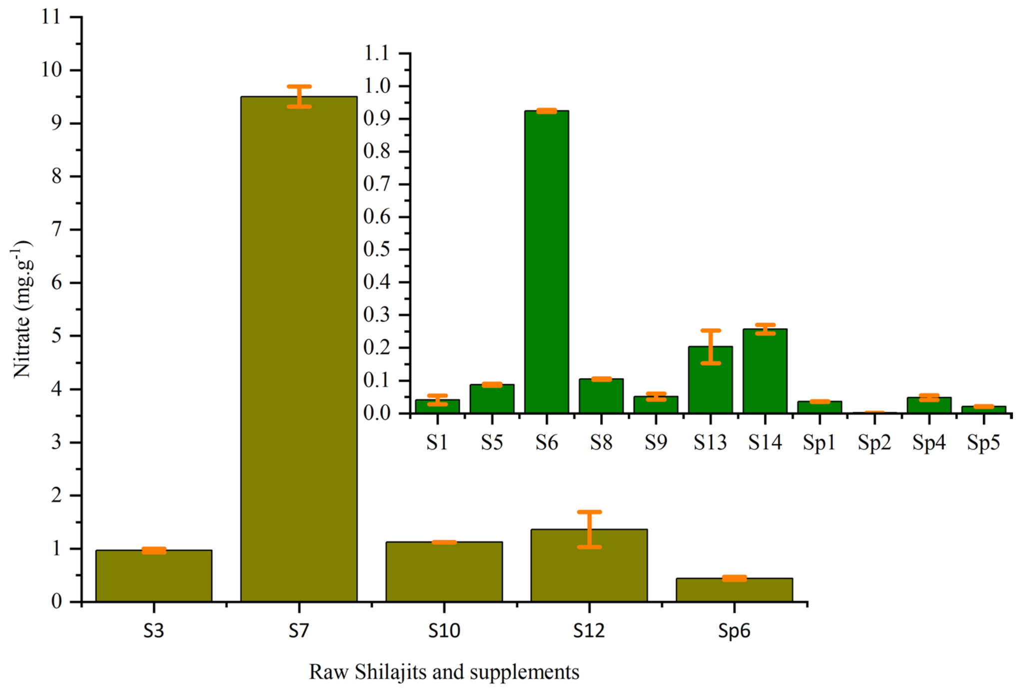

Fig. 8 Nitrate content of raw Shilajits and supplements

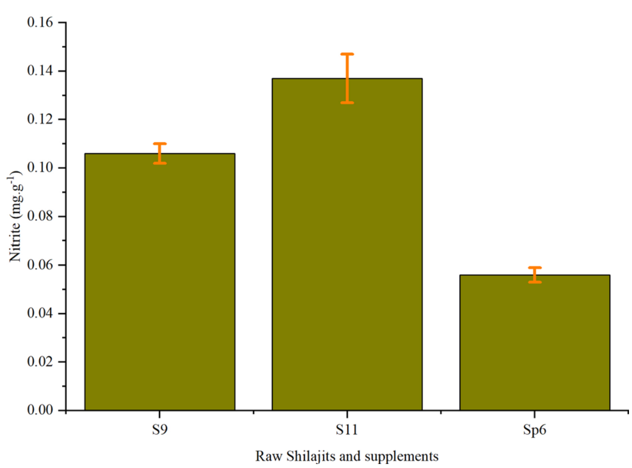

Fig. 9 Nitrite content of raw Shilajits and supplements

and 186.140 mg.g^{-1} respectively, which are much higher than any of the samples that we analysed or in any other report in the literature (Table 1). Chloride is a common inorganic anion found in various food sources, often correlating with sodium levels, and plays a crucial role in maintaining metabolic acid-base balance. Its significance continues to key roles within the central nervous system, particularly concerning the inhibitory effects of glycine and the modulation of gamma-aminobutyric acid, which regulates inhibitory synapses in the human brain and spinal cord by facilitating chloride ion influx into specific neurons [12]. Sulphate ions were present in all samples, with the highest concentrations observed in S10, S12, and S14. Supplement samples exhibited lower sulphate concentrations overall, except for Sp5, which had a higher content than some raw Shilajits. The lowest is in Sp2 at 0.024 mg.g^{-1} and it is lower than the LOD in Sp1. Also, S2 and Sp2 have almost the same concentration of sulphate. The content of sulphate in some supplements, such as Sp5 and Sp6 is higher than some Shilajits but overall sulphate content in Shilajit is more than supplements. Sulphate ions have caused several adverse reactions in hypersensitive individuals, especially asthmatics [13]. One theory regarding the source of Shilajit suggests it could stem from the decomposition of deceased organisms, including animals and plants. Fossil fuels are recognized as significant reservoirs of sulphur, with levels exceeding 10 g per million. The maximum safe intake level of sulphate hasn't been determined yet due to inadequate toxicological assessments. Nevertheless, water that contains a high concentration of sulphate of 6.7 g can cause severe diarrhoea to the consumer. Additionally, sulphur intake has been linked with ulcerative colitis, possibly due to the release of gaseous hydrogen sulphide [7]. Nitrate concentrations were notably variable across samples, with S7 showing the highest levels. For decades, dietary inorganic nitrate (NO_3^-) and nitrite (NO_2^-) were believed to be harmful to human body due to contribution to gastric cancer and other malignancies. However, the discovery of the L-arginine–nitric oxide synthase (NOS) pathway revealed their role as major sources of endogenous nitrate and nitrite, challenging the notion of their inertness. In vivo, they can be converted back to nitric oxide (NO) and other bioactive nitrogen

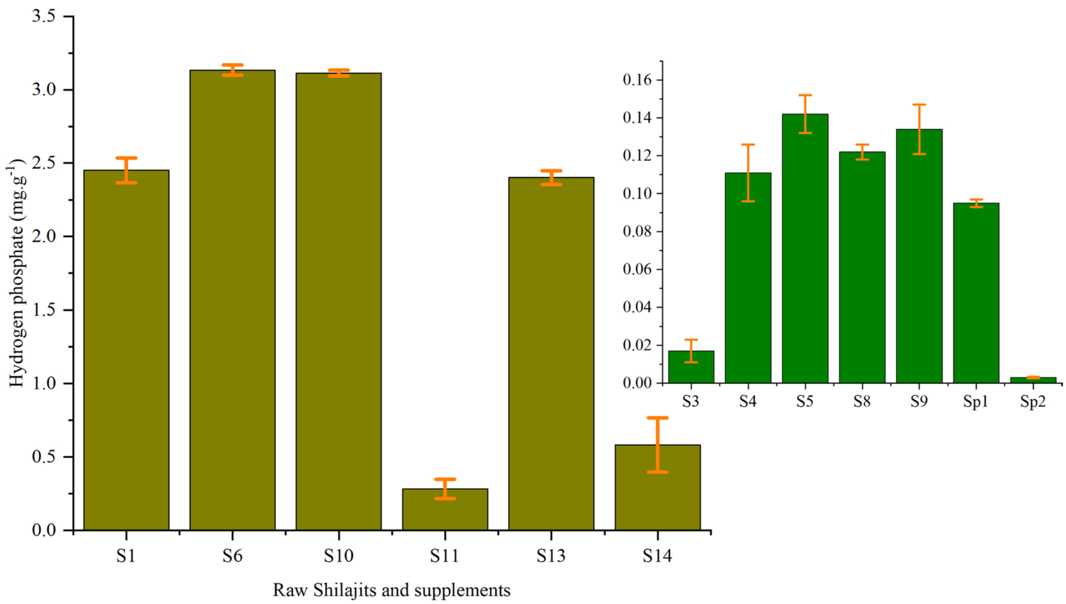

Fig. 10 Hydrogen phosphate content of raw Shilajits and supplements

oxides, with commensal bacteria playing a crucial role. Modest nitrate intake has shown benefits, including reduced blood pressure, improved platelet function, and enhanced mitochondrial function [13]. Notably, the hydrogen phosphate concentrations in S6 and S10 are almost the same, as are those in S1 and S13. Conversely, the lowest hydrogen phosphate concentration among raw Shilajits is in S3 at 0.017, whereas in Sp1 is 0.095 $mg.g^{-1}$, it is nearly fivefold higher. Inorganic hydrogen phosphates, common in consumer products, exhibit low toxicity orally, dermally, and through inhalation. Compared to food additives, systemic exposure from consumer products is minimal. Consequently, concerns regarding systemic toxicity at expected consumer product levels are minimal, given the low exposure and toxicity levels [14].

Fluoride was quantified solely in S2, with a concentration of 0.064 $mg.g^{-1}$, Fluoride plays a vital role in maintaining strong bones and good dental hygiene. It's crucial to consume the right amount of fluoride each day, as too little can lead to issues, but excessive intake can result in symptoms of acute or chronic fluoride toxicity, known as fluorosis [13].

Conclusions

This study provides analysis of inorganic anions present in Shilajit and some of its commercially available supplements. Using ion chromatography, key anions such as chloride, sulphate, nitrate, and fluoride across a diverse set of samples were quantified. The findings highlight significant variability in anion concentrations based on the geographical origin of Shilajit, with chloride and sulphate being the most abundant anions detected. Awareness of regional variations in anion content can guide manufacturers in formulating products with consistent and standardized compositions, ensuring that consumers receive reliable and predictable health benefits. Furthermore, our findings highlight that supplements exhibit variations in their inorganic anions content compared to raw Shilajit. This information enables consumers to make informed decisions, enabling them to choose supplements that align with their health goals while considering the specific inorganic anions present. Our findings indicate that on average, a single pill of a Shilajit supplement enters 0.17 mg chloride, 0.1 mg sulphate, 0.02 mg hydrogen phosphate, and 0.03 mg nitrate to our body. Traditional medicine typically prescribes two portions of 250 mg of raw Shilajit that enters 1.5 mg of chloride, 2 mg sulphate, 0.6 mg hydrogen phosphate, and 0.66 mg nitrate to the body. Based on these findings and the recommended dosage, the concentration of inorganic anions ingested through Shilajit are within the safe limits. The results highlight the necessity for stringent quality control and standardization in the production of Shilajit supplements to ensure their safety and efficacy. Moreover, the detected variation in anion content suggests that the health effects of Shilajit may vary depending on its source, which has important implications for consumers and manufacturers alike. As a result, development of standardized protocols for Shilajit extraction and supplementation will be crucial in maximizing its therapeutic potential while minimizing risks associated with contamination.

Abbreviations

IC Ion chromatographic
LOD Limit of detection
LOQ Limit of quantification

S_d Standard deviation

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Author contributions
EK: Investigation, writing, review and editing; JZ: Supervision, writing, review and editing; WL: Investigation; MK: Supervision, writing, review and editing. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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