

Ferula tadshikorum Pimenov introduction, chemical composition and use in folk medicine

D.T. Khamraeva, D.N. Tukhtaeva, O.K. Khojimatov and Rainer W. Bussmann

Correspondence

D.T. Khamraeva^{1*}, D.N. Tukhtaeva², O.K. Khojimatov¹ and Rainer W. Bussmann^{3,4}

¹Tashkent Botanical Garden, Academy of Science of the Republic of Uzbekistan, 100053, Bogishamol str., 232 B, Tashkent, Uzbekistan; khamraeva.dilovar@mail.ru

²Research institute of Forestry Uzbekistan, 111104, Tashkent region, Tashkent district, Uzbekistan; dinora1987@mail.ru

³Department of Botany, State Museum of Natural History, Erbprinzenstrasse 13, 76133 Karlsruhe, Germany. ⁴Department of Ethnobotany, Institute of Botany and Bakuriani Alpine Botanical Garden, Ilia State University,1 Botanical Str., Tbilisi, Georgia

*Corresponding Author: hamraeva.dilovar@mail.ru

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Notes on Ethnobotany

Abstract

Background: This study determined the results of the introduction of the *Ferula tadshikorum*, and comparatively studied the content of proteins and polysaccharides in leaves and roots in three age individuals.

Methods: The work used phenological, protein and polysaccharide chemical analyses, as well as a survey on the use of *Ferula tadshikorum* among local residents of Surkhandarya region of Uzbekistan.

Results: Under the conditions of introduction, phenological observations of the growth and development of *Ferula tadshikorum* were carried out. Based on 4-year experiments, we concluded that, in contrast to the natural conditions, in Tashkent Botanical Garden a shortening of the duration of stages in juvenile and immature individuals, as well as an increase in the number of virginal individuals, is observed in ontogenesis. For the first time, the chemical composition of roots and leaves was studied in three individuals of different ages: 4-year-old introducents of the virginal stage of development, and natural individuals of medium, mature virginal age. The analysis for the content of protein and protein N found that the average protein content in the roots of the *Ferula tadshikorum* plant ranged from 2.3% to 7.12%, while in the leaves it ranged from 10.23% to 25.56%. The roots and leaves of mature virginal individuals of *Ferula tadshikorum* had the greatest nutritional value in terms of total protein content. For the first time, the content of various groups of polysaccharides in the roots and leaves of 3 virginal samples of *Ferula tadshikorum* was elucidated, and alcohol-soluble sugars, water-soluble polysaccharides, pectin substances and hemicellulose were isolated. According to the monosaccharide composition, the roots contained especially to glucogalactaarabans, the leaves arabinogalactans. Some recipes of folk medicine on the use of leaves and resin for the treatment of certain human diseases are given.

Conclusion: For the medicinal plant *Ferula tadshikorum*, new prospects are opening up for the use in both folk medicine and official medicine. In the future, more in-depth phytochemical studies are needed to identify the therapeutic potential of the *Ferula tadshikorum*.

Keywords: chemical analysis, ethnobotany, Ferula tadshikorum, introduction, leaves, root, polysaccharides, proteins.

Background

The genus *Ferula* L. has 180-185 species in Europe, Asia, Africa, Australia, which are found in arid and subarid vegetation and xerophilic scrub, in sandy, salty and stony deserts, and on gypsum slopes. Used frequently in traditional oriental and folk medicine, many species are also essential fodders in arid pastures. Some species are foliar ornamentals (Plunkett *et al.* 2018).

Central Asia and Kazakhstan, with currently 100 species in the genus *Ferula*, are the distribution center of the genus (Pimenov, 2020).

Species of this genus *Ferula* are known to contain essential oils or resinous substances, coumarins, flavonoids, terpenoids, glycosides, and lactones (Su at al., 2000 a; Su at al., 2000 b; Chen *et al.* 2000 a; Chen *et al.* 2000 b; Zhou *et al.* 2000; Chen *et al.* 2001; Tamemoto *et al.* 2001; Duan *et al.* 2002; Tamemoto *et al.* 2002; Shikishima *et al.* 2002; Suzuki *et al.* 2007; Kurimoto *et al.* 2012; Kurimoto *et al.* 2012; Salehi *et al.* 2019; etc.). Most of these compounds are biologically active and can be used as medicines and food supplements.

Many Apiaceae including *Anethum graveolens* L., *Anthriscus nemorosa* (M.Bieb.) Spreng., *Chaerophyllum macropodum* Boiss., *Ferula rigiula* DC., *F. haussknechtii* H.Wolff ex Rech.f., *Heraculum persicum* Desf. *Hippomarathrum microcarpum* (M.Bieb.) B.Fedtsch., *Pimpinella aurea* DC. and *Prangos ferulacea* L. have been studied in relation to macro- and microelements (Ca, Co, Cu, Cr, Fe, K, Mn, Mg, N, Na, P, S and Zn). In addition, plant samples were evaluated for some aspects of food quality (for example, dry matter, total ash content, crude protein content, pH and crude fiber content). Of the above plants, the species *Ferula rigiula* distinguished itself with a particularly high content of crude protein (Tunçtürk & Özgökçe, 2015).

Mamadalieva *et al.* (2018) summarized important information described in the literature from 1959 to 2017, in part on ethnopharmacology, phytochemistry and pharmacological properties of some species of Apiaceae and Rutaceae collected from the Chimgan Mountains (Chatkal Ridge). For some species of Apiaceae, including *Ferula penninervis* Regel et Schmalh., *F. prangifolia* Korovin and *F. tenuisecta* Korovin, the study of the chemical composition, biological characteristics was analyzed, and pharmacological properties were established in the treatment of certain human ailments and diseases. For example, gum (resin) obtained from *Ferula penninervis* is used to treat seizures, tuberculosis, plague, syphilis, whooping cough, toothache, bronchitis, runny nose, flu, colds, diseases of the nervous system; *F. prangifolia* is used in medicines for the treatment of asthma, cough and indigestion, as well as expectorant and anthelmintic agent. Scientists of the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan isolated biologically active compounds from *F. tenuisecta*, on the basis of which the industrial production of the allopathic drugs Tefestrol and Panoferol was supplied. Tefestrol is widely used as an estrogenic drug for ovarian dysfunction, infertility, primary and secondary amenorrhea, and Panoferol has a pronounced estrogenic effect.

Recent studies have shown that *Ferula-assa-foetida* L. has antioxidant properties and can reduce oxidative stress in various human diseases (Ahmadvand *et al.* 2013). The authors studied the essential oils of the leaves, where the main compounds were substances such as Eremophilene, δ -cadinene, Longiborneol, Dehydro aromadendrene, Isoledene, τ -Gurjunene, J-Guaiene. This study showed that this plant has good antioxidant properties. Since it is an easily accessible source of natural antioxidants, such as Eremophilene and δ -cadinene, which can be used in food and pharmaceutical industries.

Sattar & Iranshahi (2017) investigated the phytochemical and biological activity of *Ferula persica* Boiss., as a result, they provided certain indications for the use of this plant in various medicines. Due to the content of umbelliprenin (a prenylated coumarin) in the roots. *Ferula persica* can be used for the development and synthesis of new drugs due to the antidiabetic and antiviral activity of its sesquiterpene coumarins.

The isolated components of essential oils from the aboveground parts of the plant *Ferula assafoetida* included monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes

and other substances (Estekhdami, Dehsorkhi, 2019). The analysis showed that the highest content of components in essential oils included for (E)-1-propenyl sec-butyl disulfide (58.9%), (Z)- β -ocimene (11.9%), (E)- β -ocimene (9.0%), β -pinene (5.0%) and (Z)-1-propenyl sec-butyl disulfide (3.9%).

Literary reports that species of the genus *Ferula* represent a source of various biologically active substances that have a number of pharmacological properties in relation to various diseases and pathogens. However *Ferula tadshikorum* is considered the least studied plant, because data in literature is very insignificant. Earlier, we reported on the ecobiological and morphological-anatomical features of *Ferula tadshikorum* introduced in the Tashkent Botanical Garden, as well as some characteristics of the resin obtained from the roots (Khamraeva *et al.* 2019, 2021, 2022; Khojimatov *et al.* 2021). In addition, there are data in the literature on the chemical composition and biological activity of essential oils obtained from roots (Sharopov *et al.* 2018). According to the study of acute toxicity of dry gum resin extract of *Ferula tadshikorum* in doses from 50 to 4000 mg/kg of body weight in rats, it was found that the resin is not a toxic substance, since it did not cause the death of animals and can be classified as a hazard class - low-hazard substances (Khodzhaev *et al.* 2020).

The purpose of this study was to identify the effect of the introduction of *Ferula tadshikorum* in the conditions of the Tashkent Botanical Garden on the chemical composition of roots and leaves, as compared to natural individuals and in introducers, as well as to outline prospects for its use in folk medicine.

Materials and Methods

Ferula tadshikorum belongs to the family Apiaceae. The plant has been used since ancient times as a medicinal drug due to the resin obtained from the roots. In Uzbekistan the species grows in two regions - Surkhandarya and Kashkadarya regions. It grows in the middle mountain belt on loess and fine-grained-gravelly slopes, and limestones, along dry river valleys and terraces, at an altitude of 1400-1800 m above sea level.

Ferula tadshikorum seeds were sown at the experimental sites of the laboratory for the Introduction of medicinal plants of the Tashkent Botanical Garden in late November - early December 2018. In 2022, the fourth year of growth phenological observations were carried out according to methodological guidelines (Rabotnov 1950a,b). The soil and climatic conditions of the Tashkent Botanical Garden have been outlined inK hamraeva *et al.* (2019, 2022).

Ethnobotanical research

A survey of the local population of Surkhandarya region was conducted in the form of a questionnaire with the consent of the respondents. The interviews were conducted in accordance with the rules of the Code of Ethics established by the International Society of Ethnobiology (ISE Code of Ethics, 2006).

Material for chemical experiments

Leaves and roots of virginal plants of the following age were taken for chemical analyses:

- young virginal plants of 4 years of life, grown under the conditions of introduction in the Tashkent Botanical Garden;

- middle-aged 12-14-year-old and mature 24-27-year-old virginal plants collected in 2022 in Babatag district of Surkhandarya region.

Determination of protein sum content in roots and leaves of *Ferula tadshikorum* was determined after preliminary mineralization of the sample with sulphuric acid followed by determination of protein nitrogen with Nessler's reagent. For protein extraction, biological material was crushed until the cell walls were destroyed, producing a homogenate. From the prepared samples, samples were taken into heat-resistant flasks, concentrated sulfuric acid H₂SO₄ (1.84 g/cm) was added and decomposed on an electric hotplate, avoiding rapid boiling. The end of the mineralization process was to obtain a completely transparent colorless solution. In the prepared samples the protein content was determined by colorimetric method with Nessler reagent at 400 nm (Ermakov and Arasimovich, 1982).

Paper chromatography was performed using Filtrak-FN 13, 18 paper (Germany) in solvent system: n-butanol-

pyridine-water (6:4:3). The manifestants: 1) acidic aniline phthalate (5 min, 100°C), 2) 5% urea solution.

The isolation of polysaccharides was carried out according to the generally accepted methodology (Methods of carbohydrate chemistry, 1967).

Gas chromatography: The hydrolysates were analyzed on a GC Plus2010 chromatograph under the following conditions: injector temperature 250°C, total flow 60ml/min, flow through the column 0.89ml/min, carrier gas nitrogen, column-Rxi-624SI MS, column length 3 m, inner ID 0.25mm, column temperature 230°C, detector temperature 250°C.

Complete acid hydrolysis of polysaccharides was carried out with 2 n H_2SO_4 at 100 °C, water-soluble polysaccharides for 8 hours, pectin's and hemicelluloses for 24 hours, respectively. The hydrolysates were neutralized with barium carbonate, deionized with CU-2 cationic exchange resin (H+), evaporated to syrup and chromatographed.

Preparation of aldonononitrile acetates. The dry hydrolysate residue was dissolved in 2 mL pyridine, 100 mg hydroxylamine hydrochloride was added and heated at 90°C for 1 hour. The reaction mixture was cooled, 2 mL acetic anhydride was added and heated at 90°C for 1 hour. Then 30 mL water was added, stirred and extracted with chloroform, the extraction was carried out twice. The chloroform extracts were combined, treated with anhydrous Na₂SO₄, evaporated and analyzed by gas chromatography (Ermakov and Arasimovich, 1982; Azizov *et al.* 2021).

Inactivation of the raw material: 100 g of crushed raw material (root, leaves) was treated twice with boiling methanol - chloroform (1:1) to remove colorants and non-carbohydrate components, the rest of the raw material was extracted twice with boiling 82° ethanol for 2 hours. The alcohol solutions were combined, evaporated and analyzed by paper chromatography in a solvent system (n-butanol-pyridine-water), identifying glucose (acidic aniline phthalate) and traces of fructose (5% urea solution).

Extraction of water-soluble polysaccharides: The rest of the raw material was extracted with hot water at 90°C at hydro-modulus (1:5 and 1:3). The extracts were separated by filtration, combined, evaporated to a small volume and precipitated with twice the volume of alcohol. The precipitate was separated by centrifugation, the precipitate was washed and dried with alcohol. The yield of water-soluble polysaccharides ranged from -3.5 g to 10 g.

Extraction of pectin substances. After extraction with water the rest of the raw material was treated with an equal volume of 0.5% oxalic acid and ammonium oxalate solutions (1:10) at 70°C, twice for 3 h. The extracts were combined, dialyzed, evaporated and precipitated with twice the volume of alcohol. The precipitate was separated and treated as described above. The yield of the pectin substances was between 1.6 g and 5 g.

Extraction of the hemicellulose. The rest of the raw material was extracted with a 5% alkali solution (1:5) at room temperature for 4 hours twice. The alkaline extract was neutralized with CH₃COOH and dialyzed against running water, evaporated and precipitated with alcohol (1:2). The precipitate was separated, washed and dried. The yield of the hemicellulose was 2.1 g to 6.0 g.

Results and Discussion

Introduction of Ferula tadshikorum

This study summarizes the results of 4-year observations on the study of the ontogenesis of the promising medicinal plant *Ferula tadshikorum* in the conditions of the Tashkent Botanical Garden at the Institute of Botany of the Academy of Sciences of Uzbekistan.

In 2022, the months of January and February were wetter and cooler than in previous years of observations, and plants began to appear only from the third decade of February. In this growing season, in general, all individuals remained viable. As it was noted in previous works, all plants of different ages of the fourth growing season also have scaly leaves, juvenile individuals are among one, and the remaining individuals are 1-3 (Khamraeva *et al.* 2019, 2022).

In the experimental territory, immature plants were the dominant individuals accounting for almost 70-75% of plants. Juvenile and virginal plants had a slightly different ratio in the number of individuals, the first age with about 9%, the second 16% of the total number of experimental plants.

In juvenile plants, 1-2 rosette leaves were formed on the rosette shoot (Fig. 1 a). According to the structure of the leaf and underground organs, juvenile individuals were similar to the same individuals of previous years, and the size of the leaf was almost equal to the individuals of the third growing season. The leaf reached 23-29 cm in length, the leaf blade was 11-17 cm long, 4-4.5 cm wide, the petiole 11-15 cm long (Fig. 1a).

In immature individuals, the diversity of leaves was similar to plants of the third growing season. These individuals had either triple-dissected or 5-6-lobed leaves, or one plant had two varieties of leaves, and lacked simple leaves (Fig. 1b). Triple-dissected leaves with ellipsoidal lobesshowed lobes 17-23 cm long, 4.5-6.5 cm wide, and petioles 12-16 long. 5-6-lobed leaves had ellipsoidal or broadly ellipsoidal segments, with leaves up to 48 cm long, segments 16-22 cm long, 4-6 cm wide, primary segments on short petioles, the rest sessile, petiole up to 22 cm long. Naturally, the root system of these plants also became deeper and somewhat enlarged. In virginal plants, pinnately dissected leaves were formed once or twice in the rosette and were 56-72 cm long (Fig. 1 in). The leaf blade was broadly triangular in outline, the leaf segments elongated-oval, broadly ellipsoidal, 8-31 cm long, 3-7 cm wide, the primary segments on short petioles the rest sessile, the petiole 14-20 cm long.

Virginal plants were characterized by a deep-rooted root system (Fig. 1 g). The main root penetrated deep into the soil, the thickened as is more than 50 cm long, spindle-shaped, the bark dark brown, the diameter in the thickest zone 3.5-4 cm. The thin apical part, due to deep penetration into the soil, as difficult to dig out. The apical part of the root retained remnants of petioles of last years' leaves. Virginal individuals had a single more or less thickened lateral root. The main root accumulates organic substances, from which gum-resin can be obtained in the future. Plants go to rest starting from the end of May and for a long time until mid-June.

According to the results of four growing seasons, juvenile plants had only simple leaves, immature individuals two varieties of leaves, trifoliate or 5-6-lobed, and virginal plants once or twice pinnately dissected leaves in different numbers, accompanied by an increase in the size of the root system. As we have noted in previous work (Khamraeva *et al.* 2019, 2022), under the conditions of introduction, the phases of ontogenesis development in *Ferula tadshikorum* passed much faster compared to natural specimens. Experiments on the cultivation of *Ferula tadshikorum* and *Ferula foetida* (Bunge) Regel in the Arnasai district of the Jizzakh region were studied (Halkuzieva *et al.* 2022).

According to our results, *Ferula tadshikorum* plants entered the immature period in the third year of ontogenesis, and the virginal phase of development was observed in the fourth year of life. However, our studies in the Tashkent oasis showed a faster rate of development of ontogenetic stages in *Ferula tadshikorum*, which were associated with greater moisture content of the soil and atmospheric air, while the Arnasai district is characterized by a sharply dry arid climate.

Protein content of *Ferula tadshikorum*. According to our data, the average protein content in the roots of *Ferula tadshikorum* ranges from 2.3% to 7.12%, while in the leaves it can reach up to 25.56%. That is, the roots and leaves of mature virginal individuals of *Ferula tadshikorum* have the highest nutritive value in terms of total protein content (Table 1).

Polysaccharides of Ferula tadshikorum

In the results of the study to identify the content of different groups of polysaccharides in roots and leaves of 3 virgin samples of *Ferula tadshikorum*, alcohol soluble sugars (ASS), water-soluble polysaccharides (WSPs), pectin substances (PS) and hemicellulose (HMC) were identified (Table 2). By chromatographic analysis it was identified that ASS consisted of fructose, glucose and sucrose.

As it is seen from Table 2 in leaves of a plant *Ferula tadshikorum* water-soluble polysaccharides were dominating and ranged from 4.2 to 10.0 % (from air-dry raw material). The basic monosaccharides were galactose and arabinose. Water-soluble polysaccharides, according to paper chromatography, included uronic acid in addition to neutral monosaccharides. Pectins and hemicelluloses were also characterized, with a higher content of arabinose and xylose. In the water-soluble polysaccharides extracted from the root, the predominant monosaccharides were arabinose, glucose and small amounts of galactose. Pectin substances included glucose, arabinose and galactose. The monosaccharide composition of hemicellulose is characterized by the main sugars, xylose and arabinose. It should be noted that high amounts of glucose, arabinose and xylose were present in all samples. Monosaccharides in the roots belonged to glucogalactaarabans and the leaves to arabinogalactans.

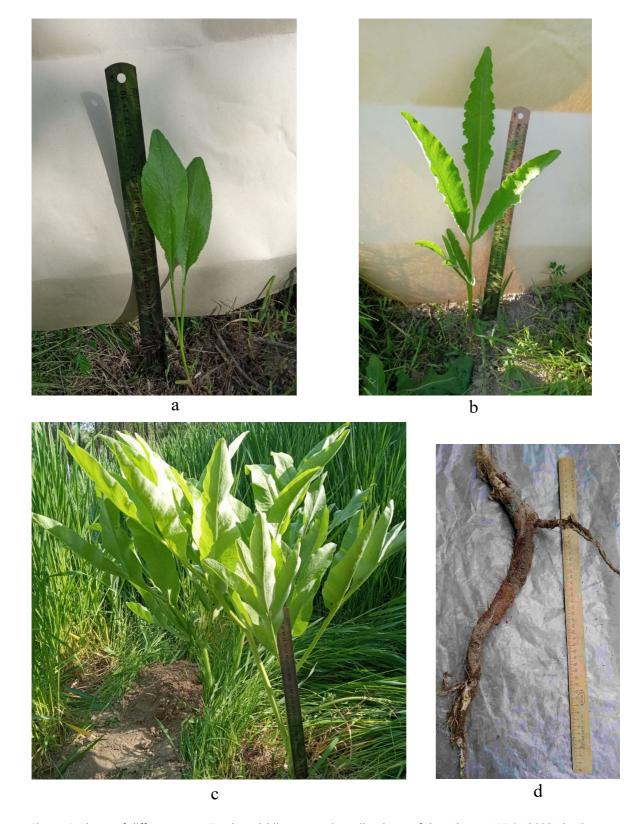


Figure 1. Plants of different ages *Ferula tadshikorum*. a - juvenile plants of the 4th year (15.04.2022), b - immature plant of the 3rd year (15.04.2022), c - virginal plant of the 2nd year (15.04.2022), d - underground organs of the virginal plant of the 2nd year (15.04.2022).

Age of virginal plants/organ studied		Protein %, number of trials	Average protein content %	Protein N content %		
4 years old		1,81	2,3	0,36		
-		2,80				
12-14 years		4,9	5,0	0,80		
		5,10				
24-27 years	s	6,49	7,12	1,16		
	rots	7,74				
4 years old		9,24	10,23	1,55		
		11,22				
12-14 years		15,59	16,39	2,62		
		17,18				
24-27 years	leaves	24,40	25,56	4,00		
	lea	26,72				

Table 1. Total protein content of roots and leaves of *Ferula tadshikorum*

Table 2. Content and monosaccharide composition of roots and leaves of Ferula tadshikorum

Age of virginal plants/organ studied		Туре	Yield %	Monosaccharide composition						UAc
		carbohydrates		Gal	Glc	Man	Ara	Xyl	Rha	
4 years		WSPs*	3,5	2,2	3,3	1,0	2,0	1,5	-	+
		PS	2,7	5,0	7,0	1,0	4,0	2,0	1,0	+
		НМС	4,4	-	2,9	1,0	1,8	2,6	-	+
12-14		WSPs	5,6	2,5	2,5	1,0	5,0	1,0	1,5	+
years		PS	3,8	4,0	4,0	1,3	7,0	4,0	1,0	+
		НМС	4,7	1,1	1,2	1,1	1,7	3,3	1,0	+
24-27		WSPs	3,9	2,3	2,1	1,4	2,7	1,3	-	+
years st	ts	PS	5,0	4,6	6,3	1,1	5,1	2,5	1,7	+
	5	НМС	5,4	1,7	1,5	1,3	2,1	5,4	1,3	+
4 years		WSPs	4,2	6,2	1,0	1,1	3,7	1,5	-	+
		PS	4,5	5,0	1,5	-	5,0	-	1,0	+
		НМС	6,0	1,8	1,0	-	7,1	4,3	-	+
12-14		WSPs	10,0	1,7	1,3	1,0	3,3	1,0	-	+
years		PS	3,2	3,0	1,0	-	4,0	2,0	-	+
		НМС	4,1	1,0	1,5	-	3,7	6,2	-	+
24-27	s	WSPs	6,0	3,7	1,8	1,2	4,4	4,5	1,0	+
years	leaves	PS	1,6	1,7	1,2	-	1,0	1,0	-	+
	le	НМС	2,1	2,5	1,0	-	1,2	7,7	-	+

^{*}WSPs - water-soluble polysaccharides, PS - pectin substances, HMC - hemicellulose, Gal - galactose, Glc - glucose, Man - mannose, Ara - arabinose, Xyl - xylose, Rha - Rhamnose, UAc - uronic acid.

Ethnobotany

Ferula tadshikorum has been used for many years by locals to treat digestive disorders. For this purpose, the freshly grown leaves of this plant are cut and dried in early spring and then drunk on an empty stomach, added to sour milk or suzma. This activates gastric secretion and improves its function. The resin of the plant is also used in the treatment of colds, severe coughs, chronic bronchitis, and to improve immunity. To do this, dissolve some resin in warm water and drink after meals 2-3 times a day. *Ferula tadshikorum* resin is also used for toothache. A tincture is used to treat foot pain. Preferably the roots of medium-aged virginial plants are harvested, then cleaned, cut into small pieces and placeed it in a dark glass container and covered with alcohol. This tincture should be kept in a dark place for 10 days.

According to Rakhimov (2010), *Ferula tadshikorum* is a fodder and food plant, and the local population of Tajikistan harvests its leaves for livestock feed in autumn and winter. In Uzbekistan, residents of Surkhandarya region also harvest hay from *Ferula tadshikorum* leaves for future use. The fodder value of the leaves is confirmed by the data we have established on the high protein content in the leaves of mature virginal individuals.

Conclusions

The introduction of *Ferula tadshikorum* in the conditions of the Tashkent Botanical Garden is proceeding satisfactorily with a reduction in juvenile and immature ontogenetic age conditions in some individuals. In the future, our results can serve as a foundation for the creation of plantations in the Tashkent region and other similar climatic territories of the republic.

For the first time, we determined the content of total protein and polysaccharide composition of leaves and roots in 3 virginal samples of *Ferula tadshikorum*. The total protein content in the roots reached up to 2-7%, and in the leaves up to 9-23%, and the highest content was found in virginal plants of 24-27 years of age. According to the results of our experiments, the dominant polysaccharide was water-soluble polysaccharides. The content of various groups of polysaccharides showed that water-soluble polysaccharides in leaves reached up to 10% and roots up to 5.6% in middle-aged virginal plants.

It can be concluded that the older the age of plants, the higher the protein content they had. For medicinal purposes it is desirable to harvest the leaves of mature virginal plants, characterized by the greatest nutritional value. Polysaccharides are most abundant in individuals of the middle-aged virginal stage of development, which is consistent with the fact that it is in this age group of plants that gum resin is collected for medicinal purposes.

Declarations

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

Consent for publication: Not applicable.

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

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

Authors' contributions: D.T. Khamraeva, D.N. Tukhtaeva, O.K. Khojimatov collected and analysed the data and drafted, the initial manuscript. R.W. Bussmann critically revised the manuscript. All authors contributed in the research, data collection, and approved the final manuscript.

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