Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1464890

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 7 - Issue 4: 649-653 / July 2024

DETERMINATION OF THE CHEMICAL CONTENT OF SHILAJIT IN TERMS OF TEN DIFFERENT POLYPHENOLIC COMPOUNDS BY UAE METHOD AND HPLC ANALYSIS

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Abstract: Shilajit (Mumio) is a complex of natural bioactive compounds that has historically been used as a therapeutic cream for many skin diseases. It is also used in traditional medicine for diseases such as diabetes Alzheimer's and cancer. In this study, to determine the polyphenolic content of Shilajit, ultrasound assisted extracts (UAE) were obtained at 45 °C and analyzed by High Performance Liquid Chromatography-Ultraviolet (HPLC-UV) for the quantitative analysis of ten different polyphenolic compounds. Such a comprehensive content analysis has not been done for Shilajit before. As a result of the experimental study, 28.99±1.23 µg/g rutin, 25.47±1.67 µg/g ferulic acid, 41.49±0.41 µg/g resveratrol and 532.19±8.21 µg/g taxifolin was determined. In this study, 532.19±8.21 µg/g of taxifolin, which is found to be very effective against many diseases in the literature, is a very high value compared to the literature. The results of this research will shed an important light to researchers working in the fields of extraction of bioactive substances, food supplement production and pharmaceutical applications.

Keywords: Shilajit, Mumio, Polyphenols, Taxifolin, Resveratrol, HPLC

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Received: April 04, 2024
Accepted: June 04, 2024
Published: July 15, 2024

Cite as: Nuralın L. 2024. Determination of the chemical content of shilajit in terms of ten different polyphenolic compounds by UAE method and HPLC analysis. BSJ Eng Sci, 7(4): 649-653.

1. Introduction

Shilajit, also known as salajit, shilajatu, mumio, mumie or mummiyo, is a brown-black sticky solid compound formed by the accumulation of plant and microbial remains in crevices of rocks, caves and cliffs over many years (Agarwal et al., 2007; Carrasco-Gallardo et al., 2012). Raw Shilajit is found in cave cracks and rock cavities about 1000 meters above sea level. It is located predominantly in the Himalayas, Southern Kazakhstan and Central Asian Alps Region (Aiello et al., 2011). The physical and chemical properties of Shilajit obtained from different regions are similar, but the percentage of inorganic and organic components may vary (Kloskowski et al., 2021; Pandit et al., 2016). Studies conducted to determine the chemical composition of Shilajit reported that the main component was 80% humic substances (Kamgar et al., 2023). Additionally, benzoic acid, fatty acids, amino acids, aromatic carboxylic acids, microelements and resin have been reported. (Agarwal et al., 2007; Ghosal et al., 1988; Schepetkin et al., 2009).

Shilajit has been used in traditional medicine for many years, especially in eastern countries. According to a World Health Organisation (WHO) report, approximately 80% of the world's population trust traditional medicine for its therapeutic and preventive properties in human health (Tran, et al., 2020). For this reason, researchers' interest in plant-based medicines is increasing globally (Mahian and Sani, 2016). Shilajit, for example, is used mainly in India to treat skin diseases and osteoporosis. It is also thought to help protect against nervous system diseases due to its neuroprotective activity. It is also recommended for anemia, diabetes and asthma patients due to its high iron content and antioxidant properties (Ghosal et al., 1988; Ghosal et al., 1991; Agarwal et al., 2007; Cornejo et al., 2011). When a general evaluation is made, it is clear that many components in the structure of Shilajit, especially the bioactive compounds, can benefit human health. However, more studies are needed to determine these components and their effects. Thus, its international use will become widespread (Schepetkin et al., 2009).

Shilajit contains a wide range of bioactive components (dibenzo-a-pyrones, phenolic acids and terpenoids) and phenolic compounds that are very beneficial for health, as well as fulvic acid, humic acid and some minerals (Pravin and Sunayana, 2022).

In this study, the presence and amounts of 10 different polyphenolic compounds in the extracts of commercial Shilajit (Figure 1) obtained by UAE method were determined using HPLC-UV. Polyphenols are secondary metabolites of plant origin and contain more than one phenol group in their structure (Tapia-Quirós et al., 2022). Polyphenols include flavonoid (e.g., taxifolin and rutin) and nonflavonoid (e.g., ferulic acid and

resveratrol) compounds (Figure 2) (Purgatorio et al., 2024). These compounds provide severe benefits to human health with their potent antioxidant, anti-inflammatory, hepatoprotective, antiviral, anti-carcinogenic and anti-mutagenic properties (Chung et al., 1998; Nijveldt et al., 2001; Purgatorio et al., 2024).

There are many studies in which bioactive compounds are extracted from various sources using different methods due to their benefits to human health (Routray and Orsat, 2012). This is the first study in the literature in which polyphenolic compounds from Shilajit were extracted using the UAE method, and qualitative and quantitative analyses were performed for ten different polyphenolic substances with HPLC-UV. Considering the benefits of bioactive compounds on human health, this study will be enlightening for researchers.

Figure 1. Commercial tablet form of Shilajit.

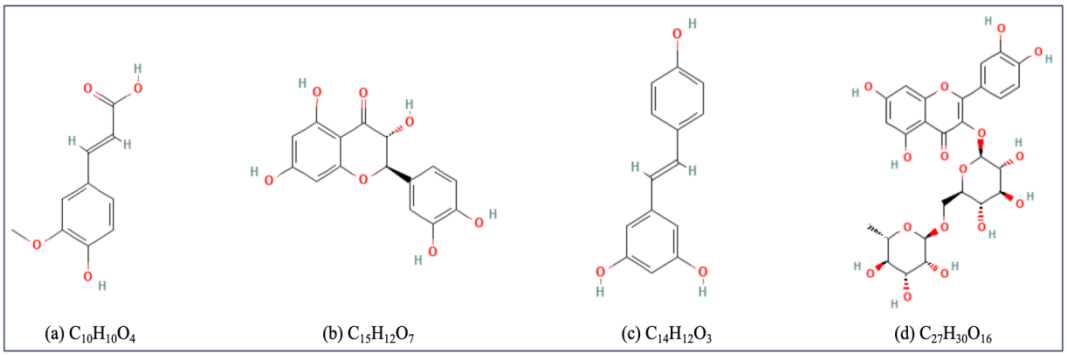

Figure 2. Chemical formulas and structures of some phenolic compounds; (a) ferulic acid, (b) taxifolin, (c) resveratrol, (d) rutin.

2. Materials and Methods

2.1. Raw Material and Chemicals

Shilajit is commercially available in 5 g packs. Shilajit sample was purchased from an herbalist in Ankara in its commercial product form. Reference substances gallic acid, caffeic acid, rutin, ferulic acid, taxifolin, resveratrol, quercetin, silibinin, apigenin (≥ 98%) were obtained from sigma aldrich Germany, kaempferol (≥ 95%) was obtained from cayman USA. Analytically pure ethanol (≥ 99.99%) was used as a solvent, and analytically pure methanol (≥ 99.99%) and acetonitrile (≥ 99.99%) were used as mobile phase was obtained from Sigma Aldrich. Analytically pure formic acid (≥ 99.99%) added to the mobile phases was obtained from Merck (Germany). Pure water used in the extraction was obtained by Heal Force Smart Ultra Water Purification device.

2.2. Sample Preparation

The samples were taken out of their commercial packages and prepared in 3 sets by dissolving two tablets (10 g) in 75 mL of distilled water.

2.3. UAE Method

The UAE method provides very efficient extractions at low temperatures and in a short time compared to traditional methods. Weightlab Instrument WF-UD6 model device was used to extract bioactive components from Shilajit. Optimum experimental conditions were determined by minor changes in the extraction conditions previously made with UAE (Nuralın, 2024). Two Shilajit tablets (5 g each) were extracted in 75 mL of pure water by ultrasonic extraction at 45 °C for 1 hour. An external circulation water bath was used for constant temperature. In this study, extractions were performed in 3 repetitions and were presented at a 95% confidence level.

2.4. HPLC Analysis

Quantitative analysis of bioactive compounds was performed with Dionex 680 HPLC system (California, USA) with a 4-channel UVD 170U detector and P680

quad pump. Thermo brand C18 type HPLC column (4.6 x 150 mm, 3µm) was used in the analysis. The mobile phase used in the analysis contains a volume of 0.1% formic acid, 79.9% deionized water and 20% acetonitrile; phase B contains 100% acetonitrile. Chromatograms were obtained at 254 nm, suitable for observing polyphenolic compounds. All absorption data were taken at 254 nm. The mobile phase was run at a constant flow rate of 1 mL/min. An elution gradient program with a constant flow rate of 1 mL/min was performed as follows: 0–18 min of 100% of A in B, 18–22.5 min 50–50% of A in B, 22.5–25min 100% of A in B. The detection wavelength of the UVD 170U detector was 254nm. The precise mixture obtained from the extraction was given to the HPLC device using a 20µl manual injection port. In order to determine the concentration of gallic acid, caffeic acid, rutin, ferulic acid, taxifolin, resveratrol, quercetin, silibinin, apigenin, and kaempferol in the samples, standard substances at different concentrations (5ppm, 10ppm, 50ppm) in ethanol were prepared, and a calibration curve was drawn. HPLC analysis was performed in duplicate, and quantification of compounds was made by averaging the two results. Concentrations were calculated using the coefficients obtained from the calibration study. In a study on Berberis vulgaris, rutin and apigenin amounts were determined with similar eluents and gradient programs (Nuralin and Gürü, 2022). In another study, the amount of taxifolin was determined using similar eluents and HPLC gradient program (Ghoreishi et al., 2016).

3. Results and Discussions

HPLC-UV system is a widely used technique to analyze the stable and highly reproducible properties of phenolic compounds and their derivatives, such as resveratrol, taxifolin and rutin (Fan et al., 2011). In this study, the identification and quantification of 10 different polyphenolic compounds were made by comparing the retention times of the reference standards and the characteristic chromatograms of the samples, and the amount was calculated according to their peak areas. Chromatograms of reference standards (10 ppm) of bioactive compounds are given in Figure 3.

The marked peak belongs to taxifolin (1st peak; Gallic acid, 2nd peak; Caffeic acid, 3rd peak; Rutin, 4th peak; Ferulic acid, 5th peak; Taxifolin, 6th peak; Resveratrol, 7th peak; Quercetin, 8th peak; Silibinin, 9th peak; Apigenin, and 10th peak; Kaempferol). The retention time of taxifolin in the 10 ppm standard mixture given in Figure 3 is 10.045 minutes.

The retention time of taxifolin, which is the labeled peak in Figure 4, is 9.997 minutes. When the retention times of rutin, ferulic acid and resveratrol are compared with the retention times of the standards in this chromatogram, these four phenolic compounds were observed in Shilajit. In order to determine the amount of rutin, ferulic acid, taxifolin and resveratrol extracted as a result of the experimental study, the peak areas obtained in the chromatograms were calculated using appropriate calibration factors, total extract volumes and sample weights used in the experiments. As a result, 28.99±1.23 µg/g rutin, 25.47±1.67 µg/g ferulic acid, 532.19±8.21 µg/g taxifolin and 41.49±0.41 µg/g resveratrol were determined from Shilajit by UAE method (Figure 5.). Compared to other polyphenolic compounds, Shilajit contains the highest amount of taxifolin using the UAE method.

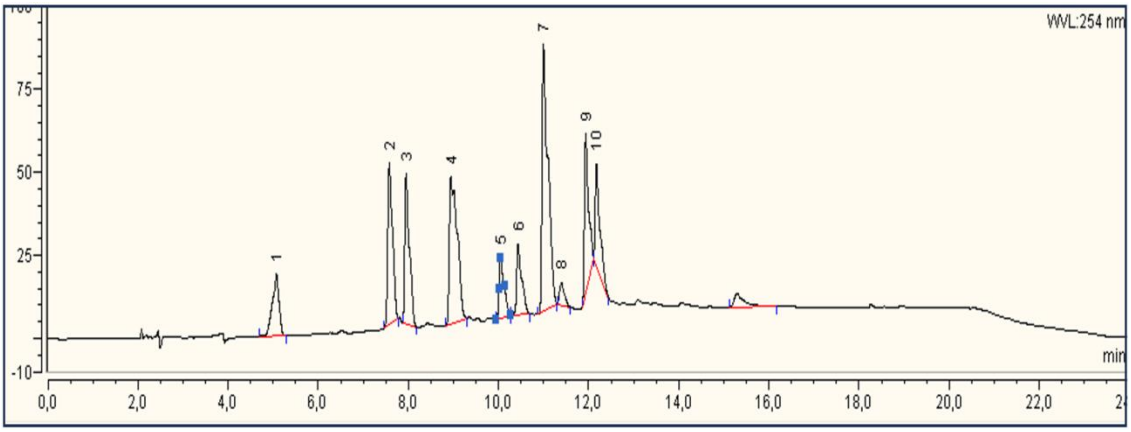

Figure 3. Chromatogram of the mixture prepared for 10 ppm concentration of 10 different reference substances.

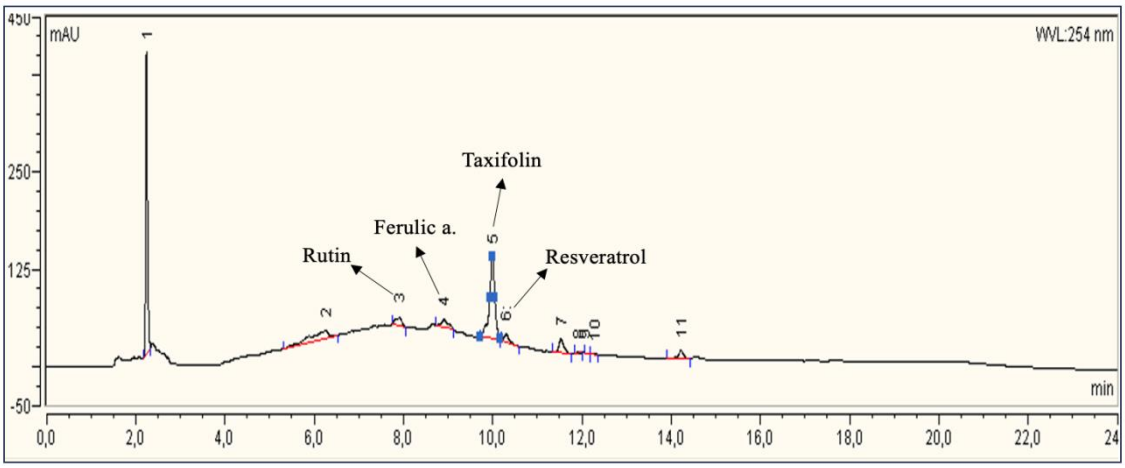

Figure 4. Peaks of rutin, ferulic acid, taxifolin and resveratrol in the chromatogram of UAE extracted Shilajit.

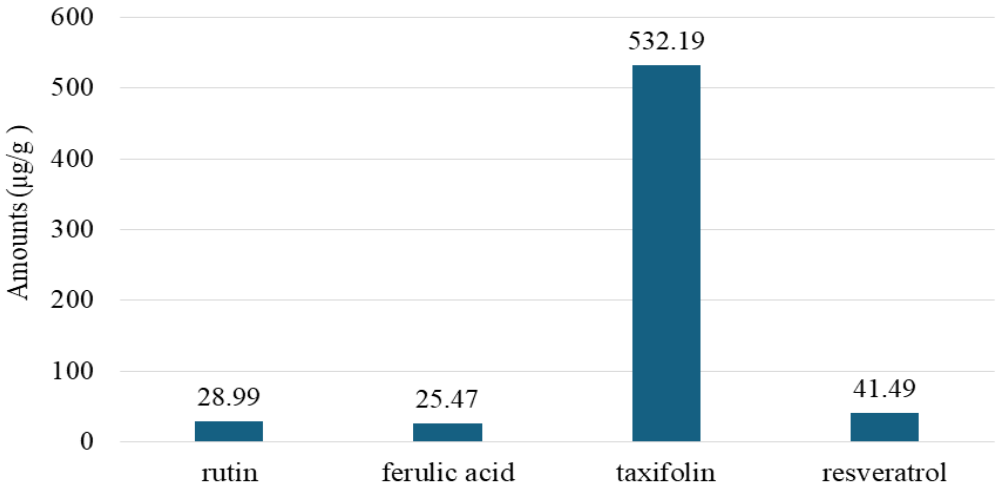

Figure 5. The amounts of rutin, ferulic acid, taxifolin and resveratrol extracted from Shilajit.

4. Conclusion

Shilajit has been used in many areas of traditional medicine for many years. It is formed due to the decomposition of substances such as plants, fungi and lichens and their combination with rock minerals. Considering the geography where it is located and the vegetation that contributes to its structure, the components it contains may vary. However, researchers have determined that many compounds have high bioavailability in their structure. In this study, ten polyphenolic compounds were investigated in order to understand the polyphenolic component content of Shilajit. The bioactive compounds extracted from Shilajit by UAE method were analyzed by HPLC-UV and 28.99±1.23 µg/g rutin, 25.47±1.67 µg/g ferulic acid, 532.19±8.21 µg/g taxifolin and 41.49±0.41 µg/g resveratrol were found. The presence of exceptionally high levels of taxifolin in Shilajit has been scientifically observed. Besides the potent antioxidant and anti-inflammatory activity of flavonoids, the unique bioactive flavonoid taxifolin is a constituent of interest to dietitians and medicinal chemists due to its wide range of health benefits. In studies, taxifolin has shown a serious inhibitory effect against inflammation, microbial infection, oxidative stress, cardiovascular disease and liver disease. In this study, flavanoids, which have important effects on human health, were obtained by UAE, a short and simple extraction technique. In this respect, the study and its results will contribute to scientific literature, pharmacological research, and RandD studies for food supplements.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	L.N.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgements

This work was supported by the Gazi University BAP project unit (Grant numbers: FGA-2023-8160 and 06/2016-11). I would like to sincerely thank Prof. Dr. Metin GÜRÜ and Cem TAŞDEMİR for their contributions to this study.

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