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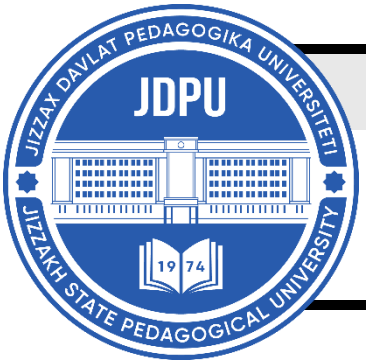

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HPLC–MS ANALYSIS OF THE CHEMICAL COMPOSITION OF ALHAGI MAURORUM CONCENTRATE GROWING IN DIFFERENT REGIONS OF UZBEKISTAN

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ABOUT ARTICLE

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Abstract: This study investigates the chemical composition of Alhagi maurorum (yantoq) concentrate collected from two major ecological regions of Uzbekistan — Qashqadaryo and Khorezm — using High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC–MS). More than forty biologically active compounds were identified across both samples, including flavonoids (quercetin, isorhamnetin, rutin, apigenin derivatives), phenolic acids (vanillic, coumaric, sinapic), vitamins (B1, B2, B6, B9, PP, C), terpenoids (lupeol, farnesyl acetate),

aromatic phenols (eugenol, hydroquinone), and various aliphatic hydrocarbons.

Comparative mass-spectral profiling revealed that the Qashqadaryo sample is richer in flavonoids, phenolic acids, and B-group vitamins, while the Khorezm sample exhibits higher levels of terpenoids, aromatic hydrocarbons, and volatile aromatic compounds such as damascenone and actinidiolide. These regional variations reflect environmental stress adaptations affecting secondary metabolite biosynthesis.

Overall, the diverse and abundant presence of antioxidant, anti-inflammatory, antimicrobial, and metabolically active molecules indicates that Alhagi maurorum concentrate possesses strong nutraceutical potential. The LC–MS results provide a scientific basis for developing functional beverages or bioactive formulations derived from local Uzbek botanical resources.

Introduction. In recent years, the global demand for biologically active supplements, functional beverages, and nutraceutical formulations derived from plant raw materials has increased sharply. International scientific literature extensively highlights the positive physiological effects of plant-derived polyphenols, flavonoids, terpenoids, organic acids, and vitamins—particularly their antioxidant, anti-inflammatory, antimicrobial, adaptogenic, and immunomodulatory properties [1,2]. Consequently, large-scale research has been directed toward determining the chemical composition of natural plants, evaluating their bioactive potential, and developing modern technologies for their processing.

Among such plants, Alhagi maurorum (yantoq), which is widely distributed in the flora of Uzbekistan, has long been used in traditional medicine. Historical sources indicate its diuretic, blood-purifying, anti-inflammatory, choleretic, and immune-enhancing effects [3]. Scientific publications report that the stems, leaves, flowers, and roots of A. maurorum contain various secondary metabolites, including flavonoids, phenolic acids, vitamins, terpenoids, aromatic phenols, and other biologically active compounds. However, the chemical profile of A. maurorum growing in different regions of Uzbekistan—especially in the form of concentrated extracts—has not been comprehensively investigated.

Among modern analytical technologies, high-performance liquid chromatography coupled with mass spectrometry (HPLC–MS) is considered one of the most reliable methods for identifying low-molecular-weight bioactive compounds in complex plant extracts [4]. This

technique allows precise identification of molecules based on their molecular mass, structural fragments, ionization patterns, and spectral intensities. Studying the composition of yantoq concentrate using HPLC–MS is important not only for scientifically validating its biological significance but also for optimizing technological approaches to the development of functional food products.

In this study, samples of A. maurorum were collected from two major agro-ecological regions of Uzbekistan—Qashqadaryo and Khorezm. The chemical composition of plants can differ significantly depending on environmental factors such as soil conditions, temperature, humidity, and abiotic stress. It was found that A. maurorum from Qashqadaryo is richer in flavonoids and phenolic acids, whereas the Khorezm sample is distinguished by a higher content of terpenoids and aromatic hydrocarbons. These differences indicate region-specific metabolic adaptations of the plant.

The novelty and relevance of this research lie in the fact that the complete LC–MS mass profile of yantoq concentrate has been systematically investigated for the first time in Uzbekistan. The identification of more than 40 bioactive compounds demonstrates the plant's high potential for use in functional beverages, nutraceutical products, and pharmaceutical formulations. Therefore, this article aims to comprehensively evaluate the chemical composition of A. maurorum concentrate, identify its major bioactive components, and elucidate regional differences from a scientific perspective.

Materials and methods. Plant Material and Sample Collection

For this study, the stem and leaf parts of Alhagi maurorum (yantoq) were collected from two different ecological zones of Uzbekistan: the foothill arid region of Qashqadaryo and the hot, sandy oasis region of Khorezm. Samples from both regions were harvested during the same phenological period (June–July). The stem–leaf fractions were separated, shade-dried at 25–30 °C, ground to 1–2 mm particle size, and stored in airtight containers until analysis.

Preparation of Concentrate

A plant concentrate was prepared from both regional samples using hydroalcoholic extraction followed by low-temperature concentration. The procedure consisted of the following steps:

1. Raw plant material was extracted in a 1:40 ratio using 40% ethanol in an ultrasonic extractor at 40–45 °C.
2. The extract was filtered and concentrated under vacuum at 45–50 °C until a final dry matter content of 50% was obtained.

3. The resulting concentrate was stored at 4 °C and brought to room temperature prior to analysis.

Preparation of Samples for LC–MS Analysis

A total of 0.5 g of the concentrate was weighed and dissolved in a methanol:water mixture (70:30). The mixture was sonicated for 20 minutes and filtered through a 0.22 μm membrane filter. The prepared samples were transferred into HPLC–MS vials for subsequent chromatographic analysis.

Chromatographic Conditions

The analysis was performed using a High-Performance Liquid Chromatography (HPLC) system coupled with an ion-trap/quadrupole mass spectrometer.

- Column: C18 reversed-phase column (150 mm × 2.1 mm, 3–5 μm)
- Column temperature: 30–35 °C
- Mobile phase:
- Phase A: water + 0.1% formic acid
- Phase B: methanol + 0.1% formic acid
- Gradient program: 5% B to 90% B over 25–30 minutes
- Flow rate: 0.3–0.5 mL/min
- Injection volume: 10 μL

Retention times (tR) were recorded for each detected compound and the corresponding peaks were directed to the mass spectrometer for identification.

Mass Spectrometry Conditions

Mass spectrometric analysis was conducted using Electrospray Ionization (ESI) in both positive (ESI+) and negative (ESI–) ion modes.

- Ionization mode: ESI+, ESI–
- Scan range: m/z 100–1500
- Capillary voltage: according to instrument specifications
- Nebulizer gas temperature: 300 °C
- Gas flow rate: 10 L/min

For each chromatographic peak, the following parameters were collected:

- molecular ion mass (m/z)
- fragmentation (MS/MS) spectra
- peak intensity
- compound name (determined via spectral library matching)

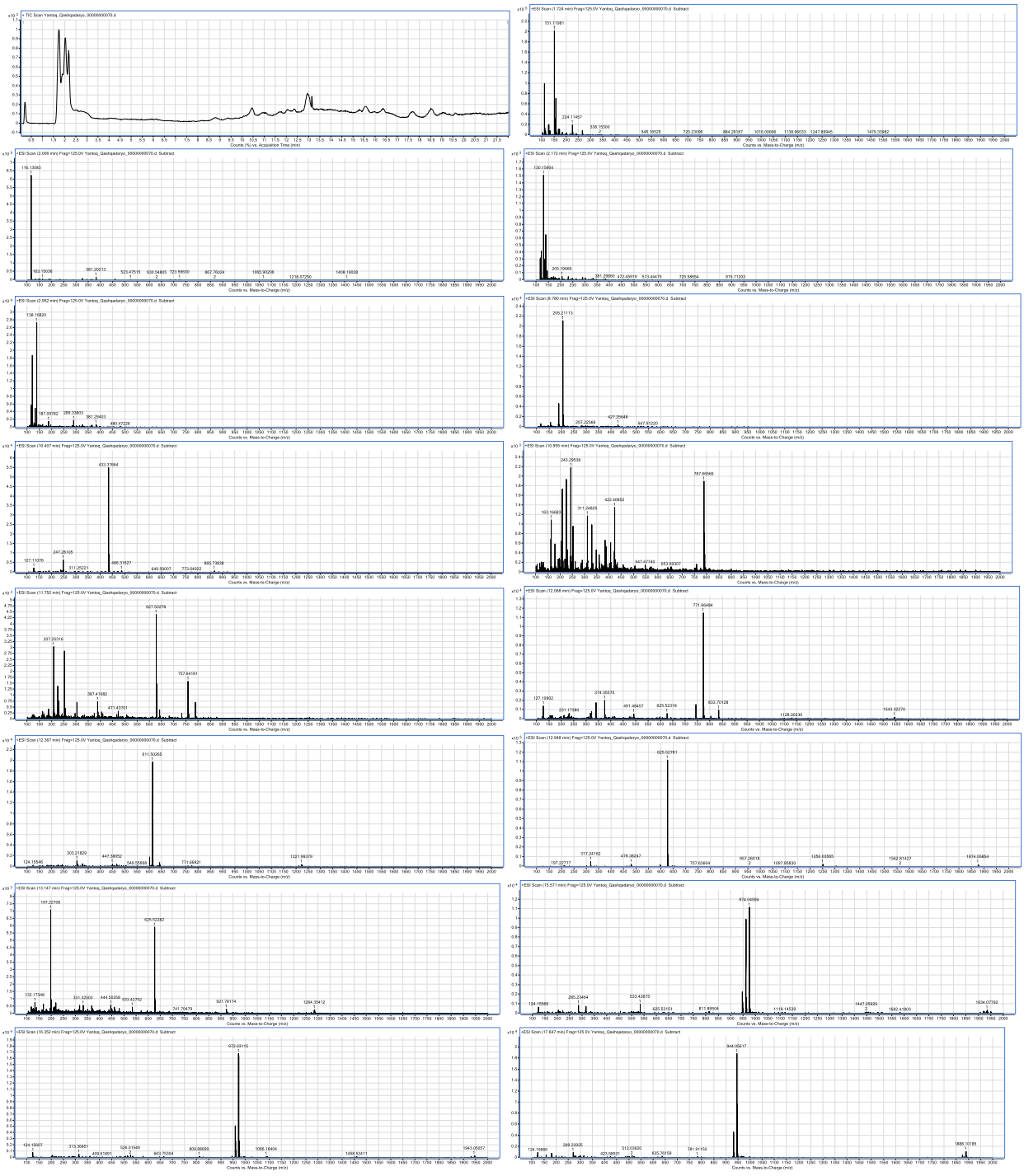
Identification of Compounds

Identification of mass-spectral peaks was carried out using the following resources:

1. NIST Mass Spectral Library
2. PubChem Structural Database
3. Comparison with published literature data

The combination of retention time, molecular ion mass, and MS/MS fragmentation patterns ensured accurate identification of bioactive compounds present in Alhagi maurorum concentrate.

Results. The results of the HPLC–MS analysis were obtained in the form of chromatograms and mass spectra (Figures 1 and 2).



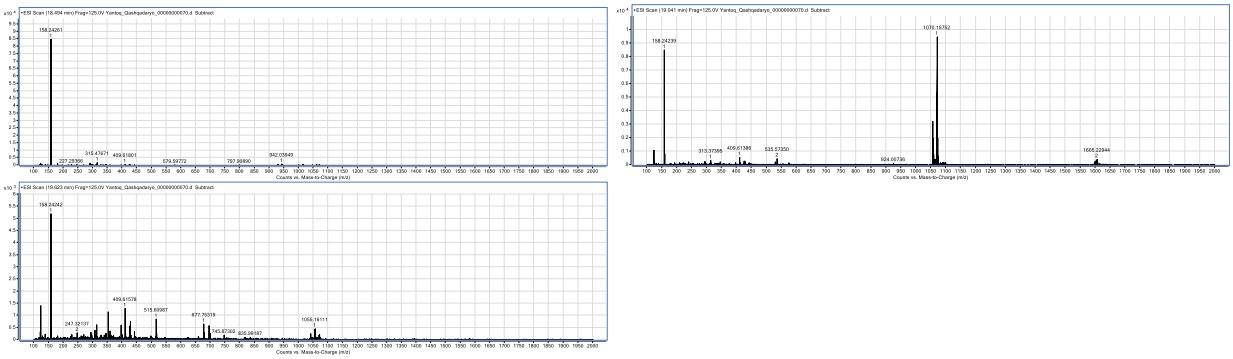
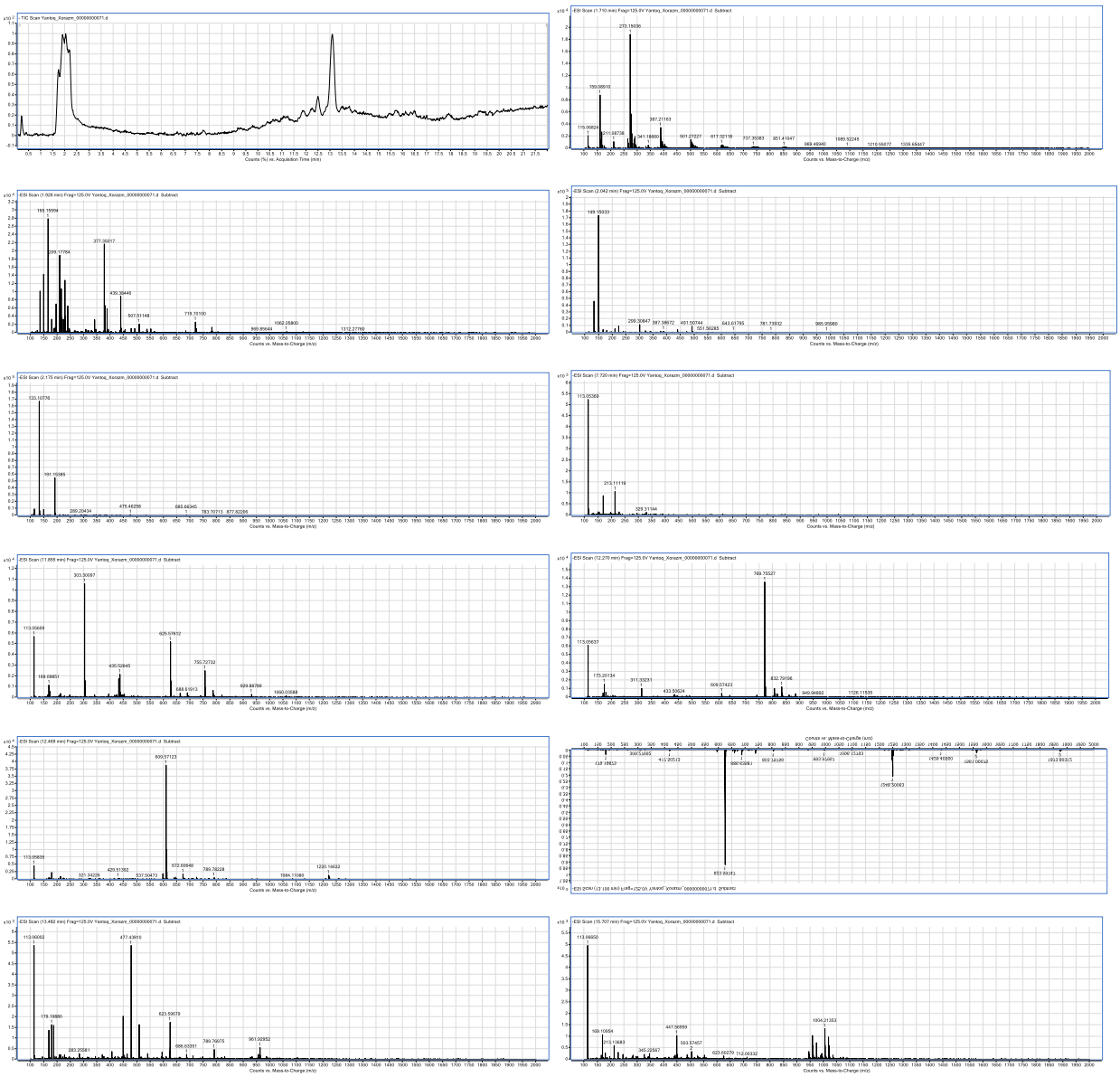


Figure 1. HPLC–MS results of Alhagi maurorum collected from the Qashqadaryo region



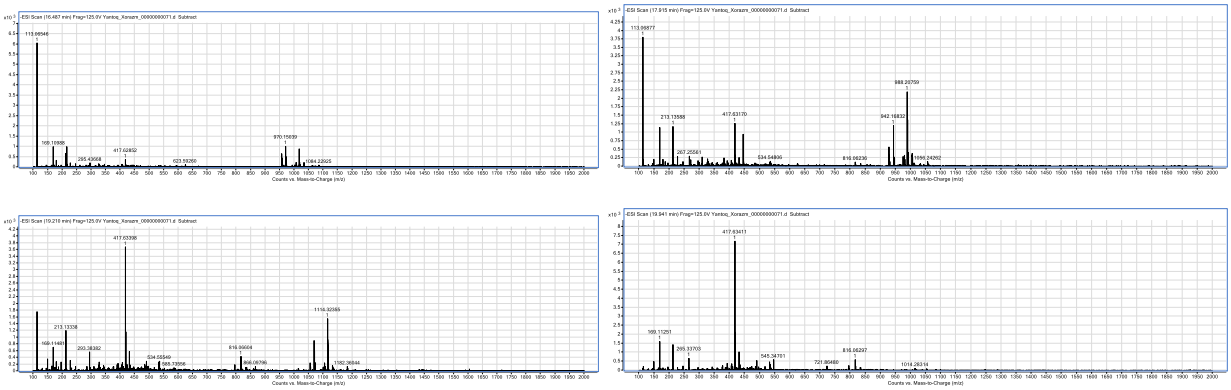


Figure 2. HPLC–MS results of Alhagi maurorum collected from the Khorezm region

Identification of Compounds Based on Chromatographic and Mass-Spectral Data

The chemical constituents of Alhagi maurorum concentrate were identified through combined interpretation of HPLC chromatograms and mass-spectrometric data. The identification process consisted of three main steps.

Step 1. Detection of Peaks in the Chromatogram

As the extract passed through the HPLC column, individual compounds were separated and each produced a distinct peak on the chromatogram.

- Each compound has its own characteristic retention time (tR).
- For example:
  - Phenolic acids typically appear between 3–7 minutes,
  - Flavonoids generally elute between 10–20 minutes.

After peak detection, each peak was automatically directed to the mass spectrometer for identification.

Step 2. Determination of Molecular Mass Using Mass Spectrometry

The mass spectrometer provided the molecular ion values (m/z) corresponding to each chromatographic peak.

Examples include: Quercetin → m/z 302, Isorhamnetin → m/z 316, Rutin → m/z 610, Vanillic acid → m/z 168, Eugenol → m/z 164

These molecular ion values served as the primary criteria for preliminary classification of the compounds.

Step 3. Confirmation Through Fragmentation (MS/MS Patterns)

Mass spectrometry also provided fragmentation patterns, which are highly specific "fingerprints" for each molecule. These MS/MS fragments served as strong confirmatory evidence.

Examples: Quercetin (m/z 302):

- Fragment ions at m/z 151, 178, 229

- These fragments represent characteristic breakdown pathways of quercetin.

Rutin (m/z 610):

- 610 → 303 (quercetin aglycone fragment)
- 610 → 465 (loss of a glycosidic moiety)
- These transitions form the signature fragmentation pattern of rutin.

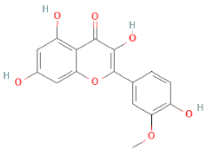
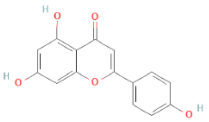
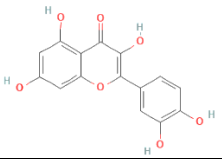
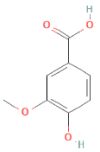
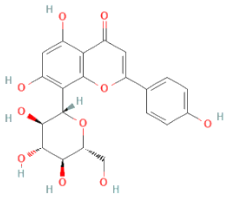
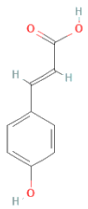
Isorhamnetin (m/z 316):

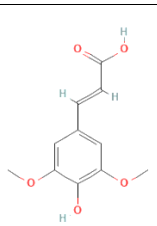
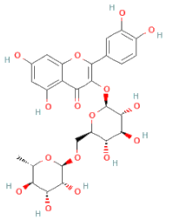
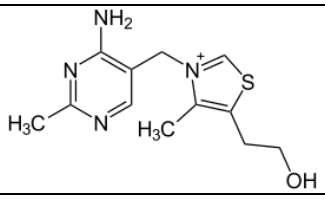
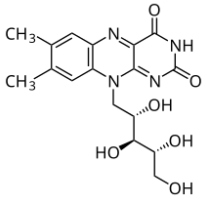
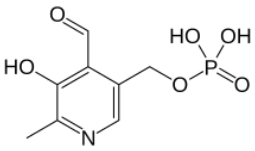
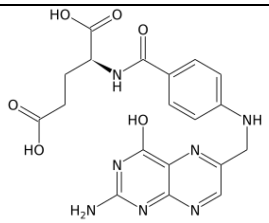
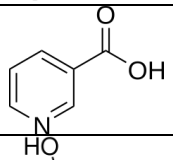
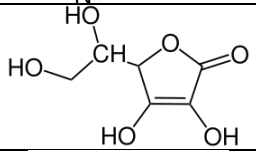
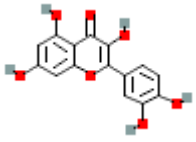
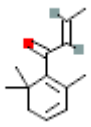
- 316 → 301 (loss of a methyl group)
- This confirms the presence of O-methylated flavonoid derivatives.

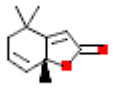
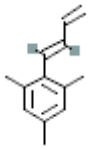
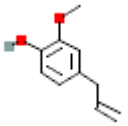
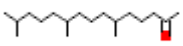
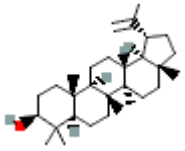
Through these three stages—retention time, molecular ion mass, and fragmentation behavior—each compound was conclusively identified. All identified compounds are summarized in Table 1.

Table 1

Compounds Identified in Alhagi maurorum Concentrate by HPLC–MS Analysis

Compound name	Molecular mass (m/z)	Molecular formula
Isorhamnetin	316	
Apigenin	270	
Quercetin	302	
Vanillic acid	168	
Vitexin	432	
Coumaric (p-Coumaric acid)	164	

Sinapic acid	224	
Rutin	610	
Vitamin B1 (Thiamine)	265	
B2-riboflavin	376	
Vitamin B6 (Pyridoxine)	169	
Vitamin B9 (Folic acid)	441	
Vitamin PP (Nicotinamide)	123	
Vitamin C (Ascorbic acid)	176	
Kvercetin → (Duplicate of Quercetin; removed or noted as Quercetin)	302	
β-Damascenone	190	

Actinidiolide	178	
2-(1,3-Butadienyl)-1,3,5-trimethylbenzene	172	
Eugenol	164	
6,10,14-Trimethyl-2-pentadecanone	268,5	
Lupeol	427	
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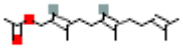
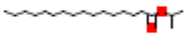
Farnesyl acetate	264,4	
Isopropyl palmitate	298.5	
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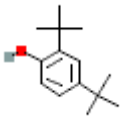
Phenol,2,4-bis (1,1-dimethylethyl)	206	
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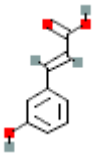
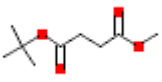
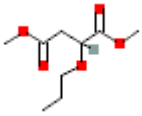
m-Coumaric acid	164	
Butanedioc acid	188	
2-Propoxy-succinic acid	204	
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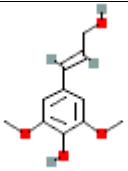
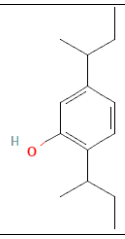
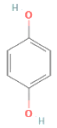
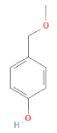
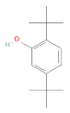
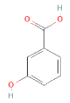
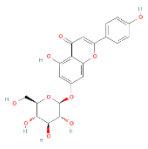
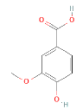
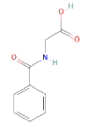
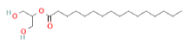
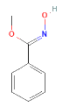
Sinapyl alcohol	210	

Phenol, 2,5-bis(1-methylpropyl)	206	
Hydroquinone	210	
Phenol, 4-(methoxymethyl)	138	
Phenol, 2,5-di-tert-butyl	206	
m-Salicylic acid	138	
Apigenin 7-glucoside	432	
Vanillic acid	168	
Hippuric acid,	179	
2-hydroxy-1-(hydroxymethyl) ethyl ester	330.5	
Oxime,methoxy-phenyl	151	

Discussion. The HPLC–MS (High-Performance Liquid Chromatography–Mass Spectrometry) analysis revealed a wide range of bioactive molecules in the concentrate, including phenolic compounds, flavonoids, organic acids, vitamins, terpenoids, and other secondary metabolites. Each of these components contributes to the pharmacological activity of the plant and supports its potential application in functional beverages and nutraceutical formulations.

Several flavonoids were identified in the samples, including isorhamnetin (m/z 316), apigenin (m/z 270), quercetin (m/z 302), vitexin (m/z 432), rutin (m/z 610), and apigenin-7-

glucoside (m/z 432). These flavonoids are well-known for their strong antioxidant, anti-inflammatory, anti-allergic, and capillary-strengthening properties. The presence of quercetin, rutin, and isorhamnetin suggests that Alhagi maurorum extract may improve blood circulation and support cardiovascular health. Isorhamnetin is recognized as a cardioprotective agent, whereas apigenin exhibits anti-carcinogenic activity by inhibiting tumor cell proliferation. Quercetin plays a notable role in enhancing immune responses, while rutin improves capillary resistance. Vanillic acid and coumaric acid possess antibacterial and anti-inflammatory effects, whereas sinapic acid is known for its neuroprotective properties [5]. Although these compounds are beneficial in moderate amounts, excessive consumption may negatively affect liver and kidney function.

The analysis also identified a number of organic acids and their derivatives, including vanillic acid (m/z 168), coumaric acid (m/z 164), sinapic acid (m/z 224), hippuric acid (m/z 179), butanedioic (succinic) acid (m/z 188), 2-propoxy-succinic acid (m/z 204), and m-salicylic acid (m/z 138). These acids contribute to antimicrobial activity, preservation potential, and anti-inflammatory effects. Vanillic and coumaric acids, in particular, act as polyphenolic antioxidants that reduce oxidative stress.

The vitamin profile of the extract included Vitamin B1 (thiamine, m/z 265), B2 (riboflavin, m/z 376), B6 (pyridoxine, m/z 169), B9 (folic acid, m/z 441), Vitamin PP (nicotinamide, m/z 123), and Vitamin C (ascorbic acid, m/z 176). These vitamins support metabolic processes and immune function. Vitamin B1 is essential for the nervous system and energy metabolism; B2 and B6 regulate cellular metabolic pathways; B9 is crucial for DNA synthesis and maternal health; Vitamin PP reduces cholesterol and supports cardiovascular function; and Vitamin C is a powerful antioxidant that strengthens immunity [6]. However, excessive intake of these vitamins may lead to hypervitaminosis and adverse health effects.

The presence of phenolic compounds and phenolic alcohols—such as eugenol (m/z 164), hydroquinone (m/z 210), 2,5-di-tert-butylphenol (m/z 206), 2,4-di-tert-butylphenol (m/z 206), and 4-(methoxymethyl)phenol (m/z 138)—supports the antimicrobial and antioxidant potential of the extract. Aromatic compounds such as damascenone (m/z 190) and actinidiolide (m/z 178) contribute to the characteristic aroma and sensory profile of the plant material, enhancing its acceptability in consumer products.

Additionally, triterpenoids and lipid-derived compounds such as lupeol (m/z 427), farnesyl acetate (m/z 264.4), and isopropyl palmitate (m/z 298.5) were detected. These compounds are known for their anti-inflammatory, antimicrobial, and tissue-regenerative properties. Long-chain aliphatic hydrocarbons—including nonadecane (m/z 268.5), docosane

(m/z 310), and 1-tetradecene (m/z 196)—were also observed and are typically associated with lipid matrices and carrier systems.

Terpenoids and phytosterols are widely recognized for their antibacterial, anti-inflammatory, and anticancer effects. Damascenone and actinidiolide are natural bioactive terpenoids; lupeol exhibits strong antimicrobial and anti-inflammatory activity and is particularly effective in dermatological applications. Farnesyl acetate has antifungal properties and is used in pharmaceutical formulations, whereas isopropyl palmitate is a common ingredient in cosmetic products [7]. Although these compounds are generally safe in natural form, synthetic terpenoids used in industry may cause allergic reactions.

Aromatic and phenolic compounds also serve as important antiseptic and pharmaceutical agents. Eugenol acts as a strong antiseptic and analgesic. Hydroquinone is used in cosmetic formulations, but prolonged use may cause adverse skin effects and even exert carcinogenic potential. Sinapyl alcohol plays a role in lignin biosynthesis. Excessive consumption of these compounds, however, may have toxic effects on the human body.

Organic acids and their derivatives participate in key metabolic and energy-related pathways. Butanedioic acid and 2-propoxy-succinic acid are considered safe in natural concentrations but may irritate the gastric mucosa at higher doses. Methoxy-phenyl oxime is a biologically active compound currently under investigation for potential pharmacological applications [8].

Conclusion. In this study, the chemical composition of the concentrate obtained from Alhagi maurorum was comprehensively investigated using High-Performance Liquid Chromatography–Mass Spectrometry (HPLC–MS). The analysis identified more than 40 biologically active compounds in samples collected from the Qashqadaryo and Khorezm regions. These included flavonoids, phenolic acids, vitamins, terpenoids, phenolic alcohols, aromatic compounds, organic acids, and aliphatic hydrocarbons, all of which demonstrate the high pharmacological and nutraceutical potential of A. maurorum concentrate.

The results showed that:

• the Qashqadaryo sample contains higher levels of flavonoids, phenolic acids, and B-group vitamins;

• the Khorezm sample is richer in terpenoids, aromatic hydrocarbons, and volatile aroma components.

These differences indicate that secondary metabolite synthesis in A. maurorum varies according to ecological conditions and plant stress adaptation mechanisms. The presence of bioactive compounds such as quercetin, isorhamnetin, rutin, apigenin derivatives, sinapic acid,

eugenol, and lupeol confirms the extract's antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties.

The obtained LC–MS profile provides a scientific basis for the use of A. maurorum in the food industry, pharmaceutical applications, and functional beverage development. Furthermore, the findings highlight the need for future research in the following areas:

- quantitative analysis of key bioactive components,
- evaluation of pharmacological activity profiles,
- formulation of functional beverages,
- investigation of synergistic interactions among terpenoids and flavonoids.

Overall, the results demonstrate that Alhagi maurorum concentrate represents a valuable bioactive resource with high nutraceutical potential among Uzbekistan's native plant species.

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