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Antimicrobial activity of the dense extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L.

In the article the results of ultrasonic extraction of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. from Central Kazakhstan were presented. The effectiveness of the process was evaluated based on the quantitative yield of total extractive substances, the content of total polyphenolic compounds, flavonoids, phenolic acids, and the antimicrobial activity of the obtained extracts. It was experimentally established that the solvent ensuring the quantitative extraction of total extractive substances is 70 % ethanol. The obtained extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. can be considered as substances for the development of antimicrobial pharmaceuticals.

Keywords: Dracocephalum, antimicrobial activity, ultrasonic extraction, HPLC, pathogenic bacteria.

Introduction

In modern conditions, there is a development of microorganism strains with multiple drug resistance and their active spread, which highlights the need for new antimicrobial and antifungal agents. In this context, plant-based medicines are becoming increasingly important alongside synthetic drugs. The biologically active compounds in these substances are similar in structure and action to the natural components of the human body, which leads to a significant reduction in side effects from their use [1–5]. Bacteria resistant to synthetic antibiotics threaten their effectiveness and limit treatment options even for common infections. Therefore, significant attention should be given to plant-based agents that can be used as highly effective antimicrobial medications.

Currently, plant-based medicines are successfully used for the treatment of most diseases, despite the fact that synthetic drugs, which emerged about a century and a half ago, have significantly advanced medicine [6].

In the flora of Kazakhstan, there are more than 1,000 essential oil plants. Of particular interest are some species from the families *Lamiaceae, Apiaceae*, and *Asteraceae*, which have either not been studied at all or have only brief information available on their chemical composition and biological properties. In this regard, the family *Lamiaceae*, which is one of the leading families in the flora of Kazakhstan, is especially noteworthy. For instance, within this family, the republic has 233 species classified into 45 genera [7].

Among them, plants of the genus *Dracocephalum* L. are the most well-known, serving as a rich and widespread source of essential oils. According to the database The Plant List (as of August 2016), the genus includes 74 species, with 20 species found in Kazakhstan [8].

Studies have shown that some species of *Dracocephalum* possess antibacterial, antitussive, antidiarrheal, antioxidant, anticancer, anti-inflammatory, antidiabetic, and soothing properties [9–12].

Experimental

The research objects are the above-ground parts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L., collected during the flowering phase from the Karkaraly Mountains (Karaganda Region, N 49^{0}43'23", E 75^{0}48'38"), in May 2022.

For obtaining dense extracts, the method of ultrasonic extraction was used for the above-ground parts of *Dracocephalum nutans* L. (nodding dragonhead) and *Dracocephalum ruyschiana* L. (Ruysch's dragonhead). The choice of the ultrasonic method is due to its ability to extract biologically active substances from plant material within a short time (15–30 minutes), whereas classical methods typically require 8–24 hours [13–15].

For the analysis, an ultrasonic bath Stegler 3DT (3 L, 20-80 °C, 120W, frequency 40 kHz) was used. The raw material was extracted using a mixture of water and ethanol (1:1) and ethanol alone, with a ratio of raw material to extractant of 1:20. Ultrasonic extraction was performed with a water: ethanol ratio of 1:1. The raw material was initially soaked for 20 minutes and then subjected to ultrasonic treatment for 30 minutes at room temperature. The extraction process was repeated three times under the same conditions, and the filtrates were combined, cooled to room temperature, and evaporated using a rotary evaporator.

For the analysis of phenolic compounds in the extracts, high-performance liquid chromatography coupled with ultraviolet (UV) detection and real-time tandem mass spectrometry (ESI-MS/MS) was used.

The content of phenolic compounds in the extracts was calculated using the external standard method according to the formula (1):

$$X=\frac{S_1 \text{ x } m_0 \text{ x}25 \text{ x } P \text{ x}100}{S_0 \text{ x } m \text{ x}25 \text{ x}100}, \quad (1)$$

where S_1 is the peak area value of the compound in the chromatogram of the test solution;

S_0 is the peak area value of the compound in the chromatogram of the standard;

m_0 is the mass of the standard compound, in grams; m_1 is the mass of the extract, in grams;

P is the content of the compound in the standard compound, in %; and 25, 25 are the dilution factors.

The antimicrobial activity of the samples was studied using the disk diffusion method.

The antimicrobial activity of the aforementioned samples was studied against Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, Gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa, and the yeast fungus Candida albicans using the disk diffusion method. The reference substances used were benzylpenicillin for bacteria and nystatin for the yeast fungus Candida albicans.

For the study, a suspension containing a standard number of viable bacterial cells was prepared and seeded as a lawn on the surface of the nutrient medium in Petri dishes. 0.01 mL of each sample was applied to sterile filter paper disks. The disks with the samples were placed on the seeded surface in a circular arrangement, 2.5 cm from the center of the dish (4 disks per dish). The inoculated plates were incubated at 36 °C for 24 hours. After incubation, zones of complete and partial bacterial growth inhibition appeared around the disks against the uniform bacterial lawn. The results were recorded by measuring the diameters of the inhibition zones. Each sample was tested in triplicate. For comparative assessment of antimicrobial activity, antibiotic solutions were used: sodium benzylpenicillin, sodium ceftriaxone, nystatin, with 70 % ethanol and DMSO in equivalent volumes used as controls.

The antimicrobial activity of the samples was assessed by measuring the diameter of the inhibition zones around the test strains (mm). An inhibition zone diameter of less than 10 mm and continuous growth in the dish were considered as indicating no antibacterial activity, 10–15 mm indicated weak activity, 15–20 mm indicated moderate activity, and over 20 mm indicated strong activity.

Statistical analysis of the data was performed using parametric statistical methods, including the calculation of the arithmetic mean and standard error.

Results and Discussion

The HPLC analysis of the chemical composition of phenolic compounds in the dense extracts of *Dracocephalum nutans* and *Dracocephalum ruyschiana* was conducted at the Research Center of the Medical University of Karaganda (Karaganda, Kazakhstan). The composition of phenolic compounds in dense extracts of *D.nutans* and *D.ruyschiana*, obtained by ultrasonic extraction, and the mass spectra for the identified compounds in negative ionization mode are presented in Table 1 and Figures 1 and 2.

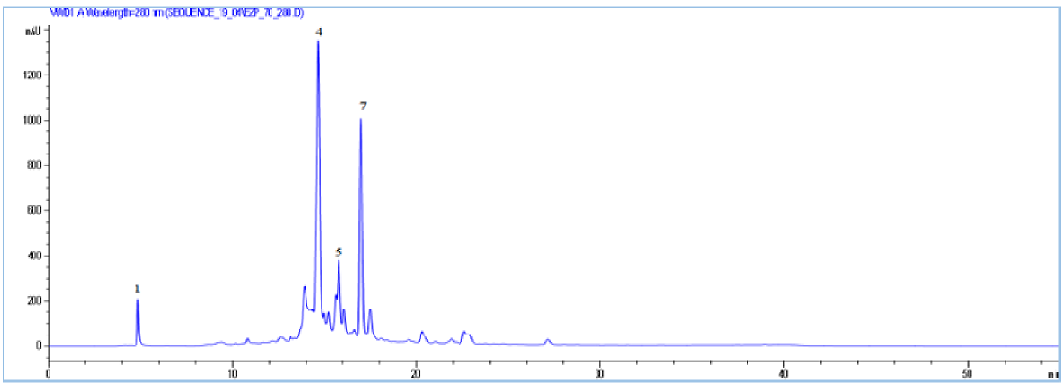

Figure 1. Chromatogram of *Dracocephalum nutans* extract at a wavelength of 280 nm

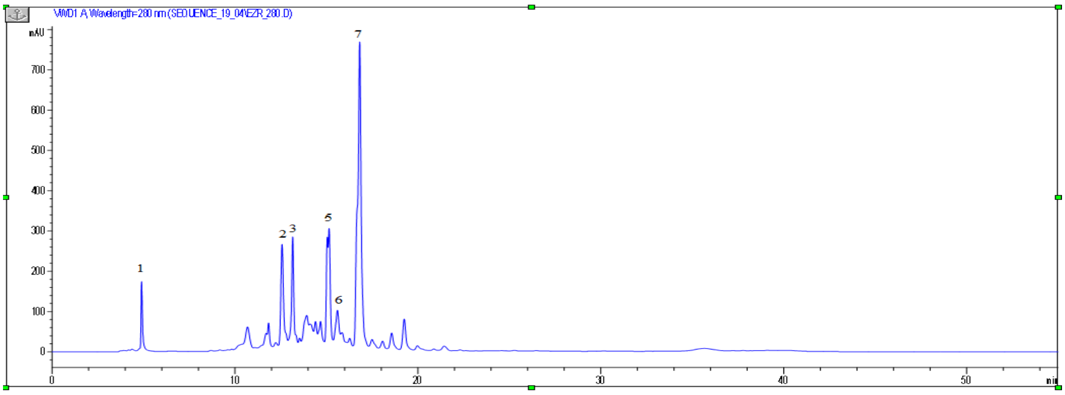

Figure 2. Chromatogram of *Dracocephalum ruyschiana* extract at a wavelength of 280 nm

Table 1

Identification and Content of Phenolic Compounds in Dense Extracts of *Dracocephalum nutans* and *Dracocephalum ruyschiana*

№ Peak	Retention Time, min	M-H − (m/z)	Identified Components	Content (mg/g of extract)	
				D. ruyschiana	*D. nutans*
1	4.985	179	caffeic acid	1.33±0.08	1.37±0.11
2	12.568	353	chlorogenic acid	12.33±0.15	-
3	13.907	163	p — couric acid	12.37±0.05	-
4	14.717	463	quercetin-3'-glucoside	-	47.95±0.19
5	15.136	574	ferulic acid	7.29±0.10	3.43±0.13
6	15.593	163	o — couric acid	3.1±0.10	-
7	16.995	359	rosmarinic acid	44.76±0.15	19.54±0.28

As shown in Table 1, a total of 7 phenolic compounds were identified and quantified in the dense extracts of *D.nutans* and *D.ruyschiana*, including 6 phenolic acids and 1 flavonoid.

The dominant phenolic compounds in the extracts are rosmarinic acid, with contents of 19.54 and 44.76 mg/g, and quercetin-3'-glucoside in *D.nutans*, with a content of 47.95 mg/g (Table 2).

Table 2

Results of the Antimicrobial Activity Study of *Dracocephalum nutans* and *Dracocephalum ruyschiana* Extracts

mm / name	Staphylococcus aureus ATCC 6538	Bacillus subtilis ATCC 6633	Escherichia coli ATCC 25922	Pseudomonas aeruginosa **ATCC 27853**	Candida albicans A TCC 10231
D.ruyschiana	15 ± 0.5	15 ± 1	10 ± 0.5	9 ± 1	12 ± 0.6
D.nutans	14 ± 1.2	12 ± 0.1	14 ± 0.3	9 ± 0.3	12 ± 0.1
Sodium benzylpenicillin	16 ± 0.1	14 ± 0.1	15 ± 0.1	12 ± 1	-
Sodium ceftriaxone	20 ± 0.3	19 ± 0.17	19 ± 0.5	19 ± 1	-
DMSO	-	-	-	-	-
(70 %) Ethanol	10 ± 0.1	10 ± 0.1	9 ± 0.1	9 ± 0.1	8 ± 0.1
Nystatin	-	-	-	-	21 ± 0.2

The extract of *D.ruyschiana* exhibits moderate antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, and weak antimicrobial activity against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, as well as against the yeast fungus *Candida albicans*.

The extract of *D.nutans* demonstrates weak antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, as well as weak activity against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, and weak activity against the yeast fungus *Candida albicans*.

Conclusion

1. For the first time, ultrasonic extraction of *Dracocephalum nutans* and *Dracocephalum ruyschiana* from the Central Kazakhstan region was successfully performed.

2. The chemical composition of the extracts of *Dracocephalum nutans* and *Dracocephalum ruyschiana* was determined using high-performance liquid chromatography (HPLC).

3. The extract of *Dracocephalum ruyschiana* can be considered as a potential substance for the development of antimicrobial agents.

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Dracocephalum nutans L. және *Dracocephalum ruyschiana* L. қою экстрактарының микробқақарсы белсенділігі

Мақалада Орталық Қазақстанда өсетін *Dracocephalum nutans* L. және *Dracocephalum ruyschiana* L. өсімдік шикізаттарының ультрадыбыстық экстракциялау нәтижелері келтірілген. Процестің тиімділігі экстрактивті заттардың жалпы шығымы, полифенолды қосылыстардың, флавоноидтардың, фенолқышқылдарының жалпы мөлшері және алынған экстрактардың микробқақарсы белсенділігі бойынша бағаланды. Эксперименталды түрде экстрактивті заттардың жалпы мөлшерін тиімді алу үшін 70 % этил спирті экстрагент ретінде қолданылды. Алынған микробқақарсы белсенділігі бар *Dracocephalum nutans* L. және *Dracocephalum ruyschiana* L. экстрактылары дәрілік заттарды әзірлеу үшін субстанция ретінде қарастырылды.

Кілт сөздер: Dracocephalum, микробқақарсы белсенділік, ультрадыбыстық экстракция, ЖТСХ, патогенді бактериялар.

А. Рамазанова, Г.А. Атажанова, Г.К. Курмантаева, Б.Б. Аширбекова

Антимикробная активность густых экстрактов *Dracocephalum nutans* L. и *Dracocephalum ruyschiana* L.

В статье представлены результаты ультразвуковой экстракции *Dracocephalum nutans* L. и *Dracocephalum ruyschiana* L., произрастающих на территории Центрального Казахстана. Эффективность процесса оценивали по количественному выходу суммы экстрактивных веществ, содержанию общего количества полифенольных соединений, флавоноидов, фенольных кислот и антимикробной активности полученных экстрактов. Экспериментально установлено, что экстрагентом, обеспечивающим количественное извлечение суммы экстрактивных веществ, является 70 %-ный спирт этиловый. Полученные экстракты *Dracocephalum nutans* L. и *Dracocephalum ruyschiana* L. можно рассматривать в качестве субстанций для разработки лекарственных средств противомикробного действия.

Ключевые слова: Dracocephalum, антимикробная активность, ультразвуковая экстракция, ВЭЖХ, патогенные бактерии.

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