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Full Length Article

Exploring the Therapeutic Potential of Shilajit from Various Regions of Gilgit-Baltistan, Pakistan: A Comprehensive Analysis

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Abstract

This research was conducted in the years 2021 and 2022. The investigation sites were Gilgit, Hunza, Nagar, and Chilas. Shilajit was collected in the spring season during the field. This study investigates the therapeutic potential of Shilajit by revealing its properties and therapeutic actions. Notably, antioxidant activity, measured through 2, 2-diphenyl-1-picrylhydrazyl (DPPH) values, ranged from 85.70 ± 0.035 mg/100 mL to 93.85 ± 0.005 mg/100 mL, with Nagar Valley samples displaying superior antioxidant properties. Total phenolic content varied across regions, with Gilgit at 17.54 ± 0.05 and Chilas at 17.07 ± 0.05 μg/100 mL, highlighting compositional variations influenced by vegetation. Physiochemical analysis, including the potential of hydrogen (pH), ranged from 5.8 ± 0.05 to 6.1 ± 0.05, with Nagar Valley exhibiting the highest at 6.1 ± 0.05. Moisture content varied from 8.02 to 8.07%, highlighting unique Shilajit characteristics in Gilgit Baltistan. Titratable acidity ranged from 0.26 to 0.29%, with Chilas samples displaying maximum acidity. Antimicrobial assessments indicated strong activity against *Escherichia coli* and *Enterococci* at concentrations of 5, 10, 15 and 20%, emphasizing superior Shilajit quality. Mineral analysis, conducted through gas chromatography (GC) and atomic absorption spectrophotometry, highlighted Nagar Valley samples with the highest mineral composition. Heavy metal concentrations, including iron, zinc, chromium, manganese, cobalt, and lead, were within permissible limits, aligning with World Health Organization standards. Additionally, Shilajit's impact on cholesterol levels in individuals demonstrated positive effects. For instance, serum triglycerides decreased from 117.6 ± 24.6 to 93.7 ± 39.12, showcasing potential hypolipidemic properties. These numeric findings contribute to a comprehensive understanding of Shilajit's diverse benefits, ranging from antioxidant activity to mineral composition, highlighting its potential in health and wellness applications

Keywords: *Asphaltum*; Phenolics; Gilgit; Shilajit; Exudate; Albuminoids

Introduction

Shilajit (*Asphaltum*) originates and is found in the different mountainous regions of the world at an altitude of between 1000 and 5000 m. It presents on the hedge of rocks and is rooted in the rocks, as it is primarily found in the different regions of Gilgit Baltistan (Pakistan), Afghanistan, Nepal, and the neighboring areas of Asia (Yeo 2018; Yaqoob *et al*. 2023). For thousands of years, dynastic Egyptians used Shilajit, a naturally occurring substance, to mummify their bodies. This is the fundamental reason for Shalajit's fame. But for thousands of years, it has also been a crucial medication and dietary supplement in both conventional and alternative medicine (Kamgar *et al.* 2023). Shilajit is also known by different names in various parts of the world, including Salajeet, Mumie or Mummiyo, and Shilajatu. It is a light brown and deep brown exudate with flexible dependability that emerges from the layers of rocks in various mountains around the world, particularly the Himalayas in the Indian subcontinent (Rana *et al.* 2021). Shilajit has been used in one form or another for thousands of years, and it is used as food, a supplement, or medicine as part of folk medicine due to its therapeutic benefits (Patel *et al*. 2023). Due to its better adaptability, it is consumed in many countries (Elahi *et al.* 2024). Earlier studies have shown that Shilajit has been extensively used in the preparation of several medicines

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because Shilajit has the maximum number of mineral constituents that are beneficial for humans as well as animals, and their effects have always been the most reliable and safe (Kumar *et al.* 2020). Shilajit has four distinct types: Tamra (copper), Blue, White Rajat (silver), Suvarna (gold), which is red and Lauha (iron), which is dark brownish. The Lauha variety is quite common and well known in the Himalayas, where it should be the most effective and beneficial, according to the treatment point of view (Shakya and Mohapatra 2020). In 1910, Shilajit was first familiarized with the scientific world, and some studies were suggested and completed by laboratories of national sciences using governmental funding in central Asian countries like Uzbekistan and Tajikistan (Singh *et al.* 2021).

Further current studies have shown and described that those mosses of *Barbula*, *Fissidenc*, *Minimum*, *Thuidium*, etc., as well as mosses of bryophytes such as *Dumortiera*, *Asterella*, *Pellia*, *Plagiochasma*, and *Marchantia*, exist and are present near the rocks that have been exuded by Shilajit. These bryophytes are responsible for and the main cause of the development of Shilajit (Rodríguez *et al.* 2022). Traditionally, Shilajit was used for stubborn diseases, which included cervical lymphadenitis, tuberculosis, type 1 diabetes, digestive system diseases, obesity, cough, jaundice, hemorrhoids, toxemia, and several other inner tumors, and it was found safe for consumption (Hadi *et al.* 2020). In the history of almost three thousand years, the natural product Shilajit has played a significant role in traditional medicine and earlier Indian traditional medicine, the Soviet Union, and Tibetan pharmacology. Due to this, it was considered the treasure of the country, and its economic value has similarly increased. It is also used as a plant growth promoter (Khanal 2018). For thousands of years, Shilajit has been used to treat a variety of different health problems. Shilajit is considered the most vital Ayurvedic drug in the traditional medicine system. Inside, in its original form, it is an asphalt substance, a compressed herbal organic matter and composed of a dark and red gelatinous medium, very bitter in terms of taste. In the Ayurvedic text, it is called silajatu or shilajatu, but it is usually called Shilajit. Its Sanskrit meaning is conqueror: the destroyer of mountains and weaknesses (Revathy 2019). Himalayan villagers discovered Shilajit when observing white monkeys migrating to the mountains during the summer. Noticing monkeys chewing a semi-soft substance between rocks, villagers, inspired by the monkeys' attributes, began consuming it. They reported enhanced energy, improved digestion, increased libido, better memory and cognition, and reduced allergies (Peralta *et al.* 2016). Shilajit results from the extended decomposition of deceased plant components and organic materials, facilitated by microorganisms. Through this process, it transforms into a sticky, pale brownish exudate influenced by temperature. This substance exhibits various significant effects on human metabolism and contributes to the treatment of diverse diseases (Aziz *et al.* 2017). Shilajit compounds exhibit magnetization inhibition, free radical scavenging, light protection, and anti-inflammatory properties and contain non-toxic elements. The mentioned properties, however, do not include cancer prevention. Shilajit, a safe oral supplement, is regularly consumed by medical professionals. Future clinical research should focus on exploring its efficacy and active components in chemoprevention and alternative medicine (Mukhtar and Ahmad 2019).

There is a noticeable research gap regarding the comparative assessment of Shilajit's therapeutic effectiveness across different localities within Gilgit-Baltistan. Furthermore, a thorough investigation into the impact of regional variations in Shilajit composition on its antioxidant and mineral content is notably absent in the different regions of Gilgit-Baltistan. No prior clinical trials have been conducted in Gilgit-Baltistan to assess the therapeutic efficacy of Shilajit. This research aims to bridge this gap and is necessary for enhancing the applications of Shilajit for targeted health and wellness involvement.

The main objectives of the study area were:

1. To investigate the therapeutic potential of Shilajit sourced from different regions of Gilgit-Baltistan, Pakistan.
2. To explore Shilajit's advantages, focusing on antioxidants and minerals for health and wellness.

Materials and Methods

Investigation sites

The research area consists of four distinct locations within Gilgit-Baltistan. District Gilgit is positioned between latitude 35°55'25" N and longitude 74°21'56" E, with elevations ranging from 1500 to 4500 m. District Hunza is situated between latitude 36°19'10"N and longitude 74°39'23"E, with altitudes ranging from 2000 to 7000 m. District Nagar is located at latitude 36°16'34"N and longitude 74°43'51"E, with elevations varying from 2000 to 7700 m. District Diamar (Chilas) is found at latitude 35°25'18"N and longitude 74°05'32"E, with altitudes ranging from 1000 to 4000 m. Map of the study area is depicted in Fig. 1.

Data collection

Shilajit was collected by random sampling technique, from various mountain sites in the study area during the field surveys, starting with a visual inspection of rock cliff faces and crevices. Sterile tools were used to prevent contamination, ensuring sample accuracy, and collected samples were properly labeled and stored in clean, airtight jars to prevent degradation during transportation for accurate analysis. The raw Shilajit contained impurities such as dirt, rubble, small stones, and plant matter. In the purification process, the raw solid rock Shilajit samples were broken down into tiny pieces and placed in a jar or

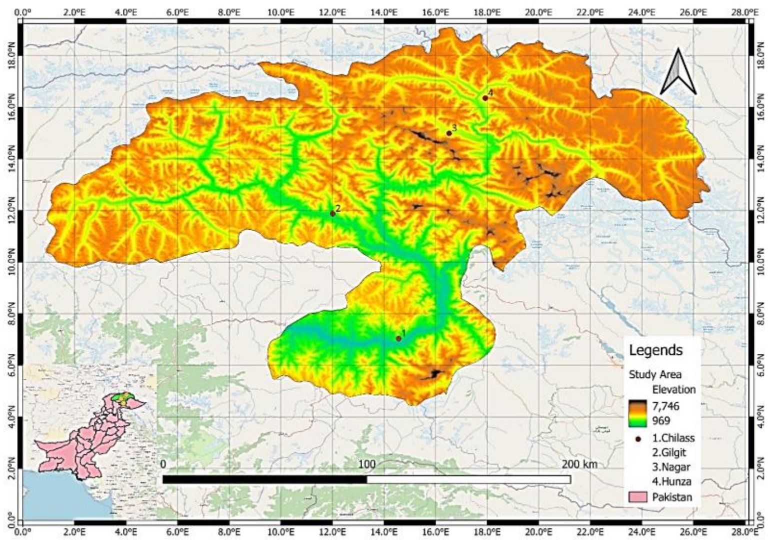

Fig. 1: Map of the study area

utensil, followed by the addition of water in an amount equal to the sample's weight. This mixture was left for 3–5 days to allow complete dissolution of Shilajit in water, resulting in the transformation of raw stone Shilajit into a dissolved state.

Data analysis

The data collected from various research sites in Gilgit-Baltistan underwent comprehensive analysis at the laboratory of the Department of Agriculture and Food Technology, Karakoram International University. After purification, Shilajit was evaluated for its impact on cholesterol and blood pressure in a 30-day trial involving 20 individuals aged 40 to 60. Blood samples were analyzed for triglycerides, cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels before and after supplementation with varying daily amounts of purified Shilajit (0.5 g, 1 g, 1.5 g and 2 g).

Determination of pH

Shilajit samples were analyzed for pH using a digital pH meter (AD 1020) following the standard AOAC (2006) method. Each treatment was placed in individual 100-mL beakers, and pH readings were recorded using a calibrated pH meter with pH 4.0, pH 7.0 and pH 9 buffer solutions. The electrode underwent cleaning with distilled water and tissue paper before each measurement.

Moisture content

The AOAC-recommended method (2006) was employed to ascertain the moisture content. Clean Petri dishes were weighed using an electric balance after oven drying. Shilajit samples were placed in the dishes, weighed together to determine the initial weight, dehydrated at 70°C for 4 h, allowed to cool in a dryer, and then reweighed. This iterative process was repeated until a stable weight was achieved.

$$\text{Moisture } \% = \frac{\text{weight of sample} - \text{weight of sample after drying}}{\text{weight of sample}} \times 100$$

Determination of titratable acidity

Total titratable acidity was calculated by the approved method given by AOAC (2006). For the determination of titratable acidity, 10 mL of Shilajit samples from each treatment were taken independently in 100 mL volumetric flasks, and volumes were made up to 100-mL with distilled water. 10 mL of the diluted sample was taken in another flask separately and 2–3 drops of phenolphthalein were to be added as an indicator. The diluted samples were titrated against a 0.1 N NaOH solution unless a light pink color is achieved, which was kept for 15–20 sec. The mL of 0.1 N NaOH used should be noted for the entire samples, and acidity can be calculated by the following formula:

$$Acidity = \frac{\mathrm{F} \times \mathrm{T} \times 0.1\mathrm{N\ NaOH}}{1.\times \mathrm{M}} \times 100$$

Where:
F = Factor of acid (citric acid).
T = mL of 0.1 N NaOH solution used.
M = mL of diluted sample taken for titration.
L = sample taken for dilution.

Determination of antioxidant activity

Antioxidant activity was assessed using the DPPH method outlined by Auribi *et al.* (2020). A DPPH solution was prepared by weighing 7.89 mg of DPPH, mixing it with 100 mL of 99.5% ethanol, and allowing it to sit in the dark for two hours. Due to the sensitivity of DPPH to light and heat, it was wrapped in aluminium foil and stored at a cool temperature. For evaluation, 5 g per treatment were homogenized and extracted with 10 mL of methanol for 48 h. From the extract, 0.1 mL was combined with 3.9 mL of DPPH solution (6×10^{-5} moles/L) in a volumetric flask and incubated at room temperature for 35 min. The absorbent mixture was measured at 517 nm after incubation with the help of a UV spectrophotometer. The antioxidant activity was measured with the help of the following formula:

$$\text{Inhabition } \% = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

Determination of total phenolic content

The Folin-Ciocalteu method, as modified by Wong and Tan (2020) was employed to determine the total phenolic content. 5 mL of Shilajit samples were homogenized and extracted with 10 mL of methanol for 48 h. 1 mL (mg/mL) of Shilajit sample extract from various treatments was independently dissolved in 4.6 mL of distilled water and 1 mL of 1 *M* Folin-Ciocalteu. After intervals of 3 min, 3 mL of 2% sodium carbonate was added to the solution and left for 120 min. The absorption of the mixture was measured at a wavelength of 760 nm using a UV spectrophotometer, with the gallic acid average curve serving as a reference.

Antimicrobial activity

Antibacterial activity in Shilajit extracts was assessed through the disc diffusion method, following the standard protocol by Hombach *et al.* (2015). For the assessment of microbial activity, two microbial agents, *Escherichia coli* (gram-negative) and Enterococci (gram-positive), were chosen for testing. A bacterial culture adjusted to the 0.5 MacFarland standard was evenly spread on Muller-Hinton agar plates. After drying for 15 min, discs saturated with various Shilajit extracts were placed on the agar surface, with each plate containing six discs: one positive control (standard antibiotic disc), one negative control, and four treated discs spaced equidistantly. The plates were incubated at 37°C for 18 to 24 h, and after incubation, the inhibition zones were measured and recorded using scale calipers. The test was repeated three times for reliability.

Mineral assessment

Shilajit samples were subjected to mineral analysis following the technique outlined by Rehan *et al.* (2017) using an atomic absorption spectrophotometer. Each 0.5 g Shilajit sample was dissolved in 25 mL of distilled water through heating. After a 20-min hold at 95°C, filtration was performed using a gravity filter. Subsequently, 1.0 mL of nitric acid was added to each sample, and the solution was diluted to 100 mL with distilled water. A 100× dilution factor was applied for further dilution, and concentrations of S, Fe, Mn, Zn, Ca, K, Mg and Na in the Shilajit samples were measured using the spectrophotometer.

Bio-evaluation studies

This study involved 20 healthy individuals aged 40–60, Shilajit was administered at various concentrations. Specifically, 5 participants received 0.5 g, another 5 received 1 g, and the remaining 10 were divided between receiving 1.5 g and 2 g doses. The supplementation period was 30 days, during which blood samples were collected before and after. Analysis for triglycerides (TG), cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels was conducted using the Micro Lab 300 LX Semi-Automatic Biochemistry Analyzer in a clinical laboratory in Gilgit Baltistan (Ojha *et al.* 2021).

Results

Antioxidant activity

Carotenoids, vitamin C, and vitamin E, along with naturally occurring antioxidants like flavonoids, tannins, phenols, and lignans, play a crucial role in preventing cellular damage from free radicals. The most reliable sources of these antioxidants are plant-based foods, including fruits, vegetables, whole grains, nuts, seeds, herbs, spices and cocoa. Descriptive mean data on DPPH antioxidant activity is provided in Table 1. The study highlights highly significant results in the antioxidant activity of Shilajit samples from various locations in Gilgit Baltistan. DPPH values ranged from 85.70 ± 0.035 mg/100 mL to 93.85 ± 0.005 mg/100 mL. The variance in antioxidant content between locations highlights possible differences in the composition of Shilajit, which may be related to different types of plants and environmental conditions. The highest values were found in samples from Nagar Valley (Fig. 2) and the lowest in samples from Chilas Valley (Fig. 3). The elevated DPPH values in Nagar samples suggest superior antioxidant properties, potentially attributed to the high altitude, favorable topography and richer vegetation in the region.

Total phenolic content

Phenolic compounds in Shilajit serve various functions, including UV screening, attracting agents, structural polymers (lignin), signaling agents (salicylic acid and flavonoids), defense responses, and antioxidants (tannins and phytoalexins). Total phenolic content data, presented in Table 1 reveals maximum phenolic content in Shilajit samples from all four localities in Gilgit Baltistan. The highest phenolic content was found in the Gilgit sample (Fig. 3), while the

Table 1: Antioxidant activity of Shilajit samples from different locations of Gilgit Baltistan

Study area	Antioxidant values	Total phenolics
Nagar	93.85 ± 0.005	17.27 ± 0.05
Gilgit	86.72 ± 0.005	17.54 ± 0.05
Hunza	92.73 ± 0.338	17.33 ± 0.06
Chilas	85.70 ± 0.035	17.07 ± 0.05

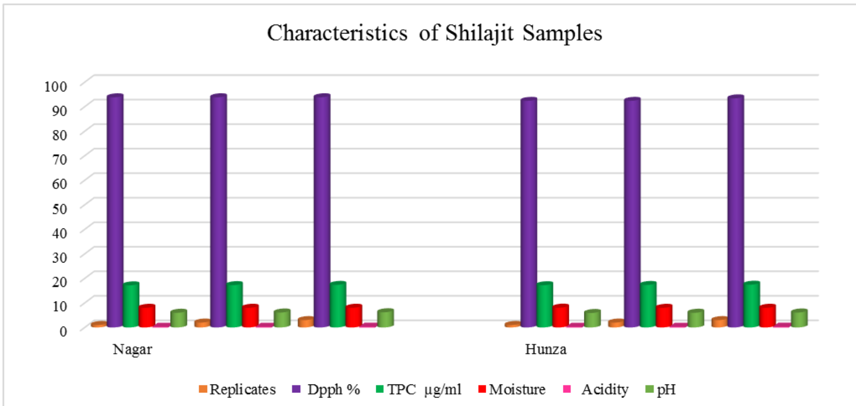

Fig. 2: The comprehensive analysis of samples from Nagar and Hunza

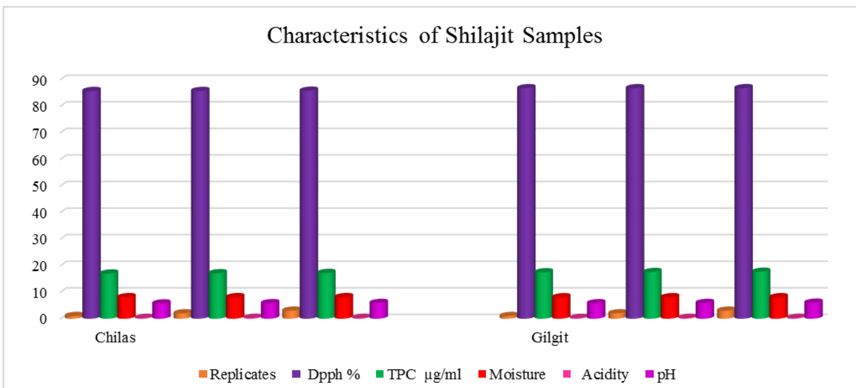

Fig. 3: The comprehensive analysis of samples from Chilas and Gilgit

minimum was in the Chilas sample (Fig. 2). The range of phenolic content across different areas underscores potential variations in Shilajit composition, possibly linked to distinct vegetation and environmental factors.

pH

The study presented pH data for collected Shilajit samples in Table 2. Results from different localities in Gilgit Baltistan ranged from 5.8 to 6.1 with Nagar Valley samples showing the highest value of 6.1 and Chilas Valley samples the lowest at 5.8. All values, including those from other localities, fell within a favorable range.

Titratable acidity

Data on titratable acidity is presented in Table 2. Shilajit samples from different localities in Gilgit Baltistan displayed highly significant results in terms of total acidity, ranging from 0.29 to 0.26%. The maximum acidity was observed in Chilas Valley samples, while the minimum was in Gilgit Valley samples. Lower acidity indicates a better profile for human consumption, reduced cancer risk and potentially longer shelf life.

Moisture content

Moisture content analysis of Shilajit samples from Gilgit Baltistan, including Nagar, Gilgit, Hunza and Chilas, revealed a range of 8.02 to 8.07%, as shown in Table 2. The lower moisture content in Nagar Valley signifies a unique and favorable Shilajit profile, distinct from other localities with slightly deviated percentages: Gilgit (8.04%), Chilas (8.07%) and Hunza (8.04%). The differences are attributed

Table 2: Physiochemical analysis of the Shilajit samples collected from different localities of Gilgit Baltistan

Study area	Moisture %	Acidity	pH
Nagar	8.02 ± 0.01	0.27 ± 0.01	6.1 ± 0.1
Gilgit	8.04 ± 0.01	0.26 ± 0.01	5.9 ± 0.34
Hunza	8.03 ± 0.028	0.28 ± 0.0105	6 ± 0.6
Chilas	8.07 ± 0.011	0.29 ± 0.011	5.8 ± 0.51

to regional vegetation variations, contributing to the novelty of Shilajit characteristics in Gilgit Baltistan.

Antimicrobial activity

The antibacterial susceptibility of Shilajit samples from research areas (Hunza, Nagar, Chilas, and Gilgit) was assessed against *E. coli* and Enterococci at concentrations of 5, 10, 15 and 20%. No bacterial growth was observed on the media, indicating strong antimicrobial activity across all locations. This high susceptibility suggests superior Shilajit quality in Gilgit Baltistan compared to other regions globally.

Mineral analysis

The study presents mineral analysis data from four Gilgit Baltistan localities in Table 3, utilizing GC atomic absorption spectrophotometry for param like S, Fe, Mn, Zn, Ca, K, Mg and Na. Results, all within safe limits, indicate Nagar Valley samples have the highest mineral composition in Gilgit Baltistan. Parameters in this locality were S 2.229 mg kg^{-1}, Fe 1.495 mg kg^{-1}, Mn 1.8346 mg kg^{-1}, Zn 36.4820 mg kg^{-1}, Ca 30.972 mg kg^{-1}, K 2.252 mg kg^{-1}, Mg 2.178 mg kg^{-1} and Na 1.62 mg kg^{-1}.

Shilajit effect on cholesterol levels of individuals

The study involved 20 healthy individuals aged 40–60, with a median age of 50. Individuals received varying doses of purified Shilajit for 30 days (0.2 g, 0.5 g, 1 g and 2 g). Notably, Shilajit showed no adverse effects on liver or kidney function. Significant improvements were observed, including increased high-density lipoprotein (HDL) cholesterol and decreased serum TG, cholesterol, low-density lipoprotein (LDL) cholesterol and VLDL cholesterol levels. Blood samples, analyzed in Gilgit's city clinical laboratory, revealed these changes using the Micro Lab 300LX, Biochemistry Analyzer. The simultaneous increase in HDL and decrease in TG and cholesterol suggest Shilajit's hypolipidemic and cardio-protective properties, indicating potential benefits for lipid profiles and antioxidant effects. Further research is needed to understand the extract's mechanisms and its impact on specific diseases affecting these biochemical parameters. Details are depicted in Table 4.

Pearson's correlation coefficient

We conducted an analysis of Shilajit samples from various locations to examine the relationship between pH, acidity, and moisture content. Using Past 4.03 software, our findings indicate a positive correlation between moisture and acidity. Conversely, there was a negative correlation between moisture content and pH, as well as between acidity and pH. The details are depicted in Fig. 4.

Discussion

A comprehensive analysis of Shilajit samples from various locations in Gilgit-Baltistan, Nagar and Hunza depicted in Fig. 2, while Gilgit and Chilas are depicted in Fig. 3. The data revealed significant antioxidant activity, with DPPH values ranging from 85.70 ± 0.035 mg/100 mL to 93.85 ± 0.005 mg/100 mL (Rahim *et al.* 2016). Notably, samples from Nagar Valley exhibit the highest antioxidant values, indicative of superior properties, potentially attributed to the region's high altitude, favorable topography, and richer vegetation. The total phenolic content further emphasizes the health benefits of Gilgit Baltistan's Shilajit, with the Gilgit sample demonstrating the highest phenolic content at 17.54, comparisons with Uspenskaya *et al.* (2021) revealed that Shilajit from Pakistan has a higher phenolic content than its Indian counterpart. The Indian samples ranged from 13.34 ± 9.90 mg/100 mL to 15.87 ± 9.90 mg/100 mL, emphasizing the superior phenolic content in Gilgit Baltistan's Shilajit. This suggested that the Pakistani region's Shilajit showed greater health benefits, including potent antioxidant properties and anti-inflammatory effects (Rahim *et al.* 2016).

These phenolic compounds, including flavonoids and tannins, contribute to the antioxidant capacity of Shilajit. The study also provides insights into the physiochemical characteristics, including pH, titratable acidity, and moisture content, ensuring the safety and nutritional value of the Shilajit samples. Compared with Mishra *et al.* (2020), the Shilajit pH values from various countries differed: India (6.2, Kumoan), Nepal (7.5, Dolpa), Pakistan (6.8, Peshawar) and Russia (8.2, Tien-Shan). The regional diversity and Shilajit varieties contribute to these variations, with Nagar Valley's pH (6.1) aligning closely with the findings of the study in Gilgit-Baltistan. Comparisons with Saristiana *et al.* (2023), showed that the acidity values in Gilgit Baltistan's samples (0.26 to 0.29%) were slightly higher than their observed range (0.24 to 0.27%). Madagascar (Indian region) exhibited 0.27%, resembling Nagar Valley's 0.27%, while Chilas samples indicated a slight acidity, emphasizing the safe and nutritious profile of Shilajit from Gilgit Baltistan.

Table 3: Mean values of mineral analysis of Shilajit samples

Minerals	Descriptive results of mineral parameters			
	Nagar	Gilgit	Chilas	Hunza
S	2.229	2.132	2.187	2.089
Fe	1.495	1.48	1.4254	1.4178
Mn	1.8346	1.7	1.709	1.689
Zn	3.6482	3.476	3.356	3.267
Ca	30.972	28.560	28.1	27.899
K	2.252	2.134	2.1	2.098
Mg	2.178	2.155	2.190	2.210
Na	1.62	1.546	1.478	1.491

Table 4: Effect of Shilajit on blood chemistry MEAN ± SD

Blood parameters	Shilajit administration dose				
	Control	0.5 g	1 g	1.5 g	2 g
Triglyceride (mg/dL)	117.6	108.7 ± 1.069	105.8 ± 0.95	101.7 ± 1.46	93.7 ± 0.903
Cholesterol (mg/dL)	160.3	151.4 ± 1.076	149.3 ± 0.84	144.3 ± 1.172	127.1 ± 0.99
HDL (mg/dL)	41.1	43.9 ± 0.96	44.6 ± 0.973	42.6 ± 1.59	44.6 ± 1.32
LDL (mg/dL)	82.8	79.3 ± 1.32	76.5 ± 1.38	71.5 ± 1.55	62.5 ± 1.57

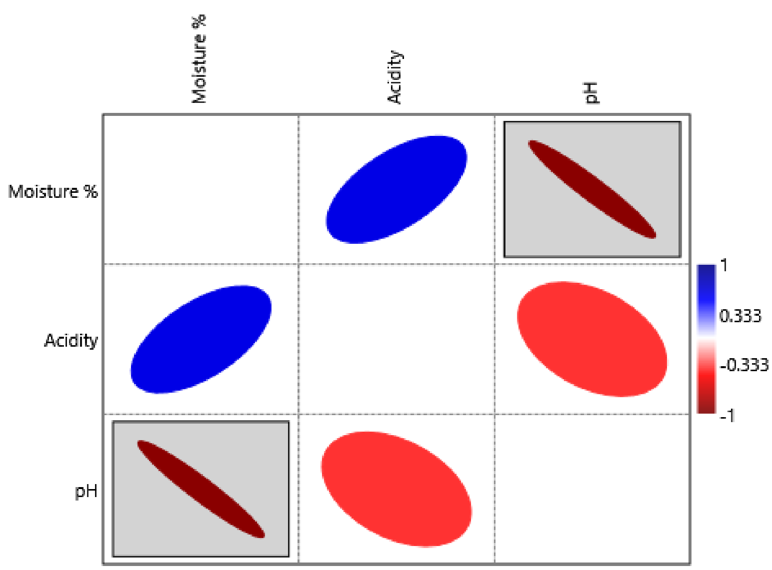

Fig. 4: Correlation Analysis of Moisture, Acidity, and pH in Shilajit Sample

The antimicrobial activity against *E. coli* and *Enterococci*, coupled with the high mineral composition in Nagar Valley samples, positions Gilgit Baltistan's Shilajit as a distinctive and potent natural supplement with diverse health-enhancing properties.

The findings align with previous studies, such as Shubaily and Jambi (2022), which demonstrated the antimicrobial properties of mumijo Shilajit. The *in vitro* study on extracted Shilajit from Gilgit Baltistan extends these findings, emphasizing the region's renowned high-quality Shilajit production. The mineral analysis further supports the superiority of Nagar Valley's Shilajit, showcasing higher percentages of essential minerals compared to Afghani Shilajit (Al-Salman *et al.* 2020). These results corroborate the safety and quality of Gilgit Baltistan's Shilajit, establishing it as a valuable natural supplement with potential health benefits. The variations in composition across different areas underscore the significance of regional factors, including vegetation and environment, in shaping the distinctive characteristics of Shilajit in Gilgit Baltistan. The novelty of these findings lies in the detailed analysis of multiple parameters, including antioxidant activity, phenolic content, physiochemical characteristics, antimicrobial activity, and mineral composition, providing a comprehensive understanding of the unique qualities of Shilajit from Gilgit Baltistan.

Shilajit, sourced from Nagar in Gilgit-Baltistan, showcases an impressive array of minerals, including sulfur,

Table 5: Comprehensive analysis of different havey metals with permisable limits by WHO and FDA

Metals	Concentrations mg kg^{-1} highest limits in Nagar	WHO& FDA daily minerals intake limits (mg/kg)
Fe	1.495	8.0
Zn	3.648	9.0
Mn	1.835	2.0
Na	1.620	2.3
Ca	30.972	57.0
Mg	2.178	3.1–3.4
S	2.229	10.0
K	2.252	2.6–3.4

iron, magnesium, manganese, zinc, calcium, potassium, and sodium, indicating its substantial nutritional profile and potential health benefits. The study also reveals heightened levels of antioxidants and phenolic compounds in Gilgit Baltistan's Shilajit, particularly notable in Nagar samples, which exhibit the highest DPPH values and phenolic content. This underscores the region's unique natural supplement with potent antioxidant properties. Additionally, the antimicrobial assessment suggests that Shilajit from Gilgit Baltistan displays notable efficacy against bacteria, implying its potential in microbial management and confirming its safety for consumption. Shilajit improves lipid profiles and antioxidant levels in individuals, demonstrating hypolipidemic and antioxidant effects, and offering promising avenues for further research into its impact on specific diseases and biochemical parameters.

The World Health Organization (WHO 1996) and the Food and Drug Administration (FDA 2019) have suggested 0.20 and 0.30 mg kg^{-1} of Cd, 1 mg kg^{-1} of Hg, 10.00 mg kg^{-1} of As and Pb, 20 mg kg^{-1} of Cu, and 50 mg kg^{-1} of Zn as the maximum amounts of heavy metals in herbal medications. With a few studies reporting exceeded amounts, the reported metal levels in Shilajit were lower than the FDA and WHO allowed limits. The permissible limits for heavy metal consumption are arranged in Table 5.

Conclusion

In conclusion, the study emphasizes the superior quality and safety of Gilgit Baltistan's Shilajit, notably in the Nagar region, which features abundant mineral, antioxidant, and phenolic content. It highlights the nutritional benefits and safety of Shilajit, with favorable mineral profiles. Furthermore, the antimicrobial assessment indicates potential health advantages. Crucially, Shilajit consumption demonstrates promising enhancements in lipid profiles without adverse effects on liver or kidney function. Future research should explore Shilajit's therapeutic effects further, focusing on its potential for managing conditions like diabetes and kidney problems.

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Author Contributions

SA and SK meticulously directed all research stages, from planning to results analysis. FF, in a supervisory role, contributed to the design and aided in specimen identification. WH actively participated in specimen collection, supported field and lab work, and assisted in professional formatting. SK managed initial draft formatting until manuscript approval. RA, AM and SD helped in collection specimens and laboratory works.

Conflicts of Interest

The authors of this paper declare no conflict of interest.

Data Availability

The corresponding author will provide access to the data from this study upon a justifiable request.

Ethics Approval

Not applicable to this paper.

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