



Ethnomedicinal use and biological properties of *Biebersteinia multifida* DC. in Uzbekistan

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Research

Abstract

Background: This article presents data on the biological and morphological features, chemical composition, and traditional medicinal use of the valuable medicinal plant *Biebersteinia multifida* DC. in Uzbekistan.

Methods: Standard methods of High-Performance Liquid Chromatography were used to study the chemical composition of the vegetative organs of *Biebersteinia multifida* DC. The nitrogen content was determined by the Kjeldahl method. Ethnobotanical data were collected through questionnaire surveys conducted during interviews with the local population. The survey was carried out with the informed consent of the respondents. The interviews were conducted in accordance with the International Society of Ethnobiology Code of Ethics.

Results: In Uzbekistan, *Biebersteinia multifida* is known as Qontepar. Since ancient times, in folk medicine, a decoction prepared from the rhizomes of a plant has been used as a hemostatic agent for postpartum bleeding and stomach diseases. Infusion of the plant in milk is used for asthma, gastritis and respiratory tract diseases. In addition, *B. multifida* are used in traditional medicine as a medicine for diseases of the musculoskeletal system, bone fractures and skin diseases. In the laboratory, 20 types of free amino acids, 44 types of macro-, micro-, ultramicroelements, water-soluble vitamins B₁, B₂, B₆, B₉, B₁₂, PP, C were determined from the vegetative organs of *Biebersteinia multifida*, and fructose, glucose, sucrose, maltose were found from carbohydrates. At the same time, the nitrogen content was determined by the Kjeldahl method, based on calculations it was found that the presence of protein in the roots, stems and leaves is 10-11%. Gallic acid was found, from flavonoids, dihydroquercetin, rutin, quercetin were found in the roots (0.0; 5.6; 2.8; 8.3 mg/kg) and in the aboveground mass (4.7; 25.2; 12.3; 11.3 mg/kg). Analysis of herbarium samples from the electronic database of the National Herbarium of Uzbekistan revealed the distribution areas of *B. multifida* in Uzbekistan (mountainous regions of Kashkadarya, Surkhandarya, Samarkand, Dzhizak and Tashkent regions). The largest number of finds of the species was noted in the Samarkand and Kashkadarya regions, where local residents mainly collect roots for medicinal purposes.

Conclusions: Therapeutic properties of *B. multifida* have been identified. It was found that the biomass of the stem, leaves and root of *B. multifida* contains 20 types of free amino acids, 44 types of macro, micro, ultramicro elements, water-soluble vitamins B1, B2, B6, B9, B12, PP, C, from carbohydrates fructose, glucose, sucrose, maltose. The protein contents in various parts of plants are 10-11%.

Keywords: *Biebersteinia multifida*, amino acids, stem, leaves, root, chemical composition, macro -, micro -, ultramicro - elements, vitamins, ethnobotany.

Background

Worldwide, the increasing demand for plant-based medicinal preparations and products derived from natural herbal raw materials has led to a reduction in the population size and, in some cases, the loss of the gene pool of rare wild medicinal plants. In this regard, the development and implementation of effective measures for the conservation of the gene pool of rare and endangered wild medicinal plant species represent one of the priority directions of modern botanical science.

At present, among the most relevant tasks are the application of modern conservation methods for wild medicinal plant species of the local flora under in situ and ex situ conditions, the identification and characterization of plant communities, the study of the stages and phases of ontogenesis, the development of cultivation techniques, and the investigation of reintroduction processes.

Leading scientific centers worldwide conduct comprehensive studies aimed at the scientifically based cultivation and processing of medicinal plants, the formulation and implementation of strategies for their rational use, as well as the investigation of the chemical composition of biologically active compounds. Particular importance is attached to plant species possessing pronounced medicinal properties, a rich chemical composition, widespread use in traditional medicine, phytoncide activity, and specific adaptive mechanisms against insect pests and pathogenic microorganisms.

Special attention is given to the study of the bioecological characteristics of promising medicinal plants, such as *Biebersteinia multifida* DC. and several other species, including their propagation and cultivation under various ecological conditions, their introduction into pharmaceutical practice, and the establishment of specialized plantations.

Materials and Methods

Distribution and Mapping

Distribution of *Biebersteinia multifida* in Uzbekistan was determined based on herbarium records from the National Herbarium of Uzbekistan, collections of the Institute of Biochemistry of Samarkand State University, and field expeditions. The species occurs in mountainous regions of Kashkadarya, Surkhandarya, Samarkand, Jizzakh, and Tashkent provinces at elevations of 500-1700 m a.s.l. The highest number of records was documented in Samarkand and Kashkadarya regions, where roots are commonly harvested for medicinal use (Figure 1).

In Uzbekistan, this species occurs in the mountainous regions of the Kashkadarya, Surkhandarya, Samarkand, Jizzakh, and Tashkent provinces at elevations ranging from 900 to 1650 m above sea level. It is most frequently found in the Samarkand and Kashkadarya provinces, where its underground parts are predominantly harvested by the local population for medicinal purposes.

Study Area and Plant Material

Samples of *Biebersteinia multifida* DC. were collected from natural populations in the Samarkand and Kashkadarya regions of Uzbekistan at elevations ranging from 550 to 1650 m above sea level. Plant material was harvested from typical habitats of the species, including rocky slopes, clay soils, and saline-like areas.

Additional experimental material was collected within the territory of the Omon-Qo'ton National Park (Urgut District, Samarkand Region). The distribution of the species was specified based on electronic records of the National Herbarium of Uzbekistan, as well as herbarium specimens from the Institute of Biochemistry of Samarkand State University.

After collection, the plants were separated into underground and aboveground organs (roots, stems, and leaves), thoroughly washed, cut into fragments of 1.0-1.5 cm, and air-dried in the shade under natural conditions, avoiding direct sunlight. The dried material was further ground and stored at +4 °C in airtight containers until analysis.

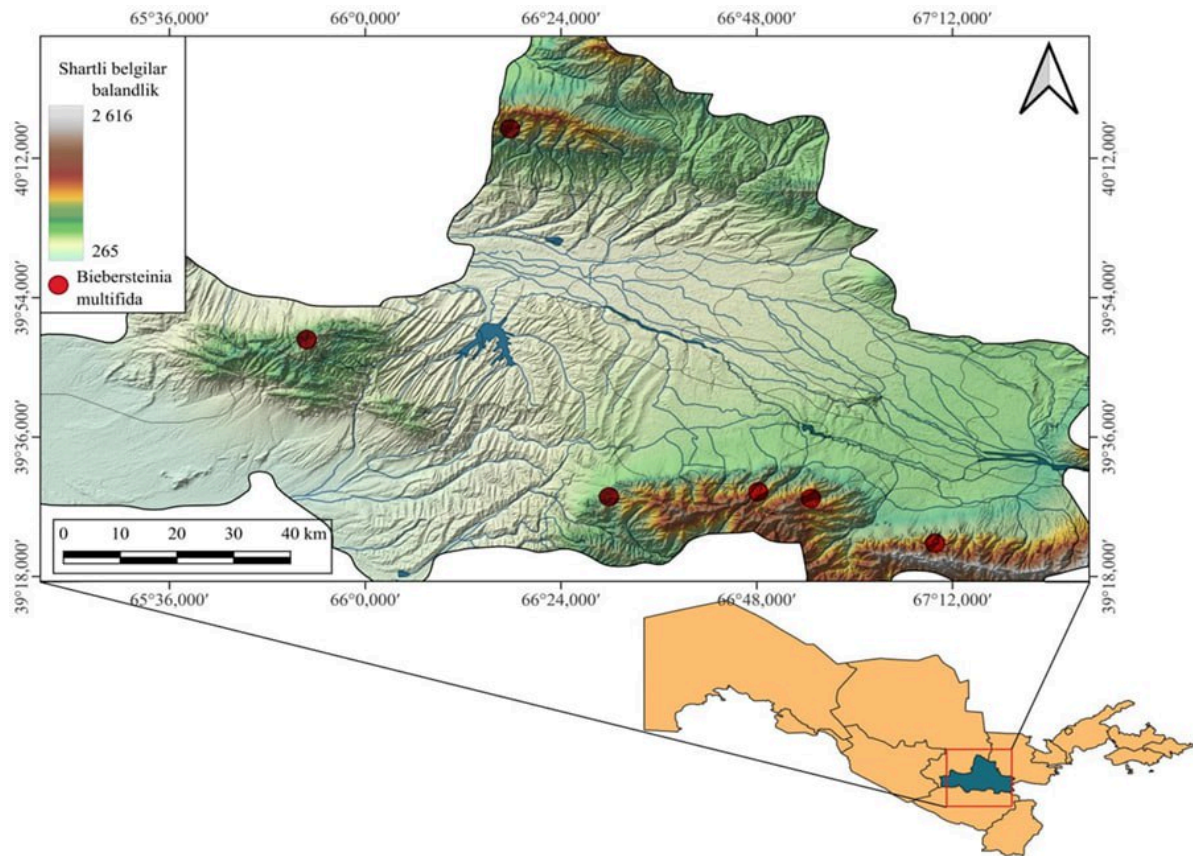


Figure 1. Map of Samarkand region of Uzbekistan - research site.

Ethnobotanical Studies

Ethnobotanical data were collected through interviews with traditional healers, herbal traders, and local residents of the mountainous regions of the Samarkand Province. All interviews were conducted after obtaining oral informed consent from the participants. The study was carried out in accordance with the Code of Ethics of the International Society of Ethnobiology (ISE 2006). Information was recorded on the traditional medicinal uses of the plant, recommended dosages, methods of preparation, and the plant organs utilized in folk medicine.

Preparation of Extracts and Determination of Free Amino Acids

Sample Preparation

Samples of powdered roots and aboveground parts of the plant were extracted with distilled water. The resulting extracts were centrifuged, proteins were precipitated, followed by a second centrifugation, and the supernatant was dried to obtain a dry residue. Free amino acids in the dried samples were derivatized using phenylthiocarbonyl (PTC) reagent to produce stable amino acid derivatives suitable for subsequent analysis.

HPLC Analysis

The analysis of free amino acids was performed at the "Protein and Peptide Chemistry" laboratory of the Institute of Bioorganic Chemistry, Academy of Sciences of Uzbekistan, using a high-performance liquid chromatography (HPLC) system with UV detection.

Chromatographic conditions:

Column: Discovery HS C18 (75 × 4.6 mm);

Mobile phases:

A — 0.14 M CN_3SOONa + 0.05% TEA, pH 6.4;

B — acetonitrile;

Flow rate: 1.2 mL/min;

Detection wavelength: 269 nm;

Elution gradient:

1-6% B (0-2.5 min),
30% B (40 min),
60% B (45-50 min),
0% B (55 min).

Identification of amino acids was performed by comparing retention times with those of standard PTC-amino acids. Quantitative determination was carried out using peak area normalization.

Determination of Water-Soluble Vitamins

B-group vitamins and vitamin C were determined by HPLC following acid extraction and sample filtration. Calibration was performed using certified standards.

Determination of Carbohydrates

The content of monosaccharides (fructose, glucose) and disaccharides (sucrose, maltose) was determined using spectrophotometric and chromatographic methods according to standard protocols for plant material analysis.

Determination of Macro-, Micro-, and Ultra-Trace Elements

The mineral composition of the studied plant organs was analyzed using inductively coupled plasma mass spectrometry (ICP-MS). Sample preparation included digestion of the dried powder with HNO₃ and H₂O₂, followed by dilution to the analytical volume. A total of 44 elements were determined, including Ca, Mg, K, Na, Fe, Mn, Zn, Cu, Li, V, Cr, Co, Ni, Rb, Zr, As, Cd, Re, U, and others.

Nitrogen and Protein Content (Kjeldahl Method)

Total nitrogen content was determined using the classical Kjeldahl method (digestion - distillation - titration). Crude protein content was calculated using the formula: Protein (%) = Nitrogen (%) × 6.25

Determination of Flavonoid Composition

The content of individual flavonoids (gallic acid, dihydroquercetin, rutin, quercetin) was determined by HPLC. Quantitative results were expressed in mg/kg of dry weight. Identification was performed using standard reference compounds.

Statistical Analysis

All experiments were conducted in triplicate. Results are presented as mean ± standard deviation (SD). Statistical analysis was performed using Microsoft Excel and Origin Pro software.

Results and Discussion

Biebersteinia multifida - Perennial; plant villous-hairy, covered with stalked glands, herbaceous; root thickened, often lobate, covered at neck with large brown scarios scales; stems sturdy, often purple below, spreading-branching in upper part, together with inflorescence 15-40 cm high; leaves lanceolate, tri-pinnatisect into linear acute lobes, subsessile, with robust rachis and scarios brown stipules; radical leaves absent. Flowers in racemiform loose inflorescences at apices of stem and branches; bracts obovate, flabellate-pinnatifid, acute, the lower shorter, the upper longer than pedicels; bracteoles close to flower, lanceolate, acuminate; sepals ovate, acute to obtuse, markedly elongating in fruit; petals yellow, nearly orange, 5-8 mm long, slightly shorter or slightly longer than sepals at onset of anthesis, glabrous or hairy-ciliate, obovate or oblanceolate or elliptic, rhombic or broadly cuneate, dissected at apex; filaments glabrous or rarely ciliate; fruit glabrous, rugose. Flowering: April. Fruiting: June. Clayey and hilly slopes and cliffs from the desert to the middle mountain zone; also, in plains but apparently always on saliferous substrata. - Caucasus: South Transcaucasus; Central Asia: Aral-Caspiy, Balkhash (Kosh-Cheku), Syr Darya, Tien-Shan, Pamir-Alai, Mountainous. Turkmenistan. General distribution: Iran (Shishkin 1949) (Fig. 2 a, b; Fig. 3).

At the mid-generative stage, the tuberous root of *Biebersteinia multifida* increases significantly in size, with a mass ranging from 62 to 166 g.

The underground parts of the plant contain carbohydrates and related compounds (33.9%), monosaccharides (2.59%), starch (4.78%), and dextrins (16.6%) (Kurbanov *et al.* 1974). Tannins account for 8.36%, and flavonoids for 0.45%. The aerial parts contain essential oils, triterpenoid saponins, alkaloids (0.06%), and tannins (Sokolov 1988).



a

b

Figure 2. *Biebersteinia multifida*. a - general view of the plant, b - part of the inflorescence.



Figure 3. Underground part of the plant

Species of the genus *Biebersteinia* are widely used in traditional medicine as important medicinal plants. Among the four species of *Biebersteinia*, only *B. multifida* occurs in Uzbekistan, where it is known as Qontepar.

Since ancient times, decoctions prepared from the plant's roots have been used in folk medicine as a hemostatic agent for postpartum hemorrhage and gastrointestinal disorders (Sakhobiddinov 1948). It has also been employed for the treatment of hypertension, flatulence, and radiculitis (lower back pain). Milk-based root decoctions are recommended for respiratory infections, asthma, jaundice, and sexual weakness (Dadabaeva 1996; Khojimatov 2008) (Fig. 4 a, b).

Literature analysis indicates that *Biebersteinia multifida* exhibits significant antimutagenic and anticancer activity: ethanol extracts of the roots inhibited cancer cell proliferation and demonstrated antimutagenic effects in the Ames test (Dabaghian *et al.* 2014). In Iran, the roots are traditionally used to treat inflammatory and musculoskeletal disorders, confirming their pharmacological activity in folk medicine (Farsam *et al.* 2000). Reviews of the *Biebersteinia* species also highlight their extensive use in traditional medicine in China and Iran, emphasizing the pharmacological potential of these plants (Zhang *et al.* 2020).

Biebersteinia multifida is used in Iran as a traditional medicinal agent, including topical ointments for the treatment of musculoskeletal disorders and bone fractures (Farsam *et al.* 2000; Amirghofran *et al.* 2005). Its use has also been reported for the management of nocturnal enuresis in children (Zhang *et al.* 2020).

Extracts of *Biebersteinia multifida* exhibit pronounced antibacterial and antifungal activities (Ahmad & Khan, 2013). Its anti-inflammatory effects have been confirmed *in vivo* (Farsam *et al.* 2000; Jahaniani *et al.* 2005). The analgesic effect of a 50 mg/kg extract dose is comparable to that of 10 mg/kg morphine (Farsam *et al.* 2000).

The anti-fatigue activity of the extract was demonstrated in a swimming test model in mice (Xu *et al.* 2014; Zhang *et al.* 2020). Coumarins such as umbelliferone, scopoletin, and ferulic acid, isolated from the roots, exhibit sedative properties (Jahaniani *et al.* 2005). These findings indicate a high potential of *B. multifida* for the development of anti-inflammatory and analgesic pharmaceutical agents.

During field expeditions to the Samarkand and Kashkadarya regions, ethnobotanical surveys were conducted among medicinal herb collectors and traders, as well as traditional healers and community elders. According to local healers and herb vendors, the rhizome of *B. multifida* has been used for medicinal purposes for many decades. Residents of the Samarkand and Kashkadarya regions utilize the underground parts of the plant (Fig.5 a, b) to treat inflammatory processes of various etiologies, bone fractures, to stop bleeding, as well as for gastrointestinal and respiratory disorders. Furthermore, healers from the Urgut district of the Samarkand region recommend decoctions of *Biebersteinia multifida* roots for treating inflammatory conditions and controlling bleeding in gynecological practice.

Preparation of *Biebersteinia multifida* Root Decoction

1. Root Preparation

Dried roots of the plant are thoroughly washed and then cut into pieces of 1-2 cm or ground into powder. When using fresh roots, they are first washed, cut into small pieces, and lightly dried.

2. Traditional Decoction Method

Take 1-2 teaspoons of the prepared root material.

Pour 1 cup of boiling water (approximately 200 mL) over the roots.

Cover and simmer over low heat for 10-15 minutes, or steep for 30-40 minutes if a decoction is preferred.

After preparation, the decoction is strained and the roots are removed.

3. Usage

Traditionally, the decoction is consumed in doses of 1/3-1/2 cup, 2-3 times per day for: digestive disorders; gastritis and peptic ulcers; rheumatic pain; general weakness or as a tonic.

Occasionally, the decoction is applied externally as a compress for joint and muscle pain.

4. Important Precautions

Dosage is approximate - no precise human data are available; it is recommended to start with small amounts.

Allergic reactions and individual intolerance may occur.

The decoction does not replace conventional medical treatment for serious conditions.

Recommended duration of use is 5-10 days.



a
Figure 4. Root of *Biebersteinia multifida*



a
b
Figure 5. *Biebersteinia multifida* roots: a - in Kitab market (Kashkadarya region) and b -Takhtakaracha pass of Samarkand region.

Local populations acquire the roots of *Biebersteinia multifida* from market vendors or directly from traditional healers, who prescribe them for the treatment of specific ailments.

In our study, we investigated not only the morphobiological characteristics of *B. multifida* but also conducted a comprehensive analysis of its chemical composition. The content of amino acids, carbohydrates, proteins, flavonoids, vitamins, mineral elements, and the plant's antiradical activity were determined.

Vegetative organs of plants grown on experimental plots were subjected to chemical analysis. Samples of roots and aerial parts (stem + leaves) of *B. multifida* were collected both from the experimental plot located within the Omon-Qo'ton National Park (Urgut District, Samarkand Region) and from natural habitats during the flowering phase (Fig. 6 a, b).



Figure 7. Experimental plot of *Biebersteinia multifida* in Omon-Qo'ton National Park.

The collected biomass was carefully cleaned, washed, cut into pieces of 1.0-1.5 cm, and shade-dried away from direct sunlight for 13 days.

Chromatograms of amino acid extracts from the roots and aerial parts of the plant demonstrate a high degree of component separation and a clean baseline, confirming the appropriateness of the derivatization and chromatographic conditions (Figs. 7, 8).

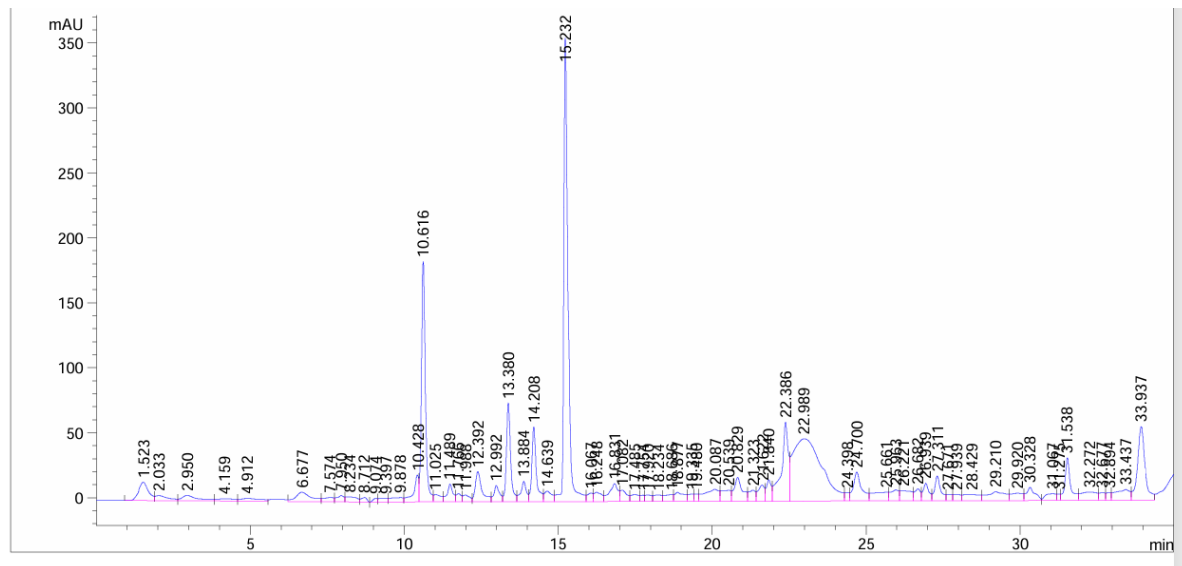


Figure 7. Chromatogram of *Biebersteinia multifida* root extract.

The chromatogram of the root extract is characterized by high peak intensities, particularly in the 10-16 min range. The most prominent peak, with a retention time of approximately 15.22 min, is dominant and significantly exceeds the intensity of the other signals, indicating a high content of one of the major amino acids in the root tissue.

Several intense peaks in the 10-12 min region also indicate substantial concentrations of certain polar amino acids associated with osmoregulation and the biosynthetic processes of the root. The retention times and peak shapes (narrow and symmetrical) suggest good separation and the absence of co-elution. In the 18-33 min range, numerous lower-intensity peaks are observed, likely corresponding to less abundant amino acids or secondary metabolism products.

The chromatogram of the aerial part is characterized by a more uniform distribution of peak intensities: the maximum peak (Rt \approx 15.11 min) is markedly lower than the corresponding peak in the root chromatogram. The overall signal intensity is reduced, consistent with the lower content of certain amino acids in the aerial organs.

The peak profile in the 10-16 min range demonstrates a variety of compounds with moderate intensity, reflecting a more complex amino acid metabolism in the photosynthetic tissues. In the 20-30 min range, a series of well-resolved peaks of medium height is observed, indicating the presence of additional compounds predominantly found in leaves, including possible amino acid derivatives and metabolites associated with stress responses.

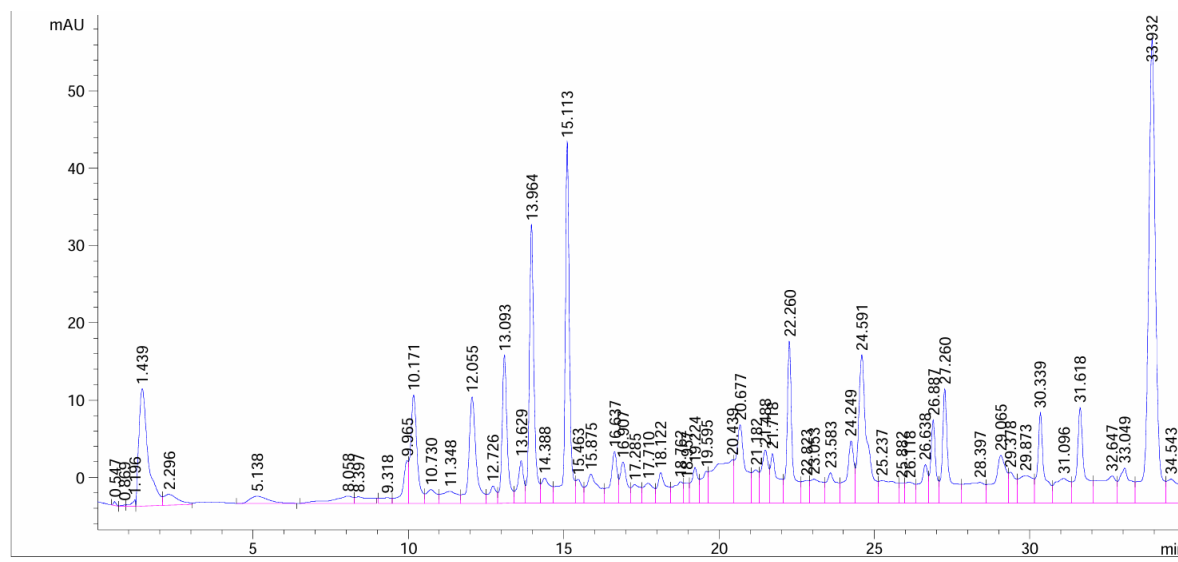


Figure 8. Chromatogram of stem+leaf extract of *Biebersteinia multifida*.

The baseline is smooth, without pronounced artifacts, confirming the absence of interfering impurities and the high quality of the extraction.

Comparison of the two chromatograms reveals pronounced differences between the underground and aerial organs of *B. multifida*:

- The root exhibits significantly higher intensities of major peaks, indicating a high content of certain dominant amino acids.
- The stem and leaves are characterized by greater amino acid diversity at lower concentrations, reflected in more uniform peaks of moderate height.
- The root profile is shifted toward polar amino acids, whereas the aerial part contains a broader range of compounds, including late-eluting, likely less polar metabolites.

These differences correspond to the physiological functions of the organs: roots accumulate amino acids involved in osmoregulation and nutrient metabolism, while leaves and stems contain compounds associated with photosynthesis, growth, and defense responses.

The results of the analysis of the composition and content of free amino acids in stem-leaf samples of *B. multifida* are presented in Table 1.

The total content of free amino acids in the stems and leaves was 3.32229 mg/kg. It was established that in the stem-leaf sample of *B. multifida*, aspartic acid, glutamic acid, cysteine, threonine, arginine, methionine, isoleucine, leucine, histidine, and phenylalanine were present at higher concentrations compared to other amino acids.

Table 1. Composition and content of free amino acids in the stem + leaf sample of *Biebersteinia multifida*

Amino Acid	Concentration, mg/g	No	Amino Acid	Concentration, mg/g
Aspartic acid	0.470847	11	Proline	0.07513
Glutamic acid	0.205581	12	Tyrosine	0.530689
Serine	0.012350	13	Valine	0.267703
Glycine	0.039242	14	Methionine	0.10341
Asparagine	0.037037	15	Isoleucine	0.107793
Glutamine	0.123502	16	Leucine	0.16762
Cysteine	0.139818	17	Histidine	0.15611
Threonine	0.251447	18	Tryptophan	0.058428
Arginine	0.19613	19	Phenylalanine	0.167343
Alanine	0.035351	20	Lysine	0.30523

The results of the analysis of the composition and content of free amino acids in the root sample of *B. multifida* are presented in Table 2.

Table 2. Free amino acids and their quantities in the root sample of *Biebersteinia multifida*

Amino Acid Name	Concentration, mg/g	No	Amino Acid Name	Concentration, mg/g
Aspartic acid	0.765677	11	Proline	0.086788
Glutamic acid	0.04328	12	Tyrosine	0.156437
Serine	0.180779	13	Valine	0.065285
Glycine	0.897497	14	Methionine	0.104877
Asparagine	0.915732	15	Isoleucine	0.016332
Glutamine	1.382491	16	Leucine	0.021557
Cysteine	0.382979	17	Histidine	0.02111
Threonine	0.332194	18	Tryptophan	0.08491
Arginine	0.32102	19	Phenylalanine	0.034281
Alanine	0.632115	20	Lysine	0.01141

According to the data presented in Table 2, all major types of amino acids were detected in the roots of *B. multifida* during the analysis of free amino acids. The total content of free amino acids in the root samples was 6.45564 mg/g, whereas in the stem-leaf organs it was 3.32229 mg/g. Overall, the results indicate that the content of free amino acids in the roots is substantially higher than in the stems and leaves, by 3.13335 mg/g. Higher concentrations of the following amino acids were identified in the roots: aspartic acid, serine, glycine, asparagine, glutamine, cysteine, threonine, arginine, alanine, tyrosine, and methionine.

In addition, the presence of water-soluble vitamins was established in the roots of *B. multifida*, including vitamin B₁ (0.803183 mg/μL), vitamin B₂ (0.01 mg/μL), vitamin B₆ (1.510252 mg/μL), vitamin B₉ (19.67826 mg/μL), vitamin B₁₂ (3.442099 mg/μL), vitamin PP (2.132668 mg/μL), and vitamin C (3.045931 mg/μL).

In roots collected from the experimental field site in the Urgut district of the Samarkand region, the contents of water-soluble vitamins B₁, B₂, B₆, and PP were comparable to those in the stems and leaves. In contrast, vitamins B₁₂ and C were present at higher levels in the stem-leaf organs (6.845809 and 11.1523 mg/μL, respectively), whereas vitamin B₉ was found at a higher concentration in the roots (19.67826 mg/μL).

The composition of macro-, micro-, and ultra-microelements in the root samples of *B. multifida* was determined using inductively coupled plasma mass spectrometry (ICP-MS).

Among the macroelements, calcium and potassium were present in the highest amounts, accounting for 2.4% and 1.01%, respectively. Sodium (0.014%), magnesium (0.17%), aluminum (0.064%), silicon (0.067%), phosphorus (0.36%), and iron (0.033%) were also detected.

Among the microelements, boron, manganese, and zinc were found in relatively high concentrations in the roots, ranging from 2.122 to 6.811 mg/kg. Other microelements, including lithium, vanadium, chromium, cobalt, nickel, copper, rubidium, and zirconium, were present in lower amounts. It was established that the total content of microelements in the roots was higher than that in the stems and leaves.

In addition, the composition of ultra-microelements was determined in the roots. Arsenic (As), niobium (Nb), cadmium (Cd), antimony (Sb), cesium (Cs), rhenium (Re), uranium (U), and other elements were detected in trace amounts. For example, the root samples contained 0.018 mg/kg of As and 0.02 mg/kg of Cd. Most other ultra-microelements were distributed relatively evenly among all plant organs; however, rhenium (Re) was absent in the stems and leaves.

Thus, in the aerial (stems and leaves) and underground (roots) parts of *B. multifida* collected from experimental fields, the following components were identified:

20 free amino acids

44 macro-, micro-, and ultra-microelements

Water-soluble vitamins B₁, B₂, B₆, B₉, B₁₂, PP, and C

Carbohydrates, including fructose, glucose, sucrose, and maltose.

Nitrogen content was determined using the Kjeldahl method, and the protein content in both the roots and the stems with leaves ranged from 10 to 11%.

Among flavonoids, gallic acid, dihydroquercetin, rutin, and quercetin were detected in the roots at concentrations of 0.0, 5.6, 2.8, and 8.3 mg/kg, respectively, while in the stems and leaves their concentrations were 4.7, 25.2, 12.3, and 11.3 mg/kg, respectively.

Conclusion

As a result of the conducted studies, data on the ethnobotanical use, distribution, morpho biological characteristics, and chemical composition of *Biebersteinia multifida* DC. growing in the territory of Uzbekistan were summarized.

It was established that *B. multifida* is widely used in the folk medicine of the Samarkand and Kashkadarya regions under the local name "Qontepar." The primary medicinal raw material is the roots of the plant, which are used in the form of decoctions and infusions for the treatment of inflammatory processes, diseases of the gastrointestinal tract and respiratory system, postpartum and gynecological bleeding, bone fractures, and disorders of the musculoskeletal system. The obtained ethnobotanical data are consistent with literature reports on the use of this species in Iran and other regions of Central Asia, confirming its high cultural and medicinal significance.

Phytochemical analysis revealed the presence of 20 free amino acids in the roots, stems, and leaves of *B. multifida*, with the total amino acid content in the roots (6.46 mg/g) being significantly higher than that in the aerial organs (3.32 mg/g). Water-soluble B-group vitamins (B₁, B₂, B₆, B₉, and B₁₂), vitamin PP, and vitamin C were detected in the plant material, along with carbohydrates such as fructose, glucose, sucrose, and maltose. The protein content in different plant organs ranged from 10 to 11%.

Mineral analysis performed using ICP-MS identified 44 macro-, micro-, and ultra-microelements. Calcium and potassium were the predominant microelements, while boron, manganese, and zinc were the most abundant microelements. The concentrations of toxic ultra-microelements (e.g., As, Cd) were found to be extremely low. The flavonoid complex included gallic acid, dihydroquercetin, rutin, and quercetin, with higher concentrations observed in the aerial parts of the plant.

Thus, *Biebersteinia multifida* represents a promising medicinal plant with a rich chemical composition and confirmed ethnomedicinal significance. However, intensive harvesting of roots from natural populations poses a threat to the sustainability of the species. The obtained results highlight the necessity of developing measures for conservation, rational use, and potential cultivation of *B. multifida* in order to preserve both this biological resource and the traditional knowledge of local communities.

Declarations

Ethics approval and consent to participate: All participants involved in the interview process gave their prior informed oral consent.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable.

Availability of data and materials: The data was not deposited in public repositories but is available from the corresponding author upon request.

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