ХИМИЯ РАСТИТЕЛЬНОГО СЫРЬЯ. 2025. №4. С. 311–316.
KHIMIYA RASTITEL'NOGO SYR'YA, 2025, no. 4, pp. 311–316.
DOI: 10.14258/jcprm.20250416338

UDC 543.544.5.068.7+581.192.2:577.112.3

ANALYSIS OF THE CHEMICAL COMPOUNDS OF THE PLANT *LAGOTIS KOROLKOWII* (PLANTAGINACEAE)

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This study is the first to analyze the flavonoids, amino acids, and vitamins present in the *Lagotis korolkowii* plant distributed in the territory of Uzbekistan. Twenty amino acids were identified in the samples, of which eight were non-essential, eight were essential, and four were conditionally essential. The total amino acid content of the roots was 9.35 mg/g, whereas that of the aerial parts was 68.35 mg/g. According to the comparative analysis, the aerial parts of *Lagotis korolkowii* were quantitatively richer in amino acids than the roots. Flavonoids in various parts of *Lagotis korolkowii* were analyzed for the first time. The results showed that the aerial parts of the plant contained kaempferol, rutin, hypolaetin, hyperoside, and apigenin. Additionally, it was found for the first time that *Lagotis korolkowii* contains large amounts of vitamins B and C and PP. The obtained data allowed us to consider *Lagotis korolkowii* as a source of flavonoids, valuable amino acids with a wide spectrum of pharmacological activity, and rich in vitamins, which can be used for the development of new food supplements and pharmaceutical substances.

Keywords: *Lagotis korolkowii*, flavonoids, hypolaetine, gallic acid, hyperoside, amino acids, cysteine, glutamine, asparagine, vitamins.

For citing: Nosirova V.M., Khujayev V.U., Turdiboev O., Matchanov A.D. *Khimiya Rastitel'nogo Syr'ya*, 2025, no. 4, pp. 311–316. (in Russ.). DOI: 10.14258/jcprm.20250416338.

Introduction

The global flora includes 30 species of the genus *Lagotis* belonging to the family Plantaginaceae, which are primarily distributed in the mountains of Europe and Asia [1]. Only one species of the *Lagotis* genus is present in the flora of Uzbekistan: *Lagotis korolkowii* (Regel &Schmalh.) Maxim [2]. *Lagotis korolkowii*, also known as Korolkov'slagotis, is primarily found in the mountains of Central Asia, particularly Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan. This species prefers alpine and subalpine meadows, growing at altitudes of approximately 2400–3200 (3500) meters above sea level [2, 3].

Some species such as *L. yunnanensis, L. glauca, L. integra,* and *L. brachystachya* are used in Tibetan folk medicine to treat fever, hypertension, and acute and chronic hepatitis. Continued studies on *L. yunnanensis*have led to the isolation of two new benzoyl esters of glucose [4]. Iridoids have been isolated from the epigeal parts of *L. integrifolia*. Extraction of the plant with methanol resulted in the isolation of aucubin (1)–3.64 g (2.6%) and aucubin (2) [5]. *L. brachystachya*is anherb widely used in traditional Tibetan medicine. Luteolin, luteoloside, and apigenin isolated from this plant have been identified as potential candidates for the treatment of hyperuricemia [6]. *L. brevitubaisis* a perennial species distributed in the highlands of China that has been used as a traditional Tibetan medicinal plant for over 2000 years. A total of 44 compounds were identified in this plant by liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS), including 19 flavonoids, 14 phenols, 8 phenylethanoid glycosides, 2 iridoid glycosides, and 1 carbohydrate [7].

L.korolkowii has not been studied previously, and its chemical composition was analyzed for the first time.

L.korolkowii is a perennial herbaceous plant that is nearly stemless, measuring 1.5–5 cm in height. The leaves are 1 to 4.5 cm long, glabrous, lanceolate, and have entire margins or occasional teeth. The inflorescence was light

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purple, bilabiate, and 12–14 mm long. The fruit has an oval capsule measuring 5 mm in length. The upper part of the plant is found at an altitude of 4500 m on the ridges of Western Tian Shan and Pamir-Alai [8].

Flavonoids are a group of natural compounds belonging to the class of polyphenols. They are widely distributed in plants and exhibit potent antioxidant activities. Flavonoids have numerous beneficial effects including reducing inflammation, preventing cancer, and supporting cardiovascular health. They may also positively influence the metabolism and strengthen the immune system. To reap these benefits, it is recommended that a variety of fruits, vegetables, and herbs be included in the diet [9].

Amino acids are organic molecules that serve as the fundamental building blocks of proteins. Each amino acid contains an amino group ($-NH_2$), carboxyl group (-COOH), and unique side chain (R) that determines its characteristics. These molecules play crucial roles in metabolic processes and are involved in the synthesis of hormones, neurotransmitters, and other biologically active compounds [10]. Vitamins are organic compounds that are essential for normal functioning of the body. They perform numerous functions such as participating in metabolic processes, supporting the immune system, and protecting cells from damage caused by free radicals [11].

This study aimed to analyze flavonoids, amino acids, and vitamins in the roots and aboveground parts of *L. korolkowii*, a plant distributed in Uzbekistan.

Experimental Section

L.korolkowii was collected from the area surrounding the Arashan Lake in the Pap district of the Namangan region (41.362382°, 70.518174°). All herbaria were maintained at the Kokand State University, Department of Biology (KD).

The plant *Lagotis korolkowii* was collected in the Arashan spring area of the Chotqol mountains at an altitude of 3000 meters in 1 August 2024. The plant (leaves and roots) was harvested during the yuvenil period, in the vegetative phase. In this period since the aerial part of the plant consisted of leaves, the leaves and roots were separated into different parts. The aerial part was dried in an open-air environment, protected from direct sunlight, while the root part was dried in open air under direct sunlight. Subsequently, the samples were delivered to the laboratory department of the Tashkent Institute of Bioorganic Chemistry for chemical composition analysis. Considering that the chemical composition of *L. korolkowii* has not been previously studied, investigating this plant holds significant promise.

The chemical analysis of flavonoids present in the plant *L. korolkowii* was performed using high-performance liquid chromatography (HPLC) on an Agilent Technologies 1260 instrument. The analytical method included isocratic elution using a diode-array detector, which ensured the accuracy and reliability of the obtained data [12].

The mobile phase consisted of a mixture of acetonitrile and buffer solution at a ratio of 35 : 75, with the pH adjusted to 2.92. The analysis time ranged from 15 to 20 min, which provided optimal separation of the components. The injection volume was 5 μL and the flow rate of the mobile phase was set at 0.75 mL/min, which is critical for achieving the necessary sensitivity and resolution.

The chromatographic column used in this study was an XDB-C18 column with dimensions of 4.6 × 250 mm and a particle size of 5.0 microns. Detection was performed at wavelengths of 254, 320, and 381 nm, allowing for collection of spectral data in the range of 200–400 nm.

The composition and quantity of free amino acids were studied using high-performance liquid chromatography according to the Cohen-Davielmethod [13]. Oxyls and peptides were precipitated from the aqueous extract by centrifugation to isolate free amino acids from the sample. For this purpose, 1 mL of the sample was mixed with 1 mL of 20% trichloroacetic acid (TCA) (purity ≥99%). After 10 min of precipitation, the precipitate was separated by centrifugation at 8000 rpm for 15 min. The supernatant (0.1 mL) was lyophilized and the hydrolysate was evaporated to dryness and subsequently dissolved in a mixture of trimethylamine (purity ≥99%), acetonitrile (purity ≥99%), and water (1 : 7 : 1) [14].

Sample Preparation for Flavonoid Analysis. To study flavonoids, dried vegetative organs of the plant were ground to a particle size of 0.1–1.5 mm. A precise sample of 1.0000 g was weighed and extracted with 99 mL of 70% ethanol at a temperature of 50–60 °C for 2 hours, with vigorous stirring in a flat-bottom flask equipped with a reflux condenser. The resulting solution, along with the flask, was transferred to an ultrasonic bath, where extraction was conducted for 15 min with a 10-minute interval, repeated twice, at a temperature of 35 °C. The extract was then cooled to room temperature.

The cooled solution was filtered first through an ash-free paper filter (blue ribbon) and subsequently, 2 mL of the filtrate was passed through a 0.2 μm membrane filter. From this solution, a 100 μL aliquot was taken and diluted to 1 mL with the eluent. The analysis was carried out using HPLC under isocratic elution mode with a diode-array detector (DAD). Acetonitrile and a buffer solution were used as the mobile phase. Spectral data were recorded within a wavelength range of 200–400 nm.

Chromatographic Conditions: Chromatograph – Agilent Technologies 1260, Mobile phase – Acetonitrile – buffer solution (30 : 70), pH – 2.92, Analysis duration – 20 minutes, Injection volume – 5 μL, Flow rate – 0.75 mL/min, Column – Eclipse XDB-C18, 5.0 μm, 4.6 × 250 mm, Detector – Diode-array detector (DAD) with detection wavelengths at 254, 320, and 381 nm.

The experiment was conducted twice to neutralize the acid. Stephen used the Cohen-Daviel method to obtain amino acids, which reacted with phenylthioisocyanate and derivatives of phenylthiocarbamyl amino acids. The obtained derivatives were analyzed by high-performance liquid chromatography (HPLC) [15].

For chromatography, an Agilent Technologies 1200 instrument equipped with a diode-array detector (DAD) and a Discovery HS C18 cartridge measuring 75 × 4.6 mm. Solvent A consisted of 0.14 M sodium acetate with the addition of 0.05% triethylamine (purity ≥99%), pH 6.4, along with CH_3CN. The flow rate was set at 1.2 mL/min, and the detection wavelength was 269 nm. The gradient was as follows: 1–6% B over 0–2.5 minutes; 6-30% B from 2.51 to 40 minutes; 30–60% B from 40.1 to 45 min. The 60–60% B mode was maintained from 45.1 to 50 min, followed by 60–0% B from 50.1 to 55 min.

The vitamin composition and quantity of *L. korolkowii* were determined using high-performance liquid chromatography (HPLC). Analysis of water-soluble vitamins was conducted using gradient elution and a diode array detector. Acetonitrile and a buffer solution were used as mobile phases. The spectral characteristics were studied in the wavelength range of 200–400 nm [16].

To analyze the water-soluble vitamins in the plant material, an initial sample of 5 g was weighed on an analytical scale and placed in a flat-bottomed flask with a volume of 300 mL. Then, 50 mL of a 40% ethanol solution was added. The resulting mixture was heated under constant stirring for 1 h using a magnetic stirrer equipped with a reflux condenser. After heating the mixture, it was maintained at room temperature for 2 h. The mixture was then cooled and filtered. To the remaining precipitate, 25 mL of 40% ethanol was added and the extraction was performed twice. The combined filtrates were brought to a volume of 100 mL using 40% ethanol. The resulting solution was centrifuged at 7000 rpm for 10 min and the upper phase of the solution was collected for further analysis.

Vitamin identification and quantitative determination were performed by comparison with phenylthiocarbamyl (PTC) derivatives of standard vitamins. Standard solutions of each vitamin were prepared at a concentration of 1 mg/ml. Phosphoric and acetate buffer systems, as well as acetonitrile (purity ≥ 99%), were used to determine water-soluble vitamins using HPLC. In our study, an acetate buffer system in combination with acetonitrile was applied, with a mobile phase flow rate of 0.75 mL/min in gradient mode: acetonitrile-buffer solution pH = 2.92 (4% / 96%) from 0 to 6 min, (10% / 90%) from 6 to 9 min, (20% / 80%) from 9 to 15 min, and (4% / 96%) from 15 to 20 min. The injection volume for the HPLC was 10 μL. Samples were passed through an Eclipse XDB S18 filter with a pore size of 5.0 microns (4.6 × 250 mm) and analyzed using a diode-array detector at wavelengths of 272, 292, 254, 297, and 360 nm.

Results and Discussion

Chemical analysis of the flavonoids in *L. korolkowii* was conducted using high-performance liquid chromatography (HPLC). The plant contained kaempferol (17.392 mg/100 g), hypolaetine (183.641 mg/100 g),rutin (87.993 mg/100 g), hyperoside (64.915 mg/100 g), and apigenin (14.231 mg/100 g).The amount of gallic acid in the phenolic compounds was determined to be (161.221 mg/100 g). The results were confirmed by table (Table 1). The results indicated that the concentrations of hypolaetine, hyperoside, kaempferol, and rutin in *Lagotis korolkowii* were higher than those of the other flavonoids. A brief overview of the most common hypolaetin and gall acids found in plants: hypolaetin is a flavonoid with several potential health benefits.

1. Antioxidant: Helps neutralize free radicals and protect cells from oxidative damage.
2. Anti-inflammatory: Reduces inflammation, which may lower the risk of chronic diseases.
3. Cardiovascular Health: Supports heart health by improving blood circulation and lowering blood pressure.
4. Cancer Prevention: May inhibit the growth of cancer cells.
5. Neuroprotection: Shows promise in protecting against neurodegenerative diseases.

6. Antibacterial & Antiviral: Helps fight infections and boost immunity [17, 18].

Gall acids are crucial for digestion and overall health:

1. Fat digestion aids in the breakdown and absorption of dietary fats and fat-soluble vitamins (A, D, E, K).
2. Cholesterol Regulation: Help regulate cholesterol levels by promoting its excretion through bile.
3. Gut Health: Support the gut microbiome balance, which is vital for digestion and immunity.
4. Liver Function: Play a role in detoxification and maintaining liver health.
5. Anti-inflammatory: Some gall acids have anti-inflammatory properties that support overall health.
6. Blood Sugar Regulation: May help improve insulin sensitivity and regulate blood sugar levels [19].

Our study showed that the total amount of free amino acids in the aerial parts of *Lagotis korolkowii* was 68.32 mg/g, whereas that in the roots was 9.35 mg/g. It has been established that among the metabolizable amino acids, there is a significant amount of aspartic acid, glycine, glutamic acid, proline, glutamine, cysteine, and asparagine (Table 2).

Total amino acids in aerial parts: 68.32 mg/g.

The total amino acid content in the roots was 9.35 mg/g. Asparagine supports the nervous system and participates in protein synthesis by strengthening the immune system, improving gut health, and reducing stress, while cysteine acts as an antioxidant, enhances skin and hair health, and helps neutralize toxins (table 3) [20].

The quantity of vitamins in the plant was analyzed using high-performance liquid chromatography, and the following results were obtained (Table 3).

Table 1. Flavonoids and phenolic compound Analysis of *Lagotis korolkowii*

Flavonoid	*Lagotis korolkowii*leaves (mg/100g)	*Lagotis korolkowii*roots (mg/100g)
Kaempferol	17.392	–
Hypolaetin	183.641	14.213
Rutin	87.993	47.251
Hypolaetin 7-O-D-Gly	6.213	–
Isoorientin	–	–
Hyperoside	64.915	28.215
Apigenin	14.231	3.741
Phenoliccompound	*Lagotis korolkowii* leaves (mg/100g*)*	*Lagotis korolkowii* roots (mg/100g)
GallAcid	161.221	15.214

Table 2. Concentration of Amino Acids in *Lagotis korolkowii*

Name	Aerial Parts (mg/g)	Roots (mg/g)
Essential Amino Acids		
Leucine	0.32	0.31
Isoleucine	2.40	0.20
Lysine	0.66	0.16
Valine	0.54	0.52
Methionine	0.44	0.47
Threonine	1.37	1.84
Tryptophan	0.73	0.20
Histidine	2.61	0.19
Phenylalanine	0.24	0.20
Non-Essential Amino Acids		
Alanine	0.85	0.71
Serine	0.65	0.17
Glycine	3.80	0.09
Asparagine	21.82	0.46
Proline	2.75	1.39
Arginine	1.29	0.32
Tyrosine	0.60	0.37
Partially Essential Amino Acids		
Cysteine	7.94	0.37
AsparticAcid	3.78	0.55
GlutamicAcid	2.53	0.24
Glutamine	13.00	0.56

Table 3. Vitamin Content of *Lagotis korolkowii*

Vitamin	*Lagotis korolkowii* leaves(mg/100g)	*Lagotis korolkowii* roots (mg/100g)
Vitamin B1	6.521	–
Vitamin B6	–	–
Vitamin B9	82.252	18.241
Vitamin PP (Niacin)	14.754	14.754
Vitamin C	67.632	28.521
Vitamin B2	25.274	–
Vitamin B12	67.562	–

As can be seen from the table, the aerial part of *Lagotis korolkowii* shows higher levels of Vitamins B9, B12, and C compared to its root. Taking this into account, these vitamins collectively ensure the overall healthy functioning of the body. Vitamin B9 and B12 complement each other in preventing anemia and nervous system disorders, while Vitamin C protects cells and aids in tissue repair. Therefore, their deficiency can lead to serious health issues [11].

Conclusions

The chemical composition of flavonoids, amino acids, and vitamins in *L. korolkowii* distributed in Uzbekistan was studied for the first time. Flavonoids in the *L. korolkowii* samples were analyzed using high-performance liquid chromatography. For the first time, we found that the concentrations of hypolaetine, hyperoside, and rutin in the aerial parts of *Lagotis korolkowii* were higher than those in the root parts. These results indicate that *Lagotis korolkowii* is rich in vitamins B, C, and PP and the concentrations of vitamins B9, B12, and C were higher than those of the other vitamins (B1, B6).

This finding is supported by the information presented above. Sufficient amounts of essential and semi-essential amino acids, including glutamine, asparagine, and cysteine, provide the potential to create biologically active supplements based on this plant.

Funding
This study was supported funding by Kokand State University and Institute of Bioorganic Chemistry. No additional grants to carry out or direct this particular research were obtained.

Conflict of Interest
The authors of this work declare that they have no conflicts of interest.

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Received November 19, 2024

Revised January 13, 2025

Accepted February 18, 2025

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