engineering proceedings

MDPI

Proceeding Paper

Quantitative Analysis of Vitamins and Amino Acids in *Alhagi* Mauro-Rum Plant Extract †

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† Presented at the 3rd International Electronic Conference on Processes—Green and Sustainable Process Engineering and Process Systems Engineering (ECP 2024), 29–31 May 2024; Available online: https://sciforum.net/event/ECP2024.

Abstract: This work focuses on the use of high-performance liquid chromatography (HPLC) for the quantitative measurement of vitamins and amino acids found in residue extracts. The goal of this study is to precisely ascertain the quantities of these vital substances in residual samples by utilizing the effectiveness of HPLC. The analytical methodology used in this study offers a reliable way to assess the nutritional value of residues, offering insightful information on their composition and uses in a range of disciplines, such as environmental studies, food science, and agriculture. The quantitative analysis results provide a basis for comprehending the chemical composition of the residue, enabling well-informed decision-making in both research and industry applications. In this work, samples from the *Alhagi maurorum* plant were subjected to a quantitative analysis of vitamins and amino acids using high-performance liquid chromatography (HPLC). Twenty amino acids, including essential amino acids and three water-soluble vitamins—riboflavin (B2), pyridoxine (B6), and folic acid (B9)—were found by the inquiry. With a total amino acid concentration of 53.08358 mg/g in the plant extract, asparagine and cysteine were the amino acids with the highest concentrations, comprising 13% and 12% of the total amino acid content, respectively. The leaves contained a notable concentration of vitamin B9, accounting for 49.34% of the total vitamin content. This study emphasizes the potential of *Alhagi maurorum* as a useful source of bioactive chemicals for application in the food and pharmaceutical industries, especially in the development of products that have anti-inflammatory, antioxidant, and digestive health-promoting qualities. Due to the plant's high nutrient content, it may be used to cure gastrointestinal issues, strengthen the immune system, and improve general health. This research adds to our knowledge of the plant's nutritional and medicinal benefits and suggests uses for it in both conventional and cutting-edge therapeutic approaches.

Keywords: *Alhagi maurorum*; HPLC; amino acids; vitamins; plant extract; quantitative analysis

Citation: Kholmurodov, B.B.o.; Jurayev, R.S. Quantitative Analysis of Vitamins and Amino Acids in *Alhagi* Mauro-Rum Plant Extract. *Eng. Proc.* **2024**, *67*, 32. https://doi.org/10.3390/engproc2024067032

Academic Editor: Dariusz Dziki

Published: 6 September 2024

1. Introduction

The world is clamoring for studies to identify new sources of natural pharmaceutical items and food these days due to the rising demand in these areas. As such, it is imperative to make prudent use of nature's blessings. It is evident that the number of new medications derived from Husuan medicinal plants will multiply many times over the course of the next ten years. Because of the strong biological activity of plants and the presence of vitamins and amino acids, medications derived from medicinal raw materials have a greater effect on the body and facilitate a smoother metabolism of substances.

Alhagi maurorum is a late-spreading wild plant that primarily grows in water-scarce places. It is a member of the leguminous family (Figure 1). Iraqi locals refer to the Yantok species, which belongs to the *Alhagi* (*Fabaceae*) family, as Aqual. This plant can grow

up to 15 m long and has the deepest root system. With intricate branches and a prickly appearance, this bush can grow to a height of 0.45–1.22 m. The plant is hairless and gray-green and has alternating leaves that range in shape from oval to lanceolate. The small, pea-like blossoms come in a variety of colors, from dark red to pinkish-purple. The leaves are located on short, spiky branches. The reddish-brown seeds are found inside a shell [1,2].

(**a**) (**b**)

Figure 1. General (**a**) and stem (**b**) view of the *Alhagi maurorum* plant.

The plant grows in the Middle East and Eurasia's temperate and tropical regions, including Cyprus, Iran, Iraq, Israel, Jordan, Kazakhstan, Kuwait, Lebanon, Mongolia, Pakistan, Syria, Tajikistan, Turkey, Turkmenistan, Uzbekistan, and Russia. It is also found in Northern India, Afghanistan, Armenia, Azerbaijan, and Northwest China. The plant is primarily found in the Xin Jiang Uyghur Autonomous Region of China. In India, it is mostly found in the dry, arid areas of Gujarat, Punjab, Uttar Pradesh, and Rajasthan [3–5].

In Asia, it is mostly found in South Kazakhstan, Afghanistan, Turkmenistan, Uzbekistan, and the foothills of the Tian Shan and southern Pamir–Aloy mountain ranges, close to the rivers Kyzyl Kum, Karakum, Syrdarya, and Amudarya [6].

It grows in forests, along rivers, canals, and irrigation ditches, on farms, in dry and salty areas, and sandy deserts, and open deserts at the base of mountains, such as Fergana, Syrdarya, Jizzakh, Bukhara, Kashkadarya, and other parts of Uzbekistan [7].

Alhagi pseudalhagi, *Alhagi maurorum*, *Alhagi canescens*, *Alhagi kirghisorum*, *Alhagi sparsifolia*, *Alhagi graecorum*, and *Alhagi persarum* are the seven principal species of alder found in the world's flora. The majority of *Alhagi maurorum*'s growth occurs in Uzbekistan.

This kind of plant has been discovered to be effective in treating stomach infections, liver illnesses, rheumatoid arthritis, and urinary tract infections. Specifically, the bilberry's alcoholic extract contains anti-diarrheal properties.

The acorn, or *Acornium maurorum*, has been utilized in traditional medicine as a successful therapy for a number of ailments, including rheumatism, liver disorders, and urinary and digestive disorders [8]. Its flowers and leaves are used to alleviate rheumatism. Aqueous preparations from its roots have been used to remove and widen the urinary tract [9]. It is still used to treat a variety of infectious disorders in Pakistan.

In southwest Iran, an aqueous extract of *A. maurorum* is still employed in traditional medicine. One instance of this is the elderberry infusion used to treat Hepatitis B. This plant has been traditionally used to treat rheumatism, peptic ulcers, and gastrointestinal disorders.

The camel thorn plant *Alhagi maurorum* has substantial amounts of flavonoids and phenolic chemicals, making it a potentially useful medicinal plant [10]. Phytochemicals that function as natural antioxidants include polysaccharides, sitosterols, glycosides, terpenoids, coumarins, saponins, carotenoids, vitamins, tannins, phenolics, and flavonoids, which are abundant in different parts of the plant, including its leaves [11–13]. This woody perennial shrub is widely distributed and contains over twelve distinct isolated flavonoids [14]. It

includes ethanolic extracts of *A. maurorum* along with flavonoids, saponins, alkaloids, organic acids, vitamins, tanning agents, sugars, resins, and wax [15]. According to chemical research, this species contains alkaloids, hydrocarbons, terpenoids, essential oils, ketones, acid derivatives, antioxidant chemicals [16], phenolic components [17], vitamins [18], fatty acids, sterols [19], coumarins [20], and flavonoids [21].

Research on a number of *Fabaceae* species has shown the existence of phenolic chemicals [22]. The broad class of phenolic chemicals found in plants is known as phenolic acids. It is composed of two main groups: derivatives of hydroxyl benzoic acid and hydroxyl cinnamic acid, which differ in the quantity and arrangement of hydroxyl and methoxy groups inside the aromatic ring. These substances have been shown to exhibit a variety of pharmacological properties, including antioxidant properties [23].

Livestock is the primary user of this plant. After this plant's composition was examined, it was discovered to contain over 300 components, including phenolic compounds, hydrocarbons, carbohydrates, terpenoids, alkaloids, and lipids (Table 1) [24,25].

Table 1. Organic matter in *A. maurorum* [24,25].

№	Groups of Organic Compounds	Name of the Organic Matter in *A. maurorum*
1	Phenolic compounds	Phenol carboxylic acids, Flavonoids, Proanthocyanidins, Xanthones, Coumarins, Hydrolyzable tannins, γ-Pyrones, Diphenyl ethers, and Naphthoquinones
2	Alkaloids	Arylethylamine, Pyrrole derivatives, and Isoquinoline alkaloids
3	Terpenoids	Mono-, Di-, and Triterpenoids and Polyterpenoids
4	Fatty acids and their esters	Caproic (hexanoic) acid, Palmitic acid, Triacontanoic acid methyl ester, and Tetradecanoic acid
5	Aldehydes	n-Hexadecanoic acid and Octadecanoic acid
6	Carbohydrates	Sucrose, Raffinose, Melesitose, Trisaccharide, 1-O-ß-D Methylglucoside, D-Pinitol, and α-D-Acetyl glucopyranose

The focus of our earlier work was on methods of organic synthesis [26–28]. This study's primary goal is to quantitatively analyze amino acids and water-soluble vitamins in plants using high-performance liquid chromatography (HPLC). The dried stems and leaves of the *Alhagi maurorum* shrub were utilized for this analysis. Consequently, vitamins and amino acids, two helpful components of the plant, were investigated.

2. Experimental Methods

The studies were conducted in the "Scientific testing laboratory" of the Shahrisabz branch of the Tashkent Institute of Chemical Technology and the "Chemistry of proteins and peptides" laboratory of the Institute of Bioorganic Chemistry, Academy of Sciences of Uzbekistan. The determination of water-soluble vitamins and the production of free amino acids using phenylthiocarbomyl (FTK) were performed using the Steven Cohen and Daviel technique [29]. For laboratory analysis, samples of *Alhagi maurorum* gathered in the fall of 2023 were utilized.

The dried material was extracted in water to determine vitamins and in 40% ethyl alcohol to determine amino acids. Next, it was filtered through filter paper with a blue ribbon (ecos-1). The filtrate was centrifuged using the DMO412 (China) centrifuge. Using the GTSonic-D6 (China) ultrasonic cleaner, the solution's clarity was improved.

Initially, a stem extract was made from the yantok plant, which grows in the Kashkadarya area. Utilizing phenylthiocarbomil (FTK) and its derivative (compound), as well as a 40% extraction of ethyl alcohol, conducted using high-performance liquid chromatography (HPLC., Agilent Technologies 1260, Waldbronn, Germany), this research was conducted on free amino acid analysis.

2.1. Method of Extraction of Alhagi maurorum

Using an analytical balance, 5–10 g of the sample was weighed out from the drawer, and it was transferred to a 300 mL flat-bottom flask. It was then mixed with 50 cc of a 40% ethanol solution. The mixture was heated for one hour while being vigorously swirled. It was then stirred at room temperature for two hours using a magnetic stirrer and a reflux condenser. After cooling, the mixture was filtered. The remaining portion was mixed with 25 milliliters of 40% ethanol and extracted two more times. A 100 mL volumetric flask containing the filtrates was filled to the brim with 40% ethanol (5–10%). After that, the mixture was centrifuged for ten minutes at 7000 rpm. For analysis, the final solution was removed from the top.

2.2. Conditions of Sample Preparation and Chromatographic Analysis for Amino Acid Determination

Using a centrifuge, the proteins and peptides that were extracted from the materials in aqueous solution were precipitated. After the material was separated, one milliliter of 20% TXUK (tetra phosphorous acetic acid) was added. Following a 10-min period, the sample was centrifuged for 15 min at 8000 rpm, and 0.1 mL of the leftover liquid was lyophilized. After evaporating the hydrolyzate, the dry residue was dissolved in a 1:7:1 solution of triethylamine, acetonitrile, and water and then dried. In order to neutralize the acid, this process was repeated twice. Using the approach of Stephen A. Cohen Daviel, amino acid phenylthiocarbamyl derivatives (FTC) were obtained by reacting with phenylthioisocyanate.

Setting up the experiment:

- High-performance liquid chromatography (Agilent Technologies 1260) was used to determine the derivatives of amino acids. Using the FTK technique, amino acids were identified.
- A 75 × 4.6 mm Discovery HS C18 column was used.
- The following combinations were utilized: B: CH_3CN, pH 6.4, with combinations of 0.14M CH_3COONa + 0.05% TEA; The wavelength was set to 2.69 and a flow rate of 1.2 mL per minute.
- The gradient percentage B/min was as follows: 1–6%/0–2.5 min; 6–30%/2.51–40 min; 30–60%/40.1–45 min; 60–60%/45.1–50 min; 60–0%/50.1–55 min.

2.3. Conditions of Sample Preparation and Chromatographic Analysis for Vitamin Determination

The samples were dried for fifteen days in a natural wind flow in a designated area free from sunlight. Using a specialized grinding mill, the dried raw materials were ground to a powder. For extraction, 40% ethyl alcohol (C_2H_5OH) was used. A sample of 0.1 g was dissolved in 10 mL of alcohol and allowed to stand for a whole day. Afterward, the solution was filtered using a filter paper and centrifuged for ten min at 8000 rpm. The resulting clear solution was placed into vials and subjected to high-performance liquid chromatography (HPLC; Agilent Technologies (Hong Kong, China), 1260).

Setting up the experiment:

- Water-soluble vitamin analysis via HPLC using Agilent Technologies 1260 liquid chromatography;
- Eclipse XDB C18 column (reverse phase), 5 μm, 4.6 × 250 mm;
- A 250 nm diode array detector (DAD);
- Solution B: CH_3CN (acetonitrile) with 0.1% trifluoroacetic acid at pH 1.7;
- Flow rate: 0.8 mL/min;
- %B/min gradient: 0–5 min/0%, 5–11 min/0:25%, 11–19 min/25:40%, 19–21 min/40:40%, 21–25 min/40–0%;
- A 250C thermostat.

3. Results and Discussion

3.1. Solvent Selection for the Extraction Process

Several studies were analyzed to select a solvent for the extraction process [30–33]. Finally, the following results were obtained (Table 2).

Table 2. Solvents used in the extraction process and the biologically active substances they release.

Groups of Extractable Substances	Solvents										
	Gasoline, Hexane	CO_2 Subcritical	Freons	Acetone	Ethyl Acetate	Alcohols	Alcoholic Aqueous Solutions	Dimethyl Sulfoxide	Water	Supercritical CO_2	Supercritical with Azeotrope CO_2
Carbohydrates	+	+/−	+	+	+	−	−	−	−	+/−	+/−
Carotenoids	+	+	+	+	+	−	−	−	−	+	+
Diacylglycerols	+	+	+	+	+	−	−	−	−	+	+
Monoacylglycerols	+	+	+	+	+	+	−	−	−	+	+
Sterols	+	+	+	+	+	+	−	−	−	+	+
Phospholipids	+	+	+	+	+	+	−	−	−	+	+
Tocopherols	+	+	+	+	+	+	+	+/−	−	+	+
Terpenoids	+	−	+	+	+	+	+	+	−	+	+
Aldehydes, ketones	+/−	−	+	+	+	+	+	+	−	+	+
Esters	−	−	+	+	+	+	+	+	−	+	+
Flavonoids	−	−	+/−	+	+	+	+	+	−	+	+
Alcohols	−	−	+	+	+	+	+	+	+	+	+
Amino acids	−	−	−	+/−	−	+	+	+	+	+	+
Organic acids	−	−	−	−	−	+	+	+	+	+	+
Carbohydrates	−	−	−	−	−	−	+	+	+	+	+
Alkaloids	−	−	−	−	−	−	+	+	+	+	+
Tannins	−	−	−	−	−	−	+	+	+	+	+
Phenolic compounds	−	−	−	−	−	−	+	+	+	+	+
Glycosides	−	−	−	−	−	−	+	+	+	+	+
Minerals	−	−	−	−	−	−	+/−	+	+	−	−
Polysacharides	−	−	−	−	−	−	−	+/−	+	−	−
Oligosaccharides	−	−	−	−	−	−	−	−	+	−	−
Proteins, peptides	−	−	−	−	−	−	−	−	+	−	−
Pectins	−	−	−	−	−	−	−	−	+	−	−

Based on the table, we believe that an aqueous alcohol solution can be used as the extractant. In aqueous solutions of alcohol, it is possible to isolate minerals, amino acids, organic acids, terpenoids, aldehydes, ketones, esters, flavonoids, alkaloids, tannins, phenolic compounds, and glycosides from the remaining solvents.

Aqueous ethyl alcohol was selected as the solvent according to the data. It was determined after multiple trials that 40% ethyl alcohol was appropriate for our study

(Table 3). The table displays the different colors of the extraction process solvents. For instance, 40% of ethyl alcohol is gray, 70% is dark blue, and 96% is pink; water is light blue. The natural substances utilized for extraction, along with the dry mass and solvent amounts chosen for the experiment, are indicated in green.

Table 3. Extraction of substances in different extractions of the *Alhagi maurorum* plant.

Solvent (Dry Mass/Solvent)	Rutin	D. Quercitin	Querstin	Exysterone	Gallic Acid	Luthionine
Water (5/200)	0.2520	0.0727	0.0856	0.0427	0.0000	0.2919
Water (5/100)	0.4070	0.0818	0.0382	0.0322	0.0042	0.0695
Water (5/75)	0.6380	0.0746	0.0289	0.0265	0.0043	0.0515
Water (5/50)	0.2047	0.0722	0.0201	0.0336	0.0030	0.0334
alcohol 40% (5/200)	1.0517	0.0713	0.0000	0.0243	0.0052	0.1543
alcohol 40% (5/100)	0.9278	0.4227	0.0000	0.0209	0.0049	0.0605
alcohol 40% (5/75)	0.8762	0.4930	0.0000	0.0229	0.0052	0.0432
alcohol 40% (5/50)	0.9090	0.2204	0.0000	0.0229	0.0052	0.0274
alcohol 70% (5/200)	0.9472	0.0678	0.0159	0.0206	0.0000	0.5059
alcohol 70% (5/100)	0.9754	0.4750	0.0000	0.0208	0.0038	0.0580
alcohol 70% (5/75)	0.9198	0.2079	0.0000	0.0192	0.0036	0.0407
alcohol 70% (5/50)	1.0350	0.2220	0.0064	0.0217	0.0036	0.0245
alcohol 96% (5/200)	0.7581	0.0000	0.0000	0.0146	0.0000	0.2491
alcohol 96% (5/100)	0.6473	0.0042	0.0000	0.0137	0.0000	0.1375
alcohol 96% (5/75)	0.5276	0.0024	0.0000	0.0139	0.0000	0.0488
alcohol 96% (5/50)	0.4918	0.0028	0.0000	0.0127	0.0000	0.0318

Therefore, ethyl alcohol as a solvent has a wider range of extracting biologically active substances compared to water, and its extraction ability depends on the concentration. When extracted with ethanol at a concentration of at least 70%, extracts without biopolymers (proteins, mucus, and pectins) are obtained.

3.2. Results of the Amino Acid Analysis of the Alhagi maurorum Plant and a Discussion of the Study

A total of 20 different basic amino acids were identified in this study's extract, including proline, tyrosine, valine, methionine, isoleucine, leucine, histidine, tryptophan, phenylalanine, and lysine. The extract also contained 19 different amino acids from these 20 basic amino acids. Among these, eight are necessary amino acids. Non-exchangeable amino acids are a sign that plant components that have been dried or extracts made with different solvents can be utilized for human purposes (Figure 2).

The following formula was used to calculate the findings and compare them with the standard sample:

$$C = \frac{S_1 \cdot V_0 \cdot P \cdot 1000}{S_0 \cdot V_1 \cdot 100}, \quad (1)$$

where S_1—sample area;

S_0—standard area (Figure 3);

V_1—sample size;

V_0—standard size;

P—amount of sample.

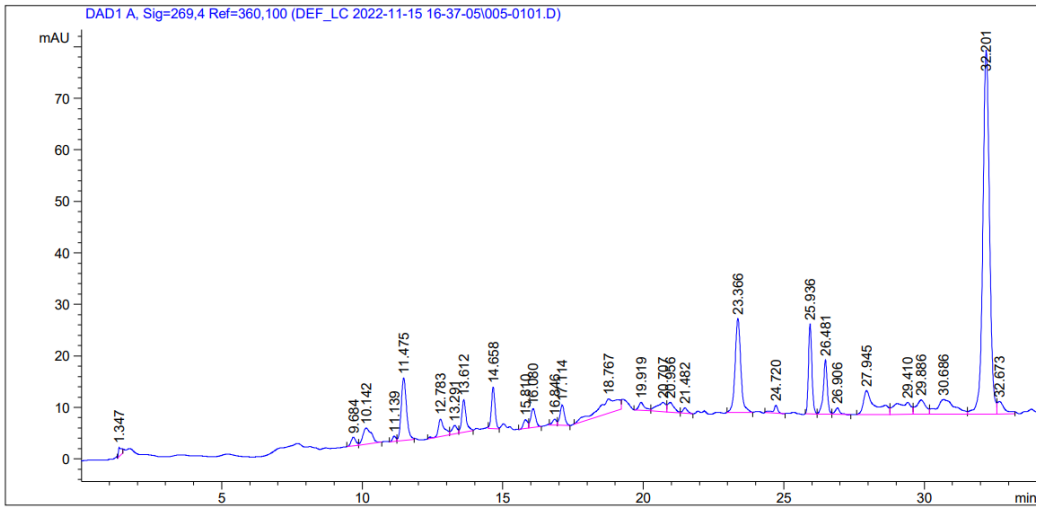

Figure 2. HPLC chromatogram of *Alhagi maurorum* stem extract at 269 nm wavelength.

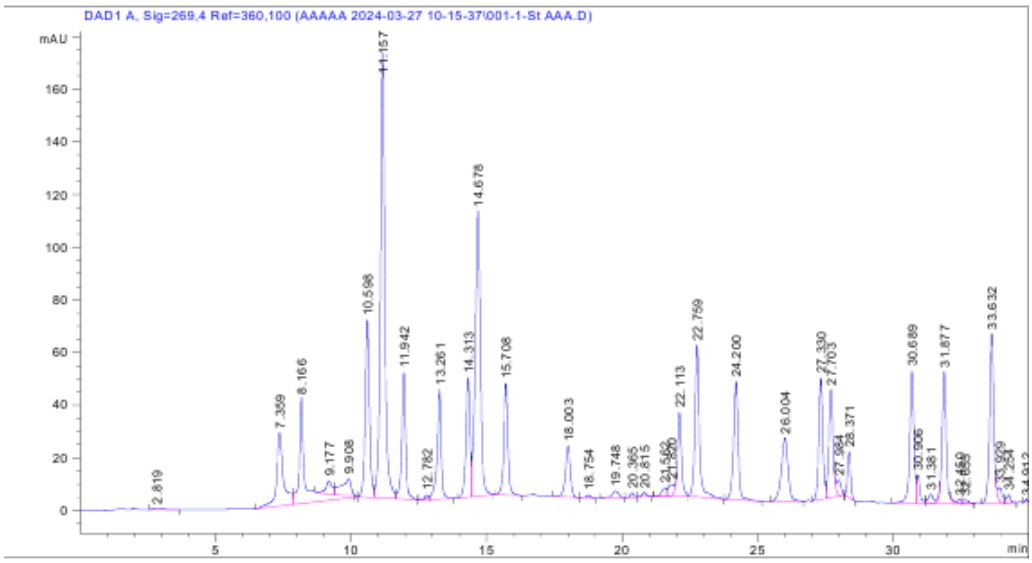

Figure 3. HPLC chromatogram of a standard solution of amino acids.

By comparing the chromatograms of the samples with the chromatograms of the standard amino acids, the amounts of amino acids were calculated in accordance with the analysis findings (Table 4).

Table 4. Amino acid content of *Alhagi maurorum* stem extract.

Name of Amino Acids	Concentration mg/g
Aspartic acid	0
Glutamic acid	1.878941
Serine	0.904018
Glycine	3.474454
Asparagine	7.006748
Glutamine	3.891102
Cysteine	6.551913
Threonine	2.807103

Table 4. *Cont.*

Name of Amino Acids	Concentration mg/g
Arginine	1.987315
Alanine	1.972477
Proline	1.90203
Tyrosine	3.698049
Valin	4.519611
Methionine	1.122176
Isolation	1.00062
Leucine	2.356721
Histidine	2.415568
Tryptophan	1.245416
Phenylalanine	1.154935
Lysine	3.194389
Jami	53.08358

It was discovered that the Yantoq (alhagi maurorom) plant extract has 53.08358 mg/g of amino acids. Among these amino acids, asparagine, at 7.006748 mg/g, demonstrated a high level, accounting for 13% in relation to the mass of all amino acids. Of it, the amino acid cysteine made up around 12%.

Serine (0.904018 mg/g, 2%) and isoleucine (1.00062 mg/g, 2%) were determined to have the lowest amounts (Figure 4).

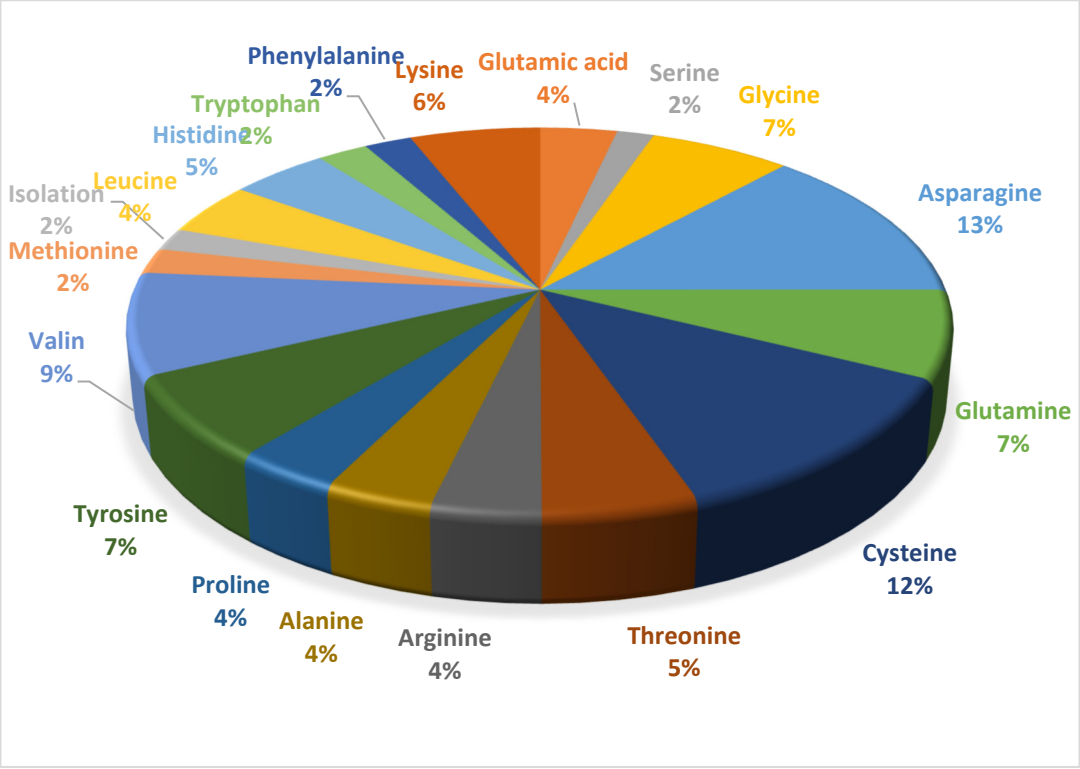

Figure 4. Mass fraction of amino acids in *Alhagi maurorum* plant extract.

3.3. Results of the Vitamins Analysis of the Alhagi maurorum Plant and a Discussion of the Study

Chromatograms were produced at a wavelength of 250 nm in order to quantify the amount of water-soluble vitamins in the leaves and stems of *Alhagi* maurorom (Figures 5 and 6).

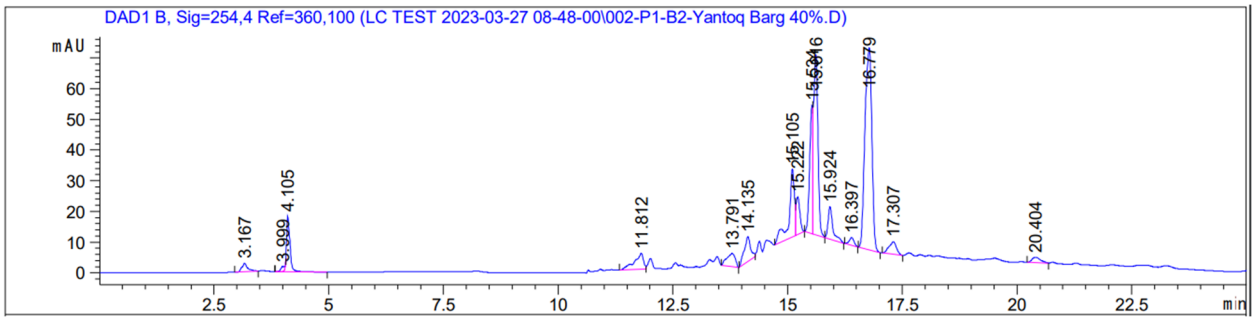

Figure 5. Chromatogram of plant leaf extract from *Alhagi maurorom* made using HPLC.

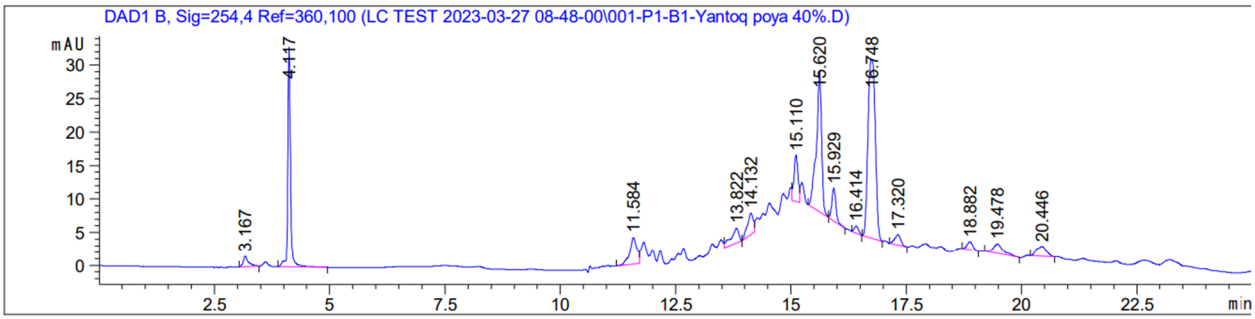

Figure 6. HPLC chromatogram of *Alhagi maurorom* plant stem extract.

The following formula was used to calculate the findings and compare them with the standard sample:

$$C = \frac{S_1 \cdot 2 \cdot 100 \cdot 1000 \cdot 1000}{S_0 \cdot V_1 \cdot 1000}, \quad (2)$$

where S_1—sample area;

S_0—standard area (Figure 7);

V_1—sample size.

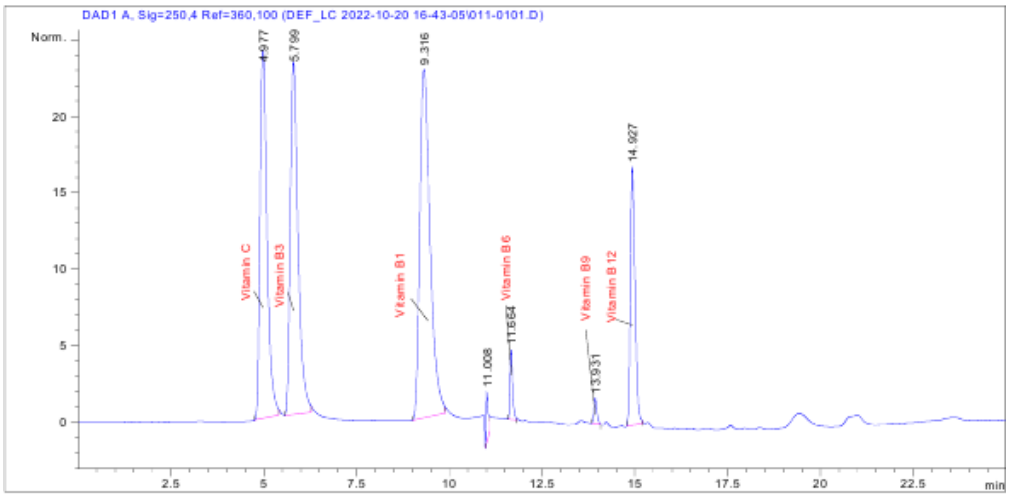

Figure 7. HPLC chromatogram of a standard solution of vitamins.

The concentration of vitamins in the solution (µg/g) was determined by comparing the acquired results with standard chromatograms (Table 5).

Table 5. Amount of water-soluble vitamins of *Alhagi* maurorom plant.

Vitamins	Amount of Vitamins Contained in the Leaf of *Alhagi* Maurorom	Amount of Vitamins Contained in the Stem of *Alhagi* Maurorom
	Concentration µg/g	
B-1	0	0
B-2	1.341662	1.190836
B-6	2.015659	1.959313
B-9	3.269345	0.769618
PP	0	0
C	0	0

Based on the findings, it was concluded that the leaf portion stores 6.62666 µg/g more water-soluble vitamins. The majority of the vitamins identified belonged to group B, and of those, the leaf had 49.34% more vitamin B9 than all other sections and vitamins combined.

The stem was discovered to have 1.7 times less vitamin than the leaf. This section showed that vitamin B6 was found to have a 49.34% higher content than other vitamins (Figure 8).

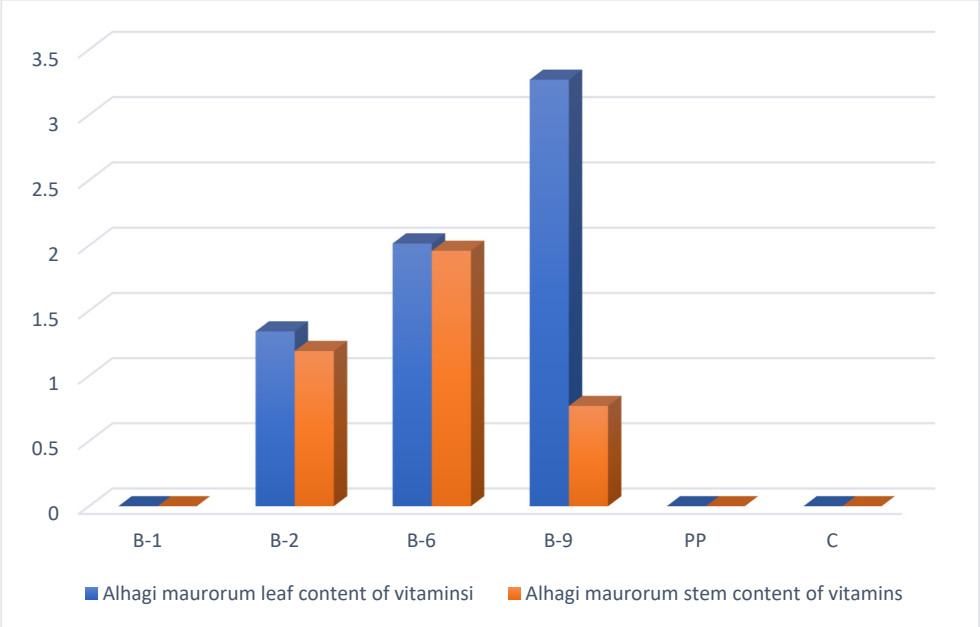

Figure 8. Graphical representation of the mass fraction of vitamins contained in alhagi maurorom extract.

Therefore, by extracting the alhagi maurorom plant in alcohol and water, the amounts of water-soluble vitamins and amino acids were found. It should be emphasized that the results obtained are peculiar to the plant growing naturally in Uzbekistan.

4. Conclusions

Twenty amino acids and water-soluble vitamins were discovered in the *Alhagi maurorom* stem throughout this study. Asparagine was discovered to have the largest amount of these amino acids (13%), but aspartic acid was found to be lacking. Vitamins from the B group were examined. The amounts of vitamins C, PP, and B1 were not determined using the approach

we employed. The findings indicate that alhagi maurorom is a biologically significant raw material because it includes valuable amino acids, including eight necessary amino acids. At the same time, vitamins boost health. When this plant is used properly in the food sector, useful items for human health can be produced. It can be employed as an anti-inflammatory drug for the human stomach and intestines, particularly in the beverage industry.

Author Contributions: Conceptualization, B.B.o.K. and R.S.J.; writing—original draft preparation, R.S.J. and B.B.o.K.; visualization, R.S.J. and B.B.o.K.; writing—review and editing, R.S.J. and B.B.o.K.; supervision, R.S.J. and B.B.o.K. contributed equally to this paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Agency of Innovative Development under the Ministry of Higher Education, science, and Innovation of the Republic of Uzbekistan (Contract No. 65).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors wish to acknowledge Shahrisabz branch of the Tashkent Institute of Chemical Technology, Shahrisabz, Uzbekistan, and the Academy of Sciences of Uzbekistan Institute of Bioorganic Chemistry, Tashkent, Uzbekistan.

Conflicts of Interest: The authors declare no conflicts of interest.

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