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Comprehensive Analysis of Flavor Compounds, Chemical Composition, and Health-Enhancing Properties of Shilajit Powder

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Abstract

Shilajit is a promising substance that has gained commercial popularity due to its therapeutic and health-enhancing properties. The chemical profile of shilajit varies based on its source and storage conditions, with an average composition found in this study of 8.75% protein, 1.95% fat, 4.23% ash, 4.59% fiber, and a moisture content of 8.66%. The key flavor compounds of pure shilajit were analyzed, revealing that the musky aroma is primarily attributed to oxacycloheptadec-8-en-2-one, which constitutes 90.84% of the volatile fraction, along with n-decanoic acid (18.26%) and meso-hydrobenzoin (6.595%). Shilajit is highly water-soluble, and its morphology was characterized by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR), confirming the presence of quartz. The FT-IR spectrum also displayed characteristic stretching vibrations of carbonyl groups (C=O), commonly found in volatile compounds and carboxylic acids. The phenolic content was quantified, with fulvic acid and humic acid levels measuring 8.39% and 26.94%, respectively. Additionally, shilajit was found to be rich in vitamins B1 and B2. Heavy metal analysis detected trace amounts of elements such as Zn, Fe, and Cu. Therefore, it is concluded that shilajit is a versatile and nutritional source of active compounds, offering promising potential for applications in the food and therapeutic industries.

Key words: Shilajit; Solubility; Flavonoids; Heavy metal; Vitamins; GC-MS technique

1. Introduction

Shilajit is a natural bioactive substance with historical significance in traditional medicine. It has been used for centuries in Ayurvedic and Unani medicine to treat various ailments and enhance overall health. The World Health Organization (WHO) recognizes traditional medicine as an important healthcare approach for disease prevention and management, often used alone or in combination with food and herbal ingredients. According to WHO estimates, approximately 80% of the global population relies on traditional remedies, including shilajit, for therapeutic purposes. Consequently, there has been growing scientific interest in investigating its potential health benefits and applications [1].

Shilajit is an organic, resinous exudate formed over centuries by the decomposition of plant material and its interaction with microbial communities, minerals, and rock surfaces under specific climatic conditions. It is predominantly found in the Himalayas, the Tibetan Plateau, and other mountainous regions. Certain plant species, such as Euphorbia royleana and Trifolium repens, contribute to its formation. This natural process results in a dark brown to black substance rich in bioactive compounds. Due to its unique composition and medicinal properties, Shilajit has been widely regarded as a valuable therapeutic agent with economic significance [2]. Shilajit has been widely used in ancient medicine as it was used to enhance immunity, raise energy levels, and

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improve body health due to its immune and therapeutic properties. Its smell is a mixture of smoke and rock. Shilajit contributes to its multiple uses in promoting health and muscle growth in cases where it may treat problems of the digestive, urinary, and reproductive systems, as well as diabetes, angina and bronchial problems, anemia, and osteoporosis [3]. The chemical composition of Shilajit varies according to the region and the processing conditions. Shilajit is characterized by its content of humic substances mainly, which may be found in high proportions in the volume of shilajit. In addition, it contains a variety of amino acids, phenolics, minerals, and trace elements. Shilajit is also rich in flavonoids, vitamins, and organic acids, which contribute to its distinctive taste and potential health benefits. Furthermore, its antioxidant properties help neutralize free radicals, thereby reducing oxidative stress and supporting overall health.

Shilajit is highly soluble in water, which enhances its versatility in both food and health applications. Its properties vary depending on factors such as purity level, extraction conditions, and the duration of the decomposition process [4]. The quality of shilajit is largely influenced by its extraction and purification methods, highlighting the need for further scientific research to fully understand its chemical composition, long-term health effects, and potential applications. Additionally, its bioactive flavor compounds contribute to its therapeutic and nutritional significance, making it a unique candidate for medicinal and functional food applications [5]. This study aims to comprehensively analyze the chemical composition and bioactive components of shilajit powder while investigating its potential health benefits. The study involves the extraction and identification of key flavor compounds using gas chromatography-mass spectrometry (GC-MS). Furthermore, the concentrations of phenolic compounds, flavonoids, and both water- and fat-soluble components were determined using high-performance liquid chromatography (HPLC). Finally, physicochemical techniques such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and atomic absorption spectroscopy were employed to assess the morphological structure and heavy metal content of shilajit powder.

2. Material and methods

2.1. Preparation of the sample

2.1.1. The sample collection

A shilajit sample was purchased from a reputable store in Baghdad, Iraq (Herbs Paradise). The raw shilajit sample was solid, dark black in color, and free of impurities. To facilitate extraction, the sample was heated in a water bath at 60°C for 15 minutes. It was then transferred to a dry container and left at 25°C for two hours. After cooling, the sample was finely ground using a grinder to obtain shilajit powder, which was then stored for further analysis.

2.1.2. Aqueous extract preparation

A 100 g sample of shilajit powder was mixed with 500 ml of distilled water and incubated at 40°C for 24 hours. The mixture was stirred on a magnetic stirrer for 30 minutes and then filtered through Whatman No. 1 filter paper. The filtrate was dried in an electric oven at 40°C, after which the dried extract was collected, scraped, and stored in bottles in a refrigerator [6].

2.1.3. Alcoholic extract preparation

For alcoholic extraction, 50 g of shilajit powder was mixed with 500 mL of 70% ethanol. The mixture was shaken in an incubator for four hours and left at room temperature for 24 hours. The filtrate was collected using Whatman No. 1 filter paper, dried at 40°C in an electric oven, and stored in airtight bottles in a freezer until further analysis [7].

2.2. Chemical composition of the shilajit sample

The chemical composition of shilajit powder was evaluated using the A.O.A.C. method [8].

2.3. Solubility analysis

The solubility of shilajit was estimated by Carrasco-Gallardo et al. [9]. Briefly, adding 2 ml of distilled water, 95% ethanol, and chloroform to 400 mg of shilajit. The progress was stirred using a mechanical shaker at a speed of 150 rpm for 45 minutes at room temperature.

2.4. Identification of flavor compounds using GC-MS

The percentages of volatile compounds in the aqueous and oily extracts of shilajit powder were estimated according to the method of Stein et al. [10]. Samples were taken from an aqueous and oily extract of shilajit powder to estimate the flavor compounds in the Shimadzu GC-MS 2010 plus gas chromatograph mass spectrometry) device. The volatile compounds of the oil extract were identified using a gas chromatograph connected to a mass spectrometer type GC-MS QP 2010 ultra-Shimadzu, Japan. After obtaining the mass spectrum of each compound, the results were processed using the GC-MS solution program, and the effective peak curves were defined based on the database of the Nstao8 machine.

The GC-MS analysis was performed under the following conditions: split mode 1:10, the separation column type is DB-5MS capillary column (5% biphenyl-95% dimethyl polysiloxane cross-section); 30 m (length) × 0.32 mm (inner diameter) with a film thickness of 0.25 ?g, the injection temperature is 280°C, the column temperature started at 80°C for 2 min, then increased by 10°C/min until it reached 280°C and continued for 6 min, the gas type used is helium 99.99%, the mass spectrometer is an electron impact ionization (EI) ion source; m/z from 40 to 800, and the sample volume at injection is 1 ?g at the mass spectrometer temperature of 200°C.

2.5. Measurement of flavonoids and vitamins using HPLC

Flavonoids and vitamins in the shilajit extract were estimated using high-performance liquid chromatography (HPLC) device of the German type Sykam according to the method mentioned in Ding et al. [11], with some modifications using a C18 separation column with a particle size of 5 microns (250 mm × 4.6 mm) and a mobile phase of 100% acetonitrile at a flow rate of 1.5 ml/min at a temperature of 25°C. An extract was taken and mixed with 5 ml of hexane, then 20 microliters were injected, and the detector was set at 285 nm. The retention time of the model compound was compared with the retention time of the standard compound. HPLC analysis was performed using external standards for quantification. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively. For the major phenolic compounds and vitamins, the LOD values were in the range of 0.05-0.10 µg/mL, while LOQ values ranged between 0.15-0.30 µg/mL, consistent with reported values for similar analytical methods. 2.6. Estimation of X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis was conducted using a Shimadzu XRD system (Japan). The sample was prepared to ensure homogeneity and purity before analysis. For XRD analysis, samples were oven-dried, finely ground, and sieved to <75 µm before mounting on the sample holder. Diffraction patterns were recorded under standard operating conditions, and each sample was analyzed in triplicate to ensure reproducibility. The sample was placed in the XRD device, and the necessary settings were adjusted, such as the X-ray wavelength, scattering angle, and scanning speed, according to the characteristics of the sample and the analytical objectives. The measurement process is carried out by sending X-rays to the sample, where the rays interact with the atoms and scatter at specific angles. On this basis, the scattering pattern is recorded and displayed on a graph showing the scattering intensity as a function of the angle. This pattern is used to analyze the crystal structure of the sample and then compare the data to accurately determine the crystal structure and provide information about the size of the grains or crystals [12].

2.7. Fourier-Transform Infrared Spectroscopy (FT-IR)

The method described by Khanna et al. [13] was followed in the characterization of the functional groups of shilajit powder by IR spectroscopy. A sample tablet was prepared with potassium bromide (KBr) by mixing 40 mg of the sample with 120 mg of KBr and crushing it well with a ceramic mortar for 10 minutes. 40 mg of the mixture was compressed by a hydraulic press of the FT-IR device at a pressure of 8 bar for 60 seconds. Then, the compressed tablet was placed in the analyzer at a frequency range of 400-4000 cm^{-1}.

2.8. Heavy metals analysis

1 g of dry and ground shilajit was taken and added to 30 ml of hydrochloric acid and 10 ml of nitric acid, and the solution was left on a hot plate at a temperature of 120 °C. The solution was filtered, then covered with a watch glass and placed on a hot plate at a temperature of 80 °C to allow gradual evaporation of the acid until a precipitate of 5-10 mL remained. After that, the volume was increased to 100 ml by adding distilled water and then analyzed by an atomic absorption spectrometer [14].

3. Results and discussion

3.1. Proximate composition of pure shilajit

Shilajit is a complex natural substance composed of various bioactive compounds, including minerals, proteins, carbohydrates, and fiber. Its chemical composition varies depending on its geographical source, environmental conditions, and processing methods. The proximate composition of pure shilajit is presented in Table 1. It was found that the moisture content of the shilajit sample was 8.66%. This result is relatively low compared to Swat and Wybieralska [15]; the moisture content in natural shilajit ranges between 5% and 12%, as does the stability of the product when stored for long periods without decomposition. As for ash, the result was 4.32%. This reflects the presence of essential minerals such as iron, calcium, and magnesium, which play an important role in enhancing biological functions. As for protein, it's around 8.75%, the protein in shilajit enhances the absorption of other nutrients. As for fiber, the result was 4.59%; this is

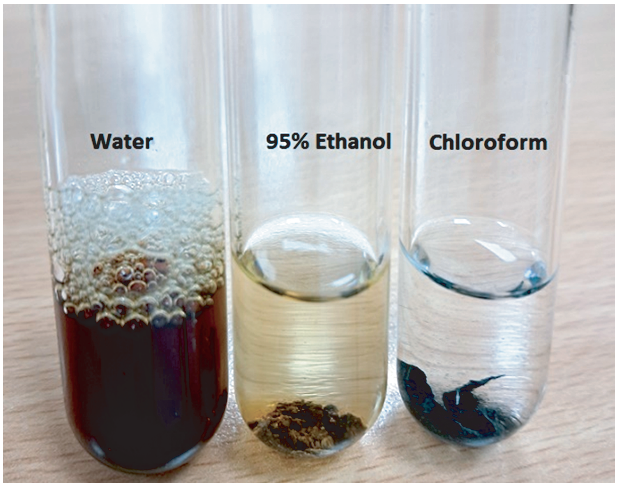
Shilajit content	Percentage (%)
Moisture	8.66
Ash	4.23
Protein	8.75
Fiber	4.59
Carbohydrate	71.82
Oil	1.95

consistent with Patel [16], who found that the fiber proportion in shilajit may reach 6-4%, and these fibers will improve digestive health and support proper absorption of nutrients. The high percentage of carbohydrates was obtained at 71.82%, which results agree with Marhoon and Abbas [17], who indicated that carbohydrates constitute the majority of shilajit components at a rate ranging between 70% and 75%. Finally, the fat was 1.95%, as these few fats help in supporting cell membranes but are not a major source of energy in shilajit. As mentioned above, the results of the pure shilajit sample are consistent with the ratios shown by previous studies, indicating that the chemical composition of this sample reflects the natural balance of shilajit, which enhances its use as a nutritional supplement to support general health [18, 19].

Table 1. Proximate composition of pure shilajit

3.2. Solubility analysis of shilajit

When studying the solubility of shilajit, as shown in Figure 1, it dissolves rapidly in water, while it is slightly soluble in alcohol and insoluble in chloroform. The results showed that shilajit is highly soluble in water, slightly soluble in alcohol, and does not dissolve in chloroform [20]. Shilajit contains a high percentage of fulvic and



this rapid dissolution in water makes it ideal for use in beverages and nutritional supplements. As for the solubility of shilajit in alcohol, it was found to be limited in solubility, which is consistent with the study by Ndwandwe [21], which indicated that non-polar organic compounds in shilajit, such as organic acids and some fats, may dissolve slowly in alcohol. Regarding its insolubility in chloroform, a non-polar organic solvent, shilajit contains polar compounds that do not react well with non-polar solvents such as chloroform. This confirms that shilajit depends on its solubility in polar solvents, such as water, while it is insoluble in non-polar solvents or is only slightly soluble in them.

Figure 1.The differences between the solubility of shilajit

3.3. Flavor compounds of shilajit powder analysis.

When examining the flavor compounds in shilajit powder oil, it was noted that there is a clear chemical structure that contributes in several ways to affect the food product from a sensory perspective, which represents the flavor compounds that give shilajit its distinctive, smoky flavor in varying proportions (Figure 2).

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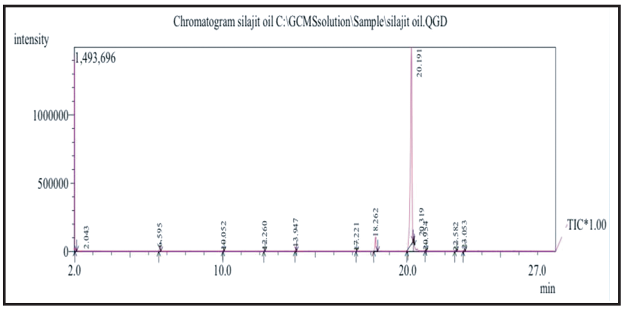


Figure 2. Flavor compounds peak for shilajit (GC-MS).

By analyzing the flavor compounds, it can be noted that there are phenolic compounds and aromatic derivatives known for their effect on the flavor and smell of the compound in which they are present. As shown in Table 2, the meso-hydro benzoin compound contains two opposite hydroxyl groups, which contribute to modifying flavors, especially in foods and beverages. When the second peak appears at 6.592 and with an area of 1.20%, as in Figure 2, they all fall within the methyl phenols known for their strong, overwhelming smell, or they can be considered undesirable at times, especially when they are present in high concentrations, as they contribute to the appearance of smoky and earthy flavors, in addition to more complex flavors, which makes them influential elements on fermented product [22]. On the other hand, other compounds, such as furan derivatives, improve flavors when they are present in moderate proportions, such as coffee and chocolate. Accordingly, it can be said that the compounds in Table 2 enhance sweet and roasted flavors as furan derivatives by adding some smoky and earthy flavors, as in the case of methyl phenols.

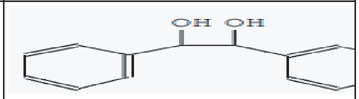
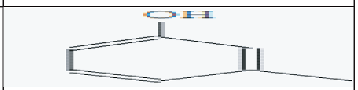
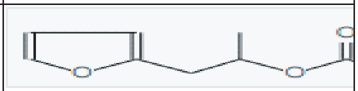
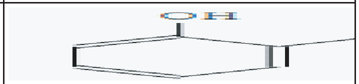
The final effect of these compounds depends to some extent on the concentration of each in the food substance. The chemical compounds in each of the seventh peaks in Figure 2 18.262 and an area of 4.37%, which consist of fatty acids as in Table 2, play an important and fundamental role in the development of flavors used in the manufacture of foods and perfumes, as they contribute to the emergence of a fatty and warm character, which gives a creamy, buttery, or fatty flavor to food products and their aromatic components.

These fatty acids have important properties that make them effective in terms of smell, as they are compounds with long chains, as in the saturated fatty acids n-Decanoic acid and nonanoic acid, and are characterized by adding a light fatty flavor and contributing to enhancing sharp and basic odors [23]. At the eighth peak (20.191) and an area of 90.84%, as in Figure 2, these compounds are widely used in the food and perfume industries due to their chemical composition consisting of aldehydes and alcohols that result in the floral and woody flavor or the smell of musk. These compounds play an important role in developing rare flavors and perfumes, and the compounds mentioned in Table 2 relate to unsaturated fatty acids in certain numbers.

The presence of the compound Oxacycloheptadec-8-en-2-one was observed, which contains in its chemical composition a single ring of oxygen composed of a double bond at a specific site.

This compound gives, in addition to fruit flavors, the flavor of lavender or jasmine flowers in certain cases due to the presence of carbon and oxygen elements among its components. Previous studies have indicated the possibility of linking the light floral scent with the compounds present in the structural structure of oxygen and used to improve the flavors and tastes of expensive foods and perfumes [24].

The unsaturated aldehyde compound 9,17-Octadecadienal contains a long chain of carbon and oxygen, which in turn affects the flavor through the appearance of the fruity and herbal flavor, as well as the appearance of woody flavors in the saturated aldehydes of this compound in foods and perfumes. On the other hand, there is a long-chain compound that is considered one of the important.

Compound name	Chemical formula	Chemical structure
meso-Hydrobenzoin	$C_{14}H_{14}O_2$	
p-Cresol	C_7H_8O	
Phenol, 3-methyl-	C_7H_8O	
2-Furanethanol, alpha. -methyl-, acetate	$C_9H_{12}O_3$	
Phenol, 2-methyl-	C_7H_8O	
n-Decanoic acid	$C_{10}H_{20}O_2$	
Nonanoic acid	$C_9H_{18}O_2$	
Undecanoic acid	$C_{11}H_{22}O_2$	
Tridecanoic acid	$C_{13}H_{26}O_2$	
11-Bromoundecanoic acid	$C_{11}H_{21}BrO_2$	

compounds called alcoholic fatty acids, such as Z, Z-3,13-Octadecedien-1-ol, which contributes to the formation of light and warm aromatic odors, which enhances its herbal or floral scent, which leads to improving the balance between the fatty and light flavors.

A type of aldehyde flavor compounds that contain medium chains with double bonds, and as shown in Table 2, the two compounds 7-Tetradecenal gives the fatty fruit flavor and 13-Tetradecenal gives the woody or spicy flavor, which in turn affect the natural flavor in various food or aromatic products, such as the sharp flavor [25].

Table 2. The Second, seventh, and eighth peaks of flavor compounds.

As shown in Table 2, all the flavor compounds above had the highest area proportion from the total area, as noted in the second, seventh, and eighth peaks, respectively. That led to the dominance of their flavors and smells on the flavor of the shilajit material.

As for the third peak, 10.052 and with an area of 0.24, it was noted that it may contribute to complex flavor formation that may be desirable or undesirable depending on their concentrations, as in Figure 2. The structural similarity of its chemical composition was noted, as well as its belonging to the aromatic nitrogen compounds, which are important compounds responsible for the flavor and smell of shilajit, especially for foods that undergo thermal or fermentation processes. Through Table 3, it is possible to note the compound responsible for most of the distinctive odors that may appear in fermented foods and smoked meats, as in the Indole compound, it has a clear effect on the aromatic and earthy flavor when added in precise concentrations. Recent studies have shown that the presence of this compound in foods rich in protein can contribute to the formation of flavors rich in smoky and earthy flavors, which enhances their sensory value, as is the case in fermented cheese and cured meats [26]. On the other hand, there are unique flavors and smells, such as earthy and herbal flavors and the complex flavors found in fermented products. They may be desirable or undesirable depending on their concentration, as in the flavor of Indolizine.

At the fourth peak, 12.260, and with an area of 0.20 as in Figure 2, complex flavor compounds appeared that combine fatty, mineral, and woody components with a little acidity and sweetness as a result of the interaction of oxygen-rich compounds with aromatic compounds (Table 3). This is consistent with what previous research has reached, which indicates the difference in chemical compounds in the formation of rare and distinctive flavors, which are among the main factors in the formation of flavors of natural and manufactured products. Alkenes and furins contribute to the formation of the fatty, light mineral or smoky earthy flavor, especially when they interact with other fatty compounds found in different diets, as in the compound 3-Decyne, which is a compound containing 3 alkene bonds that can affect the flavor found in foods [27].

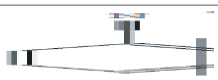
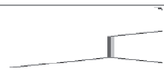
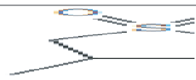
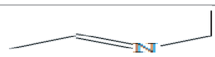
On the other hand, the table indicated that there are compounds that play an important role in stabilizing flavors and adding a distinctive aromatic character, as in cyclohexane derivatives known for floral and woody flavors similar to those found in essential oils and plant extracts [28]. In addition, there are compounds such as ketones and aldehydes, known for their strong effects on the smell, which contain chemical groups that give the final product a creamy, acidic, and lemony taste. The table indicates the presence of these compounds in ketone, isopropylidene cyclopropyl methyl, one of the cyclic ketone compounds that play an important role in giving the sweet fruit flavor [29]. With regards, it was found in the sixth peak 17.221 as shown in the Figure 2, and with an area of 0.06% nitrogenous compounds such as pyridinecarbonitrile contributing to sharp or strong flavors, there are compounds rich in esters that add floral flavor and fruit flavor that are rich in aromatic compounds, which in turn interact with other components to form a diverse mixture of flavors in Table 3, which enhances the quality of the sensory value for the consumer in products, whether aromatic or nutritional [30].

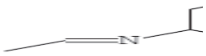
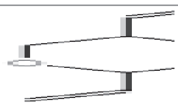
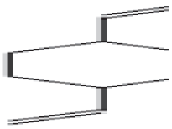
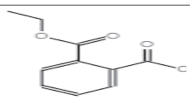
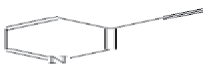
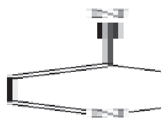
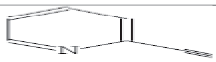
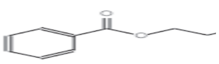
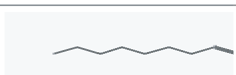
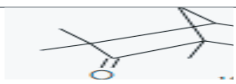
Finally, chemical compounds range from esters, alcohols, and alkenes in the eleventh peak (22.582) with an area of 0.18% to improve the flavor in food products and perfumes, as there are sharp flavors that may be aromatic or natural that differ according to their chemical structure (Figure 2). Current studies have indicated that the thiophene compound improves sharp and smoky compounds that may be useful in the flavors of meat or cooked foods such as the compound. Accordingly, these compounds help the flavor of food products and perfumes, such as sharp and aromatic flavors and natural flavors that differ according to their chemical structure. The compound in Table 3 is thiophene, tetrahydro-3-methyl-1,1-dioxide, which contains a thiophene ring with a methyl and oxidation group and has warm flavor properties similar to the flavors of nuts or spices. Research indicates that thiophene compounds enhance smoky or sharp flavors and are useful in meat or cooked food flavors. Previous studies indicated esters might enhance fruity flavors and raise the aromatic level of food flavors. As shown in Table 3, the compound butenoic acid, ethyl ester, has a somewhat mild fruity and acidic flavor and is usually used in the manufacture of fruit or sweet flavors, as it is an ester compound consisting of butenoic acid and an ethyl group, where it can be used to enhance flavors derived from citrus or fruits, as it gives a refreshing and acidic taste like pineapple or mango flavor. At the same time, there is an alcoholic compound known to have an herbal and natural flavor, similar to the flavor of fresh herbs or vegetables, such as the compound 3-Hexen-1-ol, as it contains a hydroxyl group in the hexane position.

Accordingly, previous studies have indicated the possibility of using those above-mentioned compounds in natural green flavors and using them in simple and quick-to-prepare foods such as salads and natural drinks [31]. In addition, there is a compound called 1-Pentene (Table 3), which is an alkene compound consisting of double bonds between carbon atoms that contribute to the emergence of sharp and aromatic flavors together, as it can be used in the manufacture of flavors for essential oils or flowers. In addition, the compound 1-Octyne, 8-iodo, which consists of a triple bond with an iodide group, may contain strong flavor properties that can be sharp and aromatic. Previous research has indicated that it gives strange flavors that may improve or enhance the aromatic composition of preparations related to essential oils and chemical compounds [32].

Flavor compounds in the tenth peak (20.954) with an area of 0.15%, such as aldehydes, alcohols, and esters, play an important role in the flavor and perfume industry, as shown in the Figure 2, as they cause the appearance of a hot, woody, and dried fruit taste, which contributes to improving the final product as in the food or perfume industry. Fenchone contains a compound ring with groups of ketones. Fenchone is widely used in the flavor and perfume industry due to its aromatic properties that give a slightly hot taste with a woody and balsamic flavor, as in the preparation of herbal or spice flavors. In addition, the above compound has distinctive aromatic properties that give a complementary flavor to food products such as soft drinks and juices [33]. Table 3 shows the presence of flavor compounds called enantiomers (manual) of the same compound, such as D-Fenchone and L-Fenchone, as they differ in the spatial arrangement of the chemical groups. These isomers are typically used in the same applications but may exhibit slightly different sensory properties. D-fenchone is commonly used in alcohol and spirits flavors, while L-fenchone is used in essential oils and plant-derived flavors. N-Butylfuran has a warm, mild, fruity, or nutty flavor and is used in baking and confectionery flavors. Research by Zweers and Vene [34] has shown that furans, such as 2-n-butylfuran, contribute to caramel and nutty flavors in foods and beverages.

Table 3. The third, fourth, sixth, tenth and eleventh peaks of flavor compounds

Chemical structure	**Chemical formula**	**Compound name**
3-Decyne	$C_{10}H_{18}$	
Cyclohexene, 3,3,5-trimethyl-	C_9H_{16}	
Ketone, isopropylidenecyclopropyl methyl	$C_8H_{12}O$	
Furane-2-carboxaldehyde, 5-(4-nitrophenoxymethyl)-	$C_{12}H_9NO_5$	
Photocitral A	$C_{10}H_{16}O$	
Thiophene, tetrahydro-3-methyl-, 1,1-dioxide	$C_5H_{10}O_2S$	
3-Butenoic acid, ethyl ester	$C_6H_{10}O_2$	
3-Hexen-1-ol	$C_6H_{12}O$	
1-Pentene	C_5H_{10}	
1-Octyne, 8-iodo-	$C_8H_{13}I$	
2-Ethylideneamino-propionitrile	$C_5H_8N_2$	

2-(Butane dinitrile, ethylide neamino)-	$C_6H_7N_3$	
p-Dioxane, 2,5-divinyl-	$C_8H_{12}O_2$	
Cyclopentanecarbonitrile	C_6H_9N	
2H-Pyran, 2,5-dietheny ltetrahy dro-	$C_9H_{14}O$	
3-Pyridine carbonit rile	$C_6H_4N_2$	
4-Pyridine carbonit rile	$C_6H_4N_2$	
2-Pyridine carbonit rile	$C_6H_4N_2$	
Benzoic acid, 2-phenylet hyl ester	$C_{15}H_{14}O_2$	
2-Methylb	$C_{15}H_{14}O_2$	
Fenchone	**$C_{10}H_{16}O$**	
D-Fenchone	$C_{10}H_{16}O$	
L-Fenchone	$C_{10}H_{16}O$	
2-n-Butyl furan	$C_8H_{12}O$	
Furan, 2-hexyl-	$C_{10}H_{16}O$	

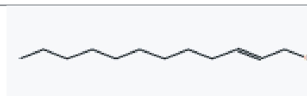
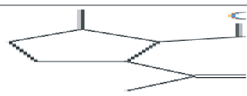
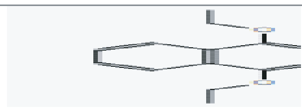
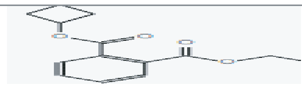
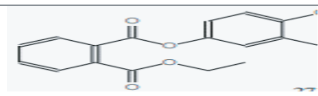
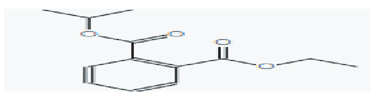
As for the fifth peak of 13.947 and an area of 0.74 as in Figure 2, we find that phthalic compounds have an important role in the development of smell and flavor by considering them important and complementary factors in stabilizing aromatic compounds used in many different industries to obtain stable and long-lasting smells with flavors, as is the case in natural and manufactured products. It was noted in Table 4 that phthalic compounds are considered aromatic and organic compounds used in various industries. For example, it was shown that the phthalic compound phthalic acid, cyclobutyl ethyl ester, contains a distinctive ester group and is a complementary factor for aromatic compounds, as in woody, oily, and floral flavors that can be used in perfumes, in addition to compounds that have a complex odor character due to their containing chemical groups such as chlorine and methyl.

At the ninth peak (20.319) and with an area of 0.69% as in Figure 2, carboxylic acids, alcohols, and fluoro-esters are combined, which help in the emergence of cold and sour flavors, which are ideal when used in modern industries such as food and aromatic industries. Table 4 indicates the presence of compounds linked to an alcoholic ester containing a long carbon chain, such as dichloroacetic acid and undec-2-enyl ester, which have acidic properties that

help improve the sour or light flavors of foods. The reason for the presence of the acid flavor is due to the association of acidic compounds such as dichloroacetic acid with flavor structures, which gives an acidic impression, which is one of the important things that balance food flavors. Studies have recently indicated that foods that consist of fluoro-compounds cause the emergence of new flavors that may last longer and have a direct effect on human senses. Some compounds cause the appearance of light flavors; they are considered sharp with cooling effects due to the presence of fluorine in their components, such as the compound (Z)-4-Decen-1-ol, chlorodifluoroacetate, which consists of unsaturated alcohols that may be linked to fluoroacetate esters as in juices or nutritional supplements to give soothing or refreshing effects. The compound also contains unsaturated alcohol but with a longer carbon chain, such as the compound trans-2-Dodecen-1-ol, chlorodifluoroacetate, which may sometimes be used in preparing special flavors such as those used in perfumes or luxury foods, as this compound gives oily, herbal, or woody flavors with a mild soothing effect. Finally, Table 4 indicates the presence of fluoro esters in some flavor compounds used in perfumes to improve their aromatic concentrations with a wonderful and distinctive character due to their effect on the smell [35]. The first peak in the figure above shows compounds that play an important role in the stability and consistency of the flavor by modifying its physical and chemical properties, as they are ester compounds known for their role in affecting the flavor. Peak 2.043 appeared, occupying an area estimated at 0.50% of the total area, as the compound 1, 2-Benzenediol, O-(3-methylbut-2-enoyl)-O'-(pivaloyl) is derived from hydroquinone linked to ester groups, so it has antioxidant properties, as these properties play an important role in the stability of the flavor by maintaining the oxidative stability that affects the quality of the final product [36]. Esters play an important role in the emergence of various aromatic flavors, especially the flavors found in fruits and fatty compounds, as the compound 3-Methyl-2-butenoic acid, trident-2-vinyl ester, as shown in Table 4, has an ester nature that makes it possible for it to affect the flavor of shilajit because it has carbon chains that are distinguished by their activity in flavor; the carbon chains that the flavor compounds have are short compared to the compound above. When the compounds in the table are compared with previous studies, it becomes clear that most compounds are not directly related to flavor. Still, their role is only related to the stability of the flavor or physical properties modification indirectly of the final product However, the ester compounds are known for their effect on flavor, so these compounds can be considered a by-product of chemical decomposition processes rather than being primary flavor compounds [37]. The chemical composition of the compounds identified in this peak is 23.053 (the twelfth peak) with an area of 0.46%, as shown in Figure 2, as it contains a variety of chemical compounds such as amino acids and nitro compounds, as well as compounds containing phenols. Depending on their properties and characteristics, these compounds may contribute to producing different flavors and fragrances. It can be noted that the compounds found in the mentioned peak can create complex flavors characterized by acidity, sharpness, and spice at the same time, and due to their addition of a rare and deep flavor together, this makes them ideal when used in foods and perfumes. Previous studies and research have supported the contribution of nitro compounds such as 2-Ethylideneamino-propionitrile and 2-(Butanedinitrile, ethylideneamino)-as well as p-dioxane, 2,5-divinyl-in enhancing the spicy, herbal, woody, smoky, and dry herb flavors in various food compositions. They may add depth to flavors for foods rich in proteins or grains or warm and intense enhancement flavors of concentrated aromas.

The cyclopentanecarbonitrile contains a nitrile group and a cyclopentane ring that imparts a sharp, fruity, or floral flavor, respectively. It can enhance aromatic flavors that resemble fruits or flowers and, in many cosmetics [38].

Table 4. The fifth, ninth, first, and twelfth peaks of flavor compounds

Compound name	Chemical formula	Chemical structure
(Z)-4-Decen-1-ol, heptafluorobutyrate	$C_{14}H_{19}F_7O_2$	
Diethyl Phthalate	$C_{12}H_{14}O_4$	
Phthalic acid, cyclobutyl ethyl ester	$C_{14}H_{16}O_4$	
Phthalic acid, 4-chloro-3-methyl phenyl ethyl ester	$C_{17}H_{15}ClO_4$	
Phthalic acid, ethyl isopropyl ester	$C_{13}H_{16}O_4$	
Phthalic acid, ethyl 2-methylallyl ester	$C_{14}H_{16}O_4$	
Dichloroacetic acid, under-2-enyl ester	$C_{13}H_{22}Cl_2O_2$	

(Z)-4-Decen-1-ol, chlorodifluoroacetate	$C_{12}H_{19}ClF_2O_2$	
trans-2-Dodecen-1-ol, chlorodifluoroacetate	$C_{14}H_{23}ClF_2O_2$	

3.4. Phenolics, flavonoids, and vitamins in shilajit

In the HPLC screening of flavonoids and phenolic compounds in shilajit, Table 5 indicates that the results obtained there are a wide range of biologically active compounds with antioxidant and anti-inflammatory properties. These results are largely consistent with previous studies on shilajit or similar compounds, such as ginsenosides in ginseng and anthocyanins in cranberries. The kaempferol compound plays an important role in preventing cancer and reducing damage caused by free radicals, which enhances the health of cells and tissues. As for vanillic acid, which was found at a concentration of 95.103 ppm, it is one of the phenolic acids known for its antioxidant properties. Compared to other studies on blueberries, which showed the presence of vanillic acid at similar concentrations ranging between 90 and 100 ppm, it can be said that vanillic acid plays a fundamental role in supporting immune health and reducing inflammation. Caffeic acid was identified at a concentration of 9.334 ppm in shilajit and is considered one of the important phenolic acids for protecting against neuroinflammation and chronic diseases. Catechol was also found in shilajit at a concentration of 21.846 ppm, a compound known for its antibacterial and antioxidant properties. Studies on blackberry juice have shown that catechol is present in these products at similar concentrations of 20-25 ppm, promoting tissue health and protecting the body from damage caused by free radicals. Overall, the results in most studies indicated that both black and yellow shilajit have the potential to be used in fortified food products after proving their non-cytotoxicity. This is due to the high protein content and strong antioxidant and antimicrobial properties of black shilajit and the high levels of calcium and iron in yellow shilajit. The highest concentration identified in shilajit tests was 4-hydroxy benzoic acid at 162.279 ppm, a compound proven to boost immunity and protect against chronic diseases. Olive oil contains 4-hydroxy benzoic acid at a concentration of 160-170 ppm, which is a compound that supports the immune system and enhances health. As for quercetin, a concentration of 5.287 ppm in shilajit, it was found in apples at similar concentrations (4-6 ppm). As for chlorogenic acid, which was found at a concentration of 40.943 ppm in shilajit, its concentration in coffee ranges between 30 and 40 ppm, where this compound enhances protection against diabetes and improves metabolism, which is the same effect observed in the shilajit test. Cinnamaldehyde, which was identified at a concentration of 23.409 ppm in shilajit, appears at a similar concentration in cinnamon oil (25 ppm), as shown in previous studies.

Table 5. Active compounds in shilajit powder

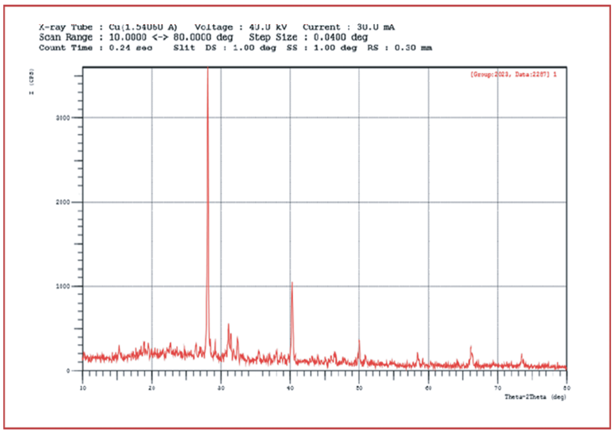
Compound	**Concentration (ppm)**
Pyrogallol	0.737
Gallic acid	0.899
Rutine	0.232
Fulvic acid	8.39
Humic acid	26.94
kaempferol	9.399
Vanillic	95.103
Caffeic	9.334
Cinnamic	7.346
Catechol	21.846
4-Hydroxy Benzoic Acid	162.279
Quercetin	5.287
Cinnamaldehyde	23.409
Eugenol	1.288
Lignin	0.221
Chlorogenic	40.943
Nigelon	0.358

The vitamin content of the shilajit sample was also analyzed using HPLC; the results showed specific concentrations of water- and alcohol-soluble vitamins, as follows: Vitamins E 0.019 ppm, A 1.833 ppm, and K 0.537 ppm were soluble in alcohol, while vitamins such as B1 0.690 ppm and B2 2.448 ppm were soluble in water (Table 6). When comparing these results with previous studies, we note that the results of vitamin concentration are very consistent with other studies that addressed the vitamin content in similar materials such as propolis and other plants.

Table 6. Concentration of vitamins in shilajit powder

Vitamin	Concentration (ppm)
E	0.019
A	1.833
K	0.537
B_1	0.690
B_2	2.448

3.5. X-ray diffraction (XRD) of shilajit



technique to study the crystalline structure of shilajit powder (Figure 3). The results showed the presence of three important peaks, the most important elements or compounds in the sample. The first peak, which appeared at 2θ=28.1447 degrees with a d-spacing value of 3.16804 Å, was the most intense in the curve (100%). This peak corresponds to quartz, a common naturally occurring compound that may be a common component of shilajit. The results indicated the presence of quartz in the sample, which contains components originating from rocks or soil that form the natural environment of shilajit.

Figure 3. X-ray diffraction patterns of the shilajit powder sample

The second peak, at 2? = 40.2974° with a d-spacing value of 2.23627 Å, has an intensity of 31%, indicating the presence of iron oxide. Since shilajit is composed of different proportions of heavy metals, it improves some of its therapeutic properties, including enhancing immunity and activating metabolic processes. This makes shilajit a good source of trace elements. It forms at 2? = 31.1299° with a d-spacing range of 2.87071 Å, where the intensity is lower than the previous two peaks, with an average total moisture content of 11%. This curve may be an indicator of organic crystals. This type of compound is an integral part of the components of shilajit and is responsible for its antioxidant activity [39].

The scanning was done using 1.54060 Å over 10°-80° to expose the fine crystalline patterns. The experimental conditions, including 40 kV and 30 mA current, led to focused, high-quality statistics that revealed the nature of the material. Comparing the results with previous studies, they showed a clear agreement with the authors in recent studies on the composition of shilajit; it contains minerals such as quartz and iron oxides. The peak 2? = 31.1299° also indicates complex organic compounds such as fulvic acid [40].

3.6. Infrared spectrum (FTIR) in shilajit powder

The infrared spectrum of shilajit was recorded from 4000 to 450 cm^{1} (Figure 4). It demonstrated various peaks representing the organic and inorganic compounds of shilajit, such as humic and fulvic acids. They are substances believed to govern many healing properties attributed to shilajit. A peak appeared at 1591 cm2, representing the stretching vibrations of carbonyl groups (C=OC=OC=O) present in volatile compounds and carboxylic acids. These peaks match the chemical structures of humic and fulvic acids, which is supported by previous studies, such as the study by Verrillo et al. [41], which reported a similar peak at 1638 cm2, reinforcing the chemical role of organic compounds in shilajit. The peak at 1391 cm2 reflects the stretching vibrations of carboxyl groups (C-OC-OC-O) associated with organic acids.

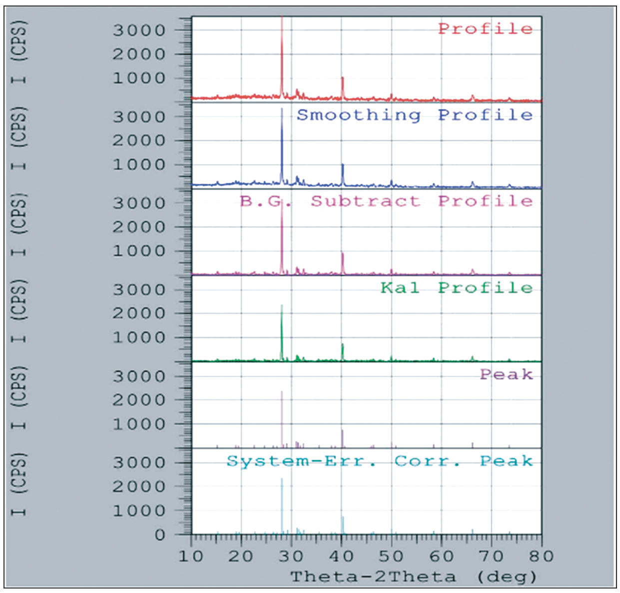


Figure 4. The data of all peaks in shilajit powder

This peak may be consistent (Figure 5) with the study conducted by Ding et al. [11], who showed similar peaks in infrared analyses of similar compounds in traditional medicine. These carboxyl groups are one of the important factors contributing to the therapeutic properties of shilajit, including improving metabolic processes and stimulating cellular interactions, which enhance the ability of shilajit to improve overall health and support it in many traditional therapies. A peak at 720 cm^1 was also observed, which shows out-of-plane bending vibrations of aromatic compounds, indicating the presence of stable aromatic bonds, which are part of the chemical structure of shilajit. This peak is consistent with the results of previous studies, such as that conducted by Jordaan [42], where it was indicated that this band corresponds to the presence of benzene groups, which are an essential part of the structure of shilajit. The presence of this benzene enhances the stability of the biological properties of shilajit, which contributes to improving its antioxidant effects. Compared to previous studies, these results are largely consistent with studies on shilajit, supporting the credibility of the results. The peaks in the range of 1500-1600 cm^1 are evidence of the presence of complex carbonyl and phenolic structures, a characteristic of organic matter in shilajit [43]. The importance of these peaks is also emphasized in determining the quality and properties of natural products such as therapeutic shilajit. These studies show clear convergence with the results above, strengthening the credibility of the spectroscopic analysis of the shilajit sample and its role in alternative medicine. These results indicate that shilajit contains complex organic and inorganic components consistent with its traditional properties described in alternative medicine. The compounds, such as humic and fulvic acids in shilajit, enhance its therapeutic value, supporting its continued use in medical applications. Although the spectroscopic analysis provided important insights into the composition of shilajit, additional analysis using techniques such as infrared spectroscopy or mass spectroscopy will be necessary to identify the chemical components and understand their subtle biological effects.

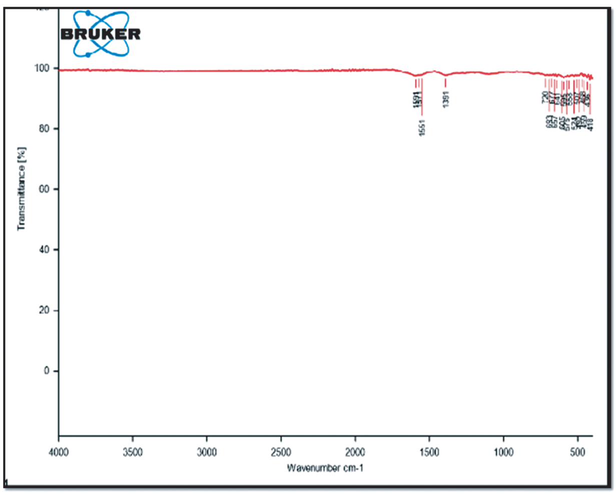


Figure 5. FTIR spectrum of shilajit powder

3.7. Heavy metals determination

The results of the minerals tested for the shilajit sample are shown in Table 7. The results indicate the absence of both cadmium (Cd) and chromium (Cr) in the shilajit sample, which is considered a positive indication. Cadmium is a toxic metal associated with some health risks, including kidney damage, immune deficiencies, and cancers. Chromium can also lead to health problems, especially with long-term exposure. According to the World Health Organization, exposure to cadmium can cause tissue and muscle damage; its absence from shilajit is a distinct factor. At the same time, Copper (Cu) concentrations of 13.5 mg/kg are considered within the safe limits for mineral consumption. Copper is an essential element for the body and is important for hemoglobin formation and overall health. However, high concentrations of copper can lead to nervous system disorders and anemia. Studies suggest that excessive exposure to copper can cause toxicity, which calls for monitoring its levels in nutritional supplements such as shilajit. Furthermore, the high iron (Fe) concentrations of 145.6 mg/kg indicate the potential of shilajit to provide iron as a dietary supplement. Iron is a vital element for hemoglobin production; it is essential for transporting oxygen in the blood. Adequate iron levels boost energy levels and help improve physical performance. Studies suggest that iron from natural sources is more usable by the body than synthetic supplements. The results indicate that the shilajit powder is free of lead (Pb) and nickel (Ni) 108 mg/kg, as lead is considered toxic when present. Nickel can cause allergies and some respiratory disorders requiring special care. The results show that the silver (Ag) concentration is 1.8 mg/kg, which is an acceptable level and does not present any significant health risks. Silver is known for its antimicrobial properties, which make it a useful material in some medical applications. As for zinc (Zn), its concentration was 24.2 mg/kg, and it is considered an essential element for health, as it contributes to enhancing immunity and helping in wound healing processes. Research suggests that zinc plays a major role in the immune system's function and hormonal balance in the body.

Table 7. Heavy metal values in shilajit

Mineral	**Concentration** (mg/Kg)
Zn	24.2
Ag	1.8
Ni	108
Pb	0
Fe	145.6
Cu	13.5
Cr	0.0
Cd	0.0

4. Conclusion

The findings of this study confirm that the chemical composition of shilajit is consistent with previous research, highlighting its quality, stability, and bioactive potential. Carbohydrates were identified as the predominant component, reinforcing shilajit's role as a natural energy source and its contribution to enhancing nutrient absorption and overall nutritional benefits. Shilajit is soluble in water, which gives it broad prospects for its use in foods and ease of use. Analysis of its physical properties using X-ray diffraction (XRD) confirmed the presence of quartz and other mineral components, which are indicative of shilajit's purity and its natural geological origin. Fourier-transform infrared (FTIR) spectroscopy further identified key functional groups associated with bioactive compounds, supporting its therapeutic and health-enhancing properties. Gas chromatography-mass spectrometry (GC-MS) analysis identified Oxacycloheptadec-8-en-2-one as the predominant volatile compound (90.84%), contributing to shilajit's unique aroma and its richness in biologically active components. These findings underscore its potential as a valuable natural supplement with significant nutritional, medicinal, and functional benefits.3- The phenolic compounds and flavonoids present in shilajit enhance its value as a healthy product that supports immunity and fights oxidative stress.

CRediT authorship contribution statement Mohammed Mustafa Fendi:

Writing-original draft, software, data curation.

Luma Khairy H:

Writing-review & editing, supervision.

Md. Suhel Mia:
Writing-review & editing, Visualization.

Wahidu Zzaman:
Writing-review & editing, Supervision.
Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. World Health Organization. (2003). WHO guidelines on good agricultural and collection practices(GACP) for medicinal plants. World Health Organization. https://doi.org/10.29171/ackupamphlethq772a332003

2. Quazi, R. S., Chougule, V., Shinde, M. U., Quazi, A. I., Gunjegaokar, S. M., & Joshi, A. A. (2023). A powerful molecule of Ayurveda science. Pharmacognosy Research, 15(3). https://doi.org/10.5530/pres.15.3.046

3. Sachdeva, V., Roy, A., & Bharadvaja, N. (2020). Current prospects of nutraceuticals: A review. Current Pharmaceutical Biotechnology, 21(10), 884-896. https://doi.org/10.2174/1389201021666200130113441

4. Kamgar, E., Kaykhaii, M., & Zembrzuska, J. (2025). A comprehensive review on Shilajit: What we know about its chemical composition. Critical Reviews in Analytical Chemistry, 55(3), 461-473.

5. Sabherwal, S., & Sheriar, N. (2022). Finding your balance: Your 360-degree guide to perimenopause and beyond. Penguin Random House India Private Limited. https://doi.org/10.1007/s13224-023-01785-5

6. Bugaev, F., Kompantsev, D., & Pogrebnyak, L. (2019). Optimization of the conditions of the extraction and purification stages of the Shilajit substance. Journal of Research in Pharmacy, 23(4), 697-700·

7. Hadi, S., Ahmed, S. H., Talib, N., Hussein, H. A., & Al-Karkhi, I. H. T. (2020). Alcoholic extract of Shilajit as anti-protein denaturation, anti-blood hemolysis, and anti-microbial. Indian Journal of Forensic Medicine & Toxicology 14(1)

8. AOAC. (2008). Official methods of analysis (18th ed.). Association of Official Analytical Chemists.

9. Carrasco-Gallardo, C., Guzmán, L., & Maccioni, R. B. (2012). Shilajit: A natural phytocomplex with potential procognitive activity. International Journal of Alzheimer's Disease, 2012, 674142. https://doi.org/10.1155/2012/674142

10. Stein, S. E. (2011). NIST/EPA/NIH mass spectral database NIST 11 and NIST mass spectral search program version 2.0g. National Institute of Standards and Technology. https://chemdata.nist.gov/mass-spc/ms-search/docs/Ver20Man 11.pdf

11. Ding, R., He, S., Wu, X., Zhong, L., Chen, G., & Gu, R. (2024). Efficient generation of HPLC and FTIR data for quality assessment using time series generation model: A case study on Tibetan medicine Shilajit. Frontiers in Pharmacology, 15, 1503508. https://doi.org/10.3389/fphar.2024.1503508

12. Yaqoob, Z., Batool, S. A., Khan, A., Hussain, R., Raza, M. A., Alqahtani, M. S., ... & Rehman, M. A. U. (2023). Characterization and medicinal applications of Karakoram Shilajit: Angiogenesis activity, antibacterial properties, and cytotoxicity. Materials Research Express, 10(10), 105403.

13. Khanna, R., Witt, M., Anwer, M. K., Agarwal, S. P., & Koch, B. P. (2008). Spectroscopic characterization of fulvic acids extracted from the rock exudate Shilajit. Organic Geochemistry, 39(12), 1719-1724.

14. Hussain, A., & Saeed, A. (2024). Hazardous or advantageous: Uncovering the roles of heavy metals and humic substances in Shilajit (phyto-mineral) with emphasis on heavy metal toxicity and detoxification mechanisms. Biological Trace Element Research, 1-21.

15. Swat, M., & Wybieralska, K. (2020). Designing functional beverages with the addition of fulvic acids for physically active people. International Journal of Food Science and Nutrition, 5(4).

16. Patel, M. J. (2010). Evaluation of antioxidant and antidiabetic effect of herbal formulations (Master's thesis). Rajiv Gandhi University of Health Sciences, India.

17. Marhoon, I. A., & Abbas, M. K. (2015). Effect of foliar application of seaweed extract and amino acids on some vegetative and anatomical characters of two sweet pepper (Capsicum annuum L.) cultivars. International Journal of Research Studies in Agricultural Sciences, 1(1), 35-44.

18. Pingali, U., & Nutalapati, C. (2022). Shilajit extract reduces oxidative stress, inflammation, and bone loss to dose-dependently preserve bone mineral density in postmenopausal women with osteopenia: A randomized, double-blind, placebo-controlled trial. Phytomedicine, 105, 154334. https://doi.org/10.1016/j.phymed.2022.154334

19. Stohs, S. J., Singh, K., Das, A., Roy, S., & Sen, C. K. (2017). Energy and health benefits of Shilajit. In Sustained Energy for Enhanced Human Functions and Activity (pp. 187-204). Academic Press.

20. Thakur, S. K., Rathi, B., Rathi, R., Tak, A., & Soni, M. (2024). Shilajeet: Classical Ayurveda texts to current research-A review. Journal of Natural Remedies,1169-1184. https://doi.org/10.18311/jnr/2024/35954

21. Ndwandwe, S. (2022). Efficacy and mechanistic study of a carbohydrate-derived fulvic acid with anti-inflammatory properties (Master's thesis). University of Pretoria, South Africa. https://doi.org/10.25403/UPresearchdata.22117130

22. Füllemann, D. (2022). Molecular background of smoky off-flavors in cocoa (Doctoral dissertation). Technische Universität München. https://doi.org/10.1021/acs.jafc.0c04633

23. Jia, Y., Wang, C., Zhang, Y., Deng, W., Ma, Y., Ma, J., & Han, G. (2024). The flavor characteristics and metabolites of three commercial dried jujube cultivars. Foods, 13(8), 1193. https://doi.org/10.3390/foods13081193

24. Wei, S., Wei, L., Xie, B., Li, J., Lyu, J., Wang, S., ... & Yu, J. (2024). Characterization of volatile profile from different coriander (Coriandrum sativum L.) varieties via HS-SPME/GC-MS combined with E-nose analyzed by chemometrics. Food Chemistry, 457, 140128. https://doi.org/10.1016/j.foodchem.2024.140128

25. Chen, X., Zhang, W., Quek, S. Y., & Zhao, L. (2023). Flavor-food ingredient interactions in fortified or reformulated novel food: Binding behaviors, manipulation strategies, sensory impacts, and future trends in delicious and healthy food design. Comprehensive Reviews in Food Science and Food Safety, 22(5), 4004-4029. https://doi.org/10.1111/1541-4337.13195

26. Hao, M., Wang, W., Zhang, J., & Chen, L. (2023). Flavor characteristics of fermented meat products in China: A review. Fermentation, 9(9), 830. https://doi.org/10.3390/fermentation9090830

27. Zhang, J., Gu, X., Yan, W., Lou, L., Xu, X., & Chen, X. (2022). Characterization of differences in the composition and content of volatile compounds in cucumber fruit. Foods, 11(8), 1101. https://doi.org/10.3390/foods11081101

28. Cagliero, C., Bechis, G., Marengo, A., Sgorbini, B., Bicchi, C., & Rubiolo, P. (2023). Essential oil components as GC-compatible hydrophobic deep eutectic solvents to extract regulated compounds from water-based fragrances. In 53rd International Symposium on Essential Oils Book of Abstracts (pp. 64-64). https://iris.unito.it/handle/2318/1933434

29. Joseph, T. M., Sathian, A., Unni, A. B., Mahapatra, D. K., Kallingal, A. K. S., & Thomas, S. (2022). Applications of flavors and fragrances in meat products. In Flavors and Fragrances in Food Processing: Preparation and Characterization Methods (pp. 405-435). American Chemical Society. https://doi.org/10.1021/bk-2022-1433.ch011

30. Guan, S., Liu, C., Ruan, M., Wang, R., Ye, Q., Wan, H., ... & Yao, Z. (2025). Detection and comparative analysis of VOCs between tomato and pepper based on GC×GC-TOFMS. Scientific Reports, 15(1), 6140. https://doi.org/10.1038/s41598-025-90796-5

31. Souleyre, E. J., Nieuwenhuizen, N. J., Wang, M. Y., Winz, R. A., Matich, A. J., I. Leperuma, N. R., & Atkinson, R. G. (2022). Alcohol acyl transferase genes at a high-flavor intensity locus contribute to ester biosynthesis in kiwifruit. Plant Physiology, 190(2),1100-1116. https://doi.org/10.1093/plphys/kiac316

32. Echeverria, G., Leclerc, C., Giné-Bordonaba, J., & Romero, A. (2021). Sensorial evaluation and aroma of vegetable oils. In Oil and Oilseed Processing: Opportunities and Challenges (pp. 245-278). https://doi.org/10.1002/9781119575313.ch12

33. Domingues, J., Delgado, F., Gonçalves, J. C., Zuzarte, M., & Duarte, A. P. (2023). Mediterranean lavenders from section Stoechas: An undervalued source of secondary metabolites with pharmacological potential. Metabolites, 13(3), 337. https://doi.org/10.3390/metabo13030337

34. Zweers, S. K. T., & Vene, K. (2021). Odour-active compounds in homemade kvass. EC Nutrition, 16, 59-73.

35. Butnariu, M. (2021). Plants as sources of essential oils and perfumery applications. In Bioprospecting of Plant Biodiversity for Industrial Molecules (pp. 261-292). https://doi.org/10.1002/9781119718017.ch13

36. Zhu, B., Chen, J., Song, G., Jia, Y., Hu, W., An, H., ... & Xue, F. (2025). Characterization of volatile flavor compounds of cigar with different aging conditions by headspace-gas chromatography-ion mobility spectrometry. Open Chemistry, 23(1), 20240129. https://doi.org/10.1515/chem-2024-0129

37. Ge, Y., Wu, Y., Aihaiti, A., Wang, L., Wang, Y., Xing, J., ... & Hong, J. (2025). The metabolic pathways of yeast and acetic acid bacteria during fruit vinegar fermentation and their influence on flavor development. Microorganisms, 13(3), 477. https://doi.org/10.3390/microorganisms13030477

38. Mahmoud, M. A., & Zhang, Y. (2024). Enhancing odor analysis with gas chromatography-olfactometry (GC-O): Recent breakthroughs and challenges. Journal of Agricultural and Food Chemistry, 72(17), 9523-9554. https://doi.org/10.1021/acs.jafc.3c08129

39. Vinola, S. M., & Mahalaxmi, S. (2024). Characterization and evaluation of physical properties of AH-Plus sealer with and without the incorporation of petasin, pachymic acid, curcumin, and Shilajit-An in vitro study. BMC Oral Health, 24(1), 352. https://doi.org/10.1186/s12903-024-