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DEVELOPMENT OF A METHOD FOR OBTAINING A FLAVONOID COMPLEX FROM THE AERIAL PART OF *FERULA SONGARICA* PALL. EX SPRENG. WITH ANTIOXIDANT ACTIVITY

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Abstract. This study presents the results of optimizing a methodology for the extraction of the flavonoid complex from the aerial parts (stems and leaves) of *Ferula songarica* Pall. ex Spreng., collected in the East of Kazakhstan during the flowering stage.

The quality indicators (moisture – 8.15%, total ash – 7.39%, ash insoluble in HCl – 0.29%) of plant materials were established according to the methods of the State Pharmacopoeia of the Republic of Kazakhstan. It was revealed that the extracts contain flavonoids by thin-layer chromatography in appropriate solvent systems using specific reagents. The optimal conditions for extracting the total flavonoid content from this raw material were determined: extractant – 50–70% ethyl alcohol; raw material-extractant ratio – 1:15; extraction time – 24 hours at room temperature using the maceration. The flavonoid yield under these conditions was 11.2–12.1%.

The antioxidant activity of water-ethyl extracts obtained from the aerial part of *Ferula songarica* Pall. ex Spreng. in the following concentrations of 0.25, 0.5, 0.75, and 1 mg/ml, respectively, was studied in vitro using the Ferric Reducing/Antioxidant

Power assay (FRAP). The concentration dependences of the optical density values for plant extracts were obtained in comparison with the standard – ascorbic acid (AA). The sequence of increasing antioxidant properties was revealed in the series: 50% ethyl extract > AA > 70% ethyl extract > 90% ethyl extract, while the activity of 50% water-ethyl extract exceeded the standard.

This is the first study on developing an antioxidant flavonoid complex from *Ferula songarica* Pall. ex Spreng.

Keywords: *Ferula songarica* Pall. ex Spreng., aerial part (stems and leaves), maceration, flavonoids, spectrophotometry, antioxidant activity (*in vitro*).

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АНТИОКСИДАНТТЫҚ БЕЛСЕНДІЛІККЕ ИЕ *FERULA SONGARICA* PALL. EX SPRENG. ЖЕР ҮСТІ БӨЛІГІНЕН ФЛАВОНОИДТЫ КЕШЕНДІ АЛУ ӘДІСІН ӘЗІРЛЕУ

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Аннотация. Мақалада Ferula songarica Pall. ex Spreng. өсімдігінің жер үсті бөлігінен (сабақтары мен жапырақтары) флавоноидты кешенді алудың оңтайлы әдістемесін әзірлеу нәтижелері келтірілген. Шикізат Шығыс Қазақстан облысында гүлдеу фазасында жиналды.

Қазақстан Республикасы Мемлекеттік фармакопеясының әдістемелері бойынша өсімдік шикізатының қатерсіздігінің көрсеткіштері (кептіру кезінде массаның жоғалуы – 8.15%, жалпы күлділігі – 7.39%, HCl-де ерімейтін күл – 0.29%) белгіленді. Арнайы реагенттерді қолдана отырып, тиісті еріткіш жүйелерінде жұқа қабатты хроматография әдісімен сығындыларда флавоноидтар бар екендігі анықталды. Жіңішке қабатты хроматография әдісімен сәйкес еріткіш жүйелерінде және арнайы реагенттерді пайдалана отырып жүргізілген зерттеулер нәтижесінде экстракттардың құрамында флавоноидтар бар екені анықталды. Зерттелген өсімдік шикізатынан флавоноидтар мөлшерін мацерация әдісімен

экстракциялаудың оңтайлы шарттары анықталды: экстрагент – 50-70% этил спирті; шикізат пен экстрагенттің қатынасы – 1:15; экстракция уақыты – бөлме температурасында 24 сағат. Осы шарттарда флавоноидтардың шығымы 11,2-12,1% құрады.

Ferric Reducing/Antioxidant Power assay (FRAP) *in vitro* темірді қалпына келтіру потенциалын анықтау әдісімен *Ferula songarica* Pall ex Spreng. Өсімдігінің жер үсті бөлігінен алынған су-спирт экстракцияларының антиоксиданттық белсенділігі келесі концентрацияларда 0.25, 0.5, 0.75 және 1 мг/мл.зерттелді. Стандартты үлгі – аскорбин қышқылымен (АҚ) салыстырғанда өсімдік сығындылары үшін оптикалық тығыздық мәндерінің концентрациялық тәуелділіктері алынды. Қатардағы антиоксиданттық қасиеттердің өсу реттілігі анықталды: 50% этил сығындысы >АҚ > 70% этил сығындысы > 90% этил сығындысы, ал 50%-дық су-этанол сығындысының белсенділігі стандарттан асып түсті.

Осы зерттеу алғаш рет антиоксиданттық белсенділікке ие Ferula songarica Pall. ex Spreng. жер үсті массасынан флавоноидтық кешен алу әдісін әзірлеуге арналды.

Түйін сөздер: *Ferula songarica* Pall. ex Spreng., жер үсті массасы (сабақтар мен жапырақтар), мацерация, флавоноидтар, спектрофотометрия, антиоксиданттық белсенділік (*in vitro*).

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РАЗРАБОТКА СПОСОБА ПОЛУЧЕНИЯ ФЛАВОНОИДНОГО КОМПЛЕКСА ИЗ НАДЗЕМНОЙ МАССЫ *FERULA SONGARICA* PALL. EX SPRENG., ОБЛАДАЮЩЕГО АНТИОКСИДАНТНОЙ АКТИВНОСТЬЮ

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Аннотация. В статье приведены результаты по разработке оптимальной методики получения флавоноидного комплекса из надземной части (стеблей и листьев) *Ferula songarica* Pall. ex Spreng., заготовленная в Восточно-Казахстанской области в фазу цветения.

По методикам Государственной Фармакопеи Республики Казахстан установлены показатели доброкачественности (потеря в массе при высушивании – 8.15%, общая зола – 7.39%, зола нерастворимая в HCl – 0.29%) растительного сырья. Методом тонкослойной хроматографии в соответствующих системах растворителей, с использованием специфических реагентов выявлено, что экстракты содержат флавоноиды. Определены оптимальные условия экстракции методом мацерации суммы флавоноидов из исследуемого растительного сырья: экстрагент – 50-70%-ный этиловый спирт; соотношение сырье-экстрагент – 1:15; время экстракции – 24 часа при комнатной температуре. Выход флавоноидов при данных условиях составил 11,2-12,1%.

Методом определения железо-восстанавливающего потенциала Ferric Reducing/Antioxidant Power assay (FRAP) *in vitro* исследована антиоксидантная активность водно-спиртовых извлечений, полученных из надземной части *Ferula songarica* Pall. ex Spreng. в следующих концентрациях 0.25, 0.5, 0.75 и 1 мг/мл соответственно. Получены концентрационные зависимости значений оптической плотности для растительных экстрактов в сравнении со стандартным образцом – аскорбиновой кислотой (АК). Выявлена последовательность возрастания антиокислительных свойств в ряду: 50%-ный этиловый экстракт >АК> 70%-ный этиловый экстракт > 90%-ный этиловый экстракт, при этом активность 50%-ного водно-этанольного экстракта превышает стандарт.

Данное исследование по разработке флавоноидного комплекса из надземной массы *Ferula songarica* Pall. ex Spreng., обладающего антиоксидантной активностью проведено впервые.

Ключевые слова: *Ferula songarica* Pall. ex Spreng., надземная часть (*стебли и листья*), мацерация, флавоноиды, спектрофотометрия, антиоксидантная активность (*in vitro*).

Introduction. Currently, one of the key directions in the development of the pharmaceutical industry in the Republic of Kazakhstan is the production of drugs derived from local raw materials through the study of natural resources with renewable industrial reserves. The flora of Kazakhstan comprises approximately 6,000 species of vascular plants, yet only 273 are officially recognized as medicinal and are used in both traditional and official medicine. One promising species is *Ferula songarica* Pall. ex Spreng., which belongs to the genus *Ferula* L. (family *Apiaceae* Lindl.). This plant is widely distributed across Kazakhstan, Western Siberia, Mongolia, and China. In local folk medicine, it has been traditionally used to treat aches, colds, and stomach disorders (Pavlov, 1963).

The chemical composition of *Ferula songarica* remains insufficiently studied. Previous research has identified essential oils, mono- and sesquiterpenoids, as well as esters of phenols and terpenoids, in its roots and aerial parts (Nazhimitdinova, et al., 1993; Turdieva, et al., 2022; Khosnutdinova, et al., 2023). The study of this species as a herbal medicinal raw material for its further use in pharmacy and medicine is highly relevant. The purpose of this study is to develop an optimal method for obtaining

flavonoids from the aerial part of *Ferula songarica* Pall. ex Spreng., and to study their antioxidant activity.

Materials and methods

The object of this study is the aerial parts (stems and leaves) of *Ferula songarica* Pall. ex Spreng., collected during the flowering phase in the East Kazakhstan region, Kazakhstan (2023). Raw materials were naturally dried under a canopy, protected from direct sunlight, until air-dry. The raw materials were ground to a particle size of 2 mm. Only carefully sorted, dried, ground, and sifted raw materials were used in all experiments.

The quality indicators of plant raw materials (moisture, total ash content, and ash insoluble in 10% HCl) were determined according to the methods described in the State Pharmacopoeia of the Republic of Kazakhstan (SF RK) (Tulegenova, 2008).

The qualitative composition of the extracts for flavonoids was determined using standard methods with the following reagents: NH_3 vapor, a 3% ethanol solution of $AlCl_3$, a 2% ethanol solution of $FeCl_3$, and a 2% solution of $Pb(CH_3COO)_2$ (Grinkevich, 1983). Thin-layer chromatography (TLC) was performed on Sorbfil plates (Russia) measuring 7 × 10 cm, and paper chromatography was carried out in organic solvent systems: (I) n-butanol – concentrated acetic acid – water, (II) butanol – concentrated acetic acid – water, (III) 6% acetic acid. After drying, the plates were treated with UV light, NH_3 vapor, and a 3% ethanol solution of $AlCl_3$.

Solvent mixtures of ethanol and distilled water (50%, 70%, and 90%) were used for extraction, with a raw material-extractant ratio of 1:10, 1:15, and 1:20. Extraction was carried out for 6 to 48 hours at room temperature.

The quantitative determination of flavonoids in the extracts was performed using a method described in the State Pharmacopoeia of the USSR (XI) (Ananyev, 1987). The spectral characteristics of the water-ethanol extracts were evaluated using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies). The test solution was prepared as follows: approximately 2 grams (accurately weighed) of the crushed raw material was placed in a 150 ml flask with a ground joint, and 30 ml of 90% ethanol containing 1% concentrated hydrochloric acid was added. The flask was connected to a reflux condenser, heated in a boiling water bath for 1 hour, then cooled to room temperature and filtered through a paper filter into a 100 ml volumetric flask. The extraction was repeated twice using the same procedure. The combined filtrates were washed with 90% ethanol, and the final volume was adjusted to the mark with the same solvent (solution A).

A 2 ml aliquot of solution A was placed in a 25 ml volumetric flask, followed by the addition of 1 ml of a 1% aluminum chloride solution in 95% ethanol. The volume was then adjusted to the mark with the same solvent. After 20 minutes, the optical density of the solution was measured using a spectrophotometer at a wavelength of 430 nm in a cuvette with a 10 mm path length.

As a reference solution, a mixture containing 2 ml of solution A, diluted to the mark with 95% ethanol in a 25 ml volumetric flask was used.

The total flavonoid content, expressed as quercetin and in terms of absolutely dry raw material (x, %), was calculated using the following formula:

$$x = \frac{D * 100 * 100 * 25 * 100}{764.6 * m * 2(100 - W)}$$

where D is the optical density of the test solution;

764.6 - specific absorption coefficient of the quercetin-aluminum chloride complex at 430 nm;

W - loss on drying of raw materials;

M - weight of the raw material (g).

The antioxidant activity of water-ethanol extracts was studied by determining the iron-reducing potential of FRAP (Ferric Reducing/Antioxidant Power assay) *in vitro*. Ascorbic acid was used as the standard of comparison.

2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% solution of potassium hexacyanoferrate (III) were added to 1 ml of the studied extract in the concentration range of 0.1 mg/ml. The reaction mixture was incubated for 25 minutes at a temperature of 50°C, and then the reaction was stopped by adding 2.5 ml of a 10% solution of trichloroacetic acid. The mixture was centrifuged for 3 minutes (1500 rpm). An aliquot of 2.5 ml from the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. Optical density was measured using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, USA) at a wavelength of 700 nm (Benzie, et al., 1996).

Statistical processing of the obtained data was carried out using the Statistica 10 software package (StatSoft, USA). The results are presented in the form X±m, where X is the average value, m is the standard error of the average. Repetition n=5.

Results and Discussion

The indicators of the quality of aerial raw materials of *Ferula songarica* Pall. ex Spreng. are within the permissible limits according to the SF RK and are as follows: loss on drying – 8.15%, total ash – 7.39%, insoluble ash in HCl – 0.29%.

Qualitative analysis of the flavonoid content of water-ethyl extracts (50, 70 and 90%) were carried out by chromatography in a thin layer (TLC) in a solvent system: *n*-butanol - concentrated acetic acid – water (5:1:1) and by two–dimensional paper chromatography in systems: butanol – concentrated acetic acid – water (40:12.5:29) and 6% acetic acid using specific reagents.

The values of retention factor (Rf) were calculated for each sample, after the detection of characteristic staining by exposure to NH_3 vapors (yellow), 3% alcohol solution $AlCl_3$ (yellow), and UV light (dark glow). This indicated the presence of flavonoids. Chromatography revealed 2 characteristic stains. The results are presented in Table 1.

Table 1. Results of chromatography of water-ethyl extracts of the aerial part of *Ferula songarica* Pall. ex Spreng.

Substance No.	Reagent	Stains	Rf		
			I	II	III
50% water-ethyl extract					
1	3% alcohol solution $AlCl_3$	Yellow	0.30	0.60	0.33
	NH_3 vapors	Yellow	0.30	0.60	0.33
2	3% alcohol solution $AlCl_3$	Yellow	0.48	0.43	0.64
	NH_3 vapors	Yellow	0.48	0.43	0.64

70% water-ethyl extract					
1	3% alcohol solution $AlCl_3$	Yellow	0.30	0.59	0.31
	NH_3 vapors	Yellow	0.30	0.59	0.31
2	3% alcohol solution $AlCl_3$	Yellow	0.48	0.51	0.67
	NH_3 vapors	Yellow	0.48	0.51	0.67
90% water-ethyl extract					
1	3% alcohol solution $AlCl_3$	Yellow	0.30	0.59	0.30
	NH_3 vapors	Yellow	0.30	0.59	0.30
2	3% alcohol solution $AlCl_3$	Yellow	0.48	0.40	0.62
	NH_3 vapors	Yellow	0.48	0.40	0.62

It was found that 50, 70, and 90% water-ethyl extracts of the aerial part of *Ferula songarica* Pall. ex Spreng. contain flavonoids. Moreover, the extracts contain both aglycones and glycosidized forms of flavonoids.

For the subsequent extraction of flavonoids, the following factors determining the yield of extractive substances were studied: extractant concentration, extraction time, and raw material-extractant ratio. Ethyl alcohol of various concentrations was used as an extractant. The choice of other optimal extraction parameters was controlled by the content of the sum of flavonoids (quantification in terms of quercetin).

It follows from the data that the largest amount of flavonoids is extracted in 70% compared to 50% and 90% water-ethyl extracts. The flavonoid content is maintained during extraction for 24 hours at room temperature and then their concentration decreases.

The raw material-extractant ratio varied from 1:10 to 1:20. It was revealed that the largest extraction of extractive substances from the studied raw materials was at a ratio of 1:15.

As a result, the optimal conditions for the extraction of flavonoids are: 50-70% water-ethyl extractants, the ratio of raw materials to extractants is 1:15, and the extraction time is 24 hours at room temperature. The yield of extractive substances of flavonoids was about 11.2-12.1%. The results of the selection of conditions for the extraction of the sum of flavonoids from the aerial part of *Ferula songarica* Pall. ex Spreng., are shown in Table 2.

Table 2. Selection of methods for the extraction of flavonoids from the aerial part of *Ferula songarica* Pall. ex Spreng.

Extraction time, hours	The content of the sum of flavonoids in terms of quercetin and absolutely dry raw materials, %		
	The raw material-extractant ratio is 1:10		
	50% water-ethyl extract	70% water-ethyl extract	90% water-ethyl extract
6	**5.682**±0.056	**8.119**±0.042	**8.358**±0.036
12	**4.201**±0.054	**5.821**±0.021	**4.471**±0.040
24	**9.127**±0.060	**6.935**±0.066	**5.391**±0.022
48	**7.877**±0.049	**5.753**±0.040	**5.055**±0.056
	The raw material-extractant ratio is 1:15		
6	**7.644**±0.025	**9.597**±0.052	**8.173**±0.036

12	**6.223**±0.027	**8.028**±0.019	**6.157**±0.033
24	**11.161**±0.045	**12.089**±0.017	**9.956**±0.041
48	**6.251**±0.010	**6.539**±0.035	**6.860**±0.041
	The raw material-extractant ratio is 1:20		
6	**9.754**±0.027	**10.116**±0.031	**8.159**±0.020
12	**6.362**±0.025	**8.014**±0.035	**6.154**±0.045
24	**9.478**±0.046	**11.050**±0.040	**8.971**±0.030
48	**5.798**±0.052	**7.248**±0.050	**6.120**±0.042

Figure 1 shows the data on the amount of flavonoids depending on the time of water-ethyl extraction and the ratio of raw materials to extractants.

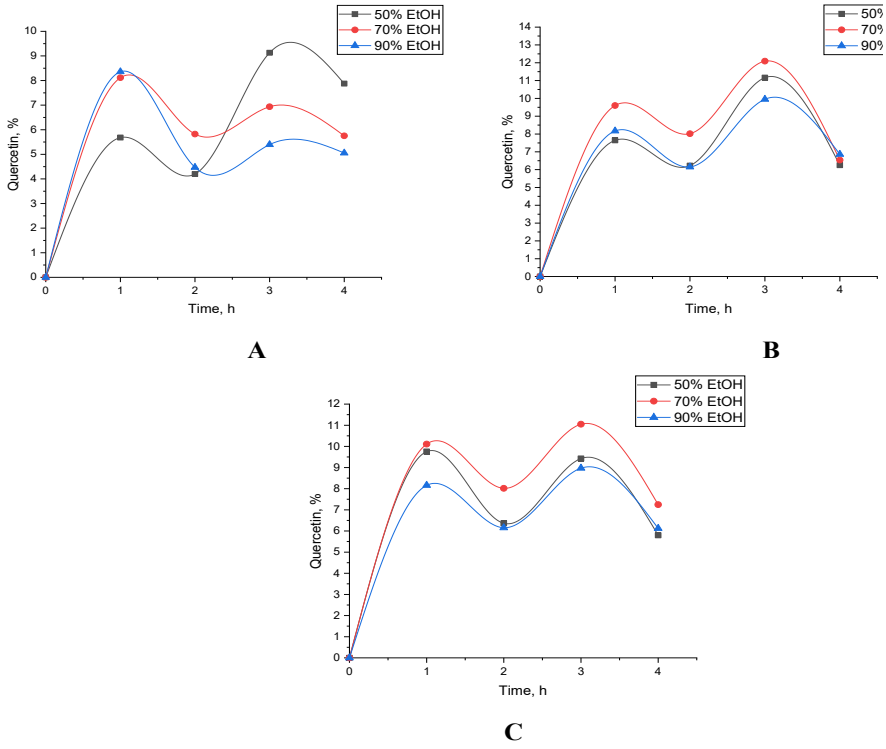

Figure 1. The content of flavonoids in water-ethyl extracts of the aerial part of *Ferula songarica* Pall. ex Spreng. depending on the extraction time (1 – 6 h; 2 – 12 h; 3 – 24 h; 4 – 48 h;) and the ratio of raw material-extractant A – 1:10; B – 1:15 and C – 1:20

The antioxidant activity of the water-ethyl extracts was studied by determining the iron-reducing potential Ferric Reducing/Antioxidant Power assay (FRAP) *in vitro*. The concentration dependences of the optical density values for the extracts were obtained in comparison with the standard substance ascorbic acid (AA). The antioxidant properties increased in the sequence: 50% ethyl extract >AA> 70% ethyl extract > 90% ethyl extract. An increase in the optical density indicates an increase in the reduction potential (Table 3, Fig.2).

Table 3. Change in optical density depends on the concentration of extracts from the aerial part of *Ferula songarica* Pall. ex Spreng.

No.	Extracts	Extract concentration, mg/ml			
		0.25	0.5	0.75	1.0
		Optical density			
1	50% water-ethyl extract	2.383 ± 0.14	2.0836 ± 0.04	2.021 ± 0.01	2.470 ±0.07
2	70% water-ethyl extract	0.0644 ± 0.01	0.1673 ± 0.15	0.4932 ± 0.09	1.0381 ± 0.08
3	90% water-ethyl extract	0.0720 ± 0.01	0.3713 ± 0.01	0.4499 ± 0.03	0.7063 ± 0.35
4	Ascorbic acid	1.569 ± 0.01	1.589 ± 0.04	1.748 ± 0.02	1.879 ± 0.04

All the studied concentrations of 50% water-ethyl extract showed higher activity compared to the standard.

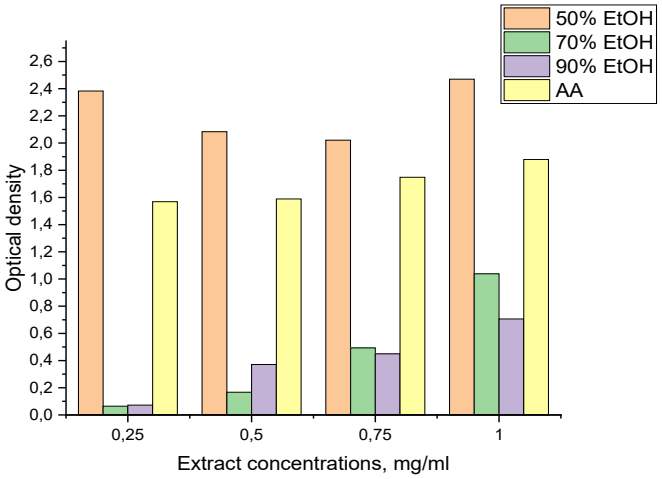

Figure 2. Comparative assessment of the antioxidant activity of water-ethyl extracts of the aerial part of *Ferula songarica* Pall. ex Spreng.: 50% EtOH – 50% water-ethyl extract, 70% EtOH – 70% water-ethyl extract, 90% EtOH – 90% water-ethyl extract, AA – ascorbic acid

According to the literature data, the standardization of *Ferula varia* herb based on flavonoid content was previously conducted, using the glycosylated flavonoid cynaroside as a standard. The authors studied the following parameters: the degree of raw material grinding (ranging from 2 mm to an unground state), ethanol concentration (60–95%), extraction time (15–90 minutes), and a temperature of 60°C. Under these conditions, the yield of cynaroside from the raw material was approximately 1% (Kotenko, et al., 2009). A distinctive feature of our study is the use of raw material with a fixed particle size, the inclusion of an additional parameter – the raw material-extractant ratio, the aqueous-ethanol extractants with concentrations ranging from 50% to 90%, an extraction time of 6 to 48 hours, and extraction at room temperature. As a result, the optimized conditions developed in our study yielded flavonoid content (quercetin) of 11.2-12.1%.

According to literary data, antioxidant activity was previously found for the species *Ferula caratavica, Ferula kuchistanica, Ferula pseudoreoselinum, Ferula samarcandica, Ferula tenuisecta* and *Ferula varia* (Youssef, et al., 2020).

It has been reported that extracts containing phenolic compounds, including flavonoids obtained from *Ferula persica* and *Ferula szovitsiana* showed high antioxidant activity (Taghinia, et al., 2019; Dehghan, et al., 2007). The ethyl acetate extract of *Ferula caspica M. Bieb*., extracts of the aerial part of *Ferula communis*, and other species showed antioxidant activity (Kahraman, et al., 2019; Rahali, et al., 2019).

Numerous studies carried out mainly *in vitro* show that flavonoids can be classified as non-enzymatic antioxidants that can directly or indirectly weaken or prevent cellular damage caused by free radicals (Procházková, et al. 2011). The antioxidant activity of water-ethyl extracts obtained from the aerial part of *Ferula songarica* Pall. ex Spreng. was studied for the first time.

Thus, the optimal conditions for obtaining an extract containing flavonoids and exhibiting high antioxidant activity are: extractant – 50% ethyl alcohol, raw material-extractant ratio – 1:15, extraction time – 24 hours at room temperature.

Conclusion

The quality indicators for the aerial part of *Ferula songarica* Pall. ex Spreng. have been determined, which correspond to the limits of permissible standards according to the State Pharmacopoeia of the Republic of Kazakhstan: moisture – 8.15%, total ash – 7.39%, ash insoluble in HCl – 0.29%.

An optimal method for obtaining the sum of flavonoids from the aerial part of *Ferula songarica* Pall. ex Spreng. has been developed: extractant – 50-70% ethyl alcohol, raw material-extractant ratio – 1:15; extraction time – 24 hours at room temperature.

The antioxidant effect of 50, 70, and 90% water-ethyl extracts has been established by the method of determining the iron-reducing potential of Ferric Reducing/Antioxidant Power assay (FRAP) *in vitro*. Moreover, the 50% water-ethyl extract showed higher activity than the standard.

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