

Research Article

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Study of the antioxidant, antiradical, and antimicrobial activities of the soft extract of *Dracocephalum ruyschiana* L.

The article presents the results of a study on the antioxidant, antiradical, and antimicrobial activities of a thick extract of *Dracocephalum ruyschiana* L. obtained by ultrasonic extraction from the aerial parts of plants growing in Central Kazakhstan. The aim of the study was to comprehensively evaluate the biological activities of the ultrasonic extract in vitro and assess its potential for further pharmaceutical applications. The results demonstrated that the thick extract exhibits pronounced antiradical activity, indicating its ability to effectively neutralize free radicals and reduce oxidative processes. The antioxidant properties of the extract may be associated with its high content of extractive substances, including biologically active phenolic compounds. Particular attention was paid to the use of ultrasonic cavitation, which facilitates more efficient extraction of active compounds compared with conventional extraction methods. The relevance of the study is related to the important role of free-radical processes in the development of various diseases, including atherosclerosis, ischemic heart disease, cancer, and inflammatory disorders. Substances with antioxidant and antiradical properties are widely investigated for their potential use in the prevention and complex treatment of these conditions. The findings indicate the pharmacological potential of *Dracocephalum ruyschiana* as a promising source of natural biologically active compounds. To the best of our knowledge, this is the first study to provide a comprehensive assessment of the antioxidant, antiradical, and antimicrobial activities of a thick extract of *Dracocephalum ruyschiana* obtained from plant material using ultrasonic cavitation.

Keywords: antioxidant activity, antiradical activity, aerial part, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), optical density, *Dracocephalum ruyschiana*, ultrasonic cavitation, antimicrobial activity.

Introduction

A systematic assessment of the biological potential of dragonhead species (genus *Dracocephalum* L.) has demonstrated promising prospects for their utilization in various domains, particularly in biomedicine. At present, considerable attention from research groups worldwide is directed toward the study of pharmacological applications of extracts derived from *Dracocephalum* plants, as well as the isolation and characterization of their secondary metabolites [1–3]. Accumulating experimental evidence confirms that representatives of this genus exhibit a broad spectrum of biological activities, including anticancer, anti-inflammatory, antioxidant, and antibacterial effects, which have been comprehensively summarized in recent review articles [4–7].

The genus *Dracocephalum* belonging to the family Lamiaceae Lindl. In the flora of Eastern Kazakhstan, the genus *Dracocephalum* occupies a leading position among the genera of the family Lamiaceae, comprising 9 species, which accounts for 12 % of the total number of species within this family. Species of *Dracocephalum* are distributed throughout Central Kazakhstan under various ecological conditions, occurring at altitudes of up to 2500 meters above sea level. They form part of diverse phytocenoses. The genus *Dracocephalum* is widely represented in the flora of Eastern Kazakhstan and is used in traditional medicine (Labiatae), commonly referred to as the mint family, is regarded as a potential source of pharmacologically relevant natural products. According to *The Plant List* (August 2020), the genus comprises 74 accepted species, 20 of which are reported in Kazakhstan [8].

Medicinal plants represent an important reservoir of bioactive secondary metabolites, and increasing research efforts are directed toward approaches that may improve their biosynthetic productivity. Within the Lamiaceae, species of *Dracocephalum*, including *D. ruyschiana*, are characterized by particularly high levels of polyphenolic constituents [9].

A considerable amount of information on the properties of *D. ruyschiana* is available in the literature [10, 11]. According to Iranian, Chinese, and Indian practitioners of traditional medicine, consumption of a cup of *D. ruyschiana* tea exerts a preventive effect against a number of health conditions. *D. ruyschiana* has also been used as a plant-based remedy with antidepressant properties [12–15].

Numerous studies report on the properties of the aerial parts of *D. ruyschiana*, including its phytochemical composition and various types of biological activity, which is associated with the widespread use of this plant in medicine.

Thus, *D. ruyschiana* is an important medicinal plant that, due to its high value, rich chemical composition, and diverse pharmacological effects, attracts considerable attention from researchers worldwide. In addition, *D. ruyschiana* represents a valuable source of phenolic compounds, flavonoids, and rosmarinic acid, and may serve as a widely available and renewable source of raw material for phytopharmacology. Given the broad spectrum of its therapeutic properties, this article presents a comprehensive overview of the composition, pharmacological activity, and methods for obtaining extracts from *D. ruyschiana* [16].

The focus of this study was *D. ruyschiana*, a species broadly distributed across the territory of Kazakhstan and considered a valuable source of raw medicinal material. Histochemical investigation of the aerial organs of *D. ruyschiana* aimed at determining the localization of biologically active compounds represents an important step for the authentication of plant material and for advancing further pharmaceutical and medical research [17].

Dracocephalum ruyschiana is an essential oil-producing plant traditionally employed in folk medicine. Its pharmacological potential is associated with the presence of diverse bioactive constituents in the aerial parts, including cardenolides, alkaloids, tannins, coumarins, and flavonoids. These metabolites underlie its therapeutic applications in the management of respiratory disorders, fever reduction, asthenic conditions, and reproductive health. Moreover, nectar of *D. ruyschiana* has been reported to exhibit antimicrobial properties against both Gram-positive and Gram-negative bacteria [18–20].

Dracocephalum ruyschiana has long been employed in traditional medicine for the treatment of respiratory disorders, as an antipyretic remedy, in cases of asthenia, and as an agent enhancing reproductive function [18]. In addition, the nectar of this species demonstrates antimicrobial efficacy against both Gram-positive and Gram-negative microorganisms [21].

Literature data confirms that Kazakhstani species of *Dracocephalum* have significant pharmacological potential [22–24], which justifies further preclinical and clinical research to develop safe and effective phytopharmaceutical products based on them.

The aim of the study is to study the antioxidant and radical scavenging activities of the dense extract of *Dracocephalum ruyschiana* (L.) obtained through ultrasonic cavitation, in vitro.

Experimental

The research objects are the above-ground parts of *Dracocephalum ruyschiana* L., collected during the flowering phase from the Karkaraly Mountains (Karaganda Region, N 49°43'23", E 75°48'38"), in May 2023 (Fig. 1).



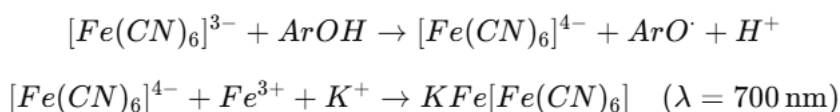
Figure 1. *Dracocephalum ruyschiana* in the flowering phase

For the first time, ultrasonic extraction was used to obtain the total extractives from the herb *Dracocephalum ruyschiana*. 20.0 g of air-dried herb were placed in an extraction vessel and covered with 400 ml of extractant—either water or a mixture of ethanol and water (1:20, v/v).

Ultrasonic extraction of the raw material was performed without pre-soaking using the ultrasonic cleaner Sonic-3 at a frequency of 40 kHz and room temperature (20–22 °C) for 30 minutes. Subsequently, the liquid extract was decanted, and extraction was repeated under the same conditions. The extract was prepared using 70 % ethanol and designated as EZR-70 %. The resulting combined liquid extracts of *Dracocephalum ruyschiana* were filtered through a paper filter. The liquid filtrate was poured into a rotary evaporator and the extractant was evaporated at a temperature of 50 °C, resulting in a thick extract of *Dracocephalum ruyschiana*. Residual solvent was removed from the thick extract in a water bath at 60 °C. The thick extract is an amorphous powder of a greenish-brown color, with a specific odor, and is very hygroscopic. The yield of the extract is 6 % [25].

Determination of Ferric Reducing Antioxidant Power (FRAP assay)

The mechanism of this method can be represented as follows:



To 1 mL of the tested extracts at concentrations ranging from 0 to 1 mg/mL, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium hexacyanoferrate(III) solution were added. The reaction mixture was incubated for 25 minutes at 50 °C. The reaction was stopped by adding 2.5 mL of 10 % trichloroacetic acid. The mixture was then centrifuged for 3 minutes (1,500 rpm). A 2.5 mL aliquot of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % FeCl₃ solution. The absorbance was measured at 700 nm.

The obtained data are presented in Table 1 and Figure 2, showing the concentration-dependent absorbance values for the studied plant extracts and the standard compound, ascorbic acid (AA).

The antiradical activity of the presented samples was studied using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)* [Brand-Williams W., Cuvelier M.E., Berset C. (1995) *Lebensm Wiss Technol* 28:25–30].

To evaluate the antiradical activity of the tested samples in the DPPH assay, a 100 µM ethanolic DPPH solution was used.

For the selection of samples with pronounced antiradical activity, 2 mL of a 100 µM ethanolic DPPH solution was mixed with 20 µL of the test sample dissolved in ethanol at a concentration of 10 mg/mL. Thus, the final concentration of the test sample in the reaction mixture was 100 µg/mL.

Ten minutes after adding the test sample solution to the DPPH radical solution, the decrease in optical density was measured at 515 nm.

For the tested samples, interaction with the DPPH radical was studied at final concentrations of the extracts ranging from 5 to 100 µg/mL. After that, the concentration of the test extract required to reduce the optical density of the 100 µM DPPH solution by 50 % was determined (IC₅₀, DPPH).

The percentage of DPPH radical inhibition was calculated using the following formula:

$$I = \frac{A_0 - A_x}{A_0} \times 100,$$

where A₀ is the control optical density of the solution containing all reagents except the test sample; A_x is the optical density of the sample [16].

The antimicrobial activity was studied using the disc diffusion method

Antimicrobial activity was evaluated against standard reference strains: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. These strains represent the most common causative agents of purulent-inflammatory diseases and were obtained from the microbial culture collection of the Department of Microbiology of the Non-Commercial Joint-Stock Company “Karaganda Medical University”.

The antimicrobial activity was evaluated using the disc diffusion method against Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and the yeast *Candida albicans* ATCC 10231. Benzylpenicillin and eucalyptus oil were used as reference drugs for bacteria, while nystatin was used for

Candida albicans. Comparative drugs are benzylpenicillin sodium salt, gentamicin for bacteria and nystatin for the yeast fungus *Candida albicans*.

For the assay, a microbial suspension containing a standard number of viable cells was prepared and uniformly inoculated onto the surface of nutrient agar in Petri dishes. Sterile filter paper discs were impregnated with 0.01 mL of the test samples and placed on the inoculated agar surface (four discs per plate) at a distance of 2.5 cm from each other. The plates were incubated for 24 h at 36 °C.

Statistical processing was carried out using parametric statistics methods with calculation of the arithmetic mean and standard error.

After incubation, zones of complete or partial inhibition of microbial growth were observed around the discs. Antimicrobial activity was assessed by measuring the diameters of the inhibition zones. Each sample was tested in triplicate [26].

Results and Discussion

For the first time, we carried out ultrasonic extraction of *Dracocephalum ruyschiana*. Given that the authors [27] used solvents with higher polarity for ultrasonic extraction to more effectively extract polyphenolic acids, we chose 70 % ethyl alcohol for ultrasonic cavitation. The result of the evaluation of the anti-radical activity of the obtained extract is presented in Table 1 and Figure 2, which shows the concentration dependence of the optical density for the studied plant extract and the standard, ascorbic acid (AC).

Table 1

Optical density values of *Dracocephalum ruyschiana* extract samples

Concentration range, mg/mL	Optical density values for the samples under study, mg/ml	
	EZR 70	AA
0.25	1.19±0.11	1.92±0.01
0.5	1.52±0.18	2.28±0.01
0.75	1.60±0.01	2.25±0.01
1	2.01±0.12	2.31±0.01
EZR 70- <i>Dracocephalum ruyschiana</i> extract, AA-ascorbic acid		

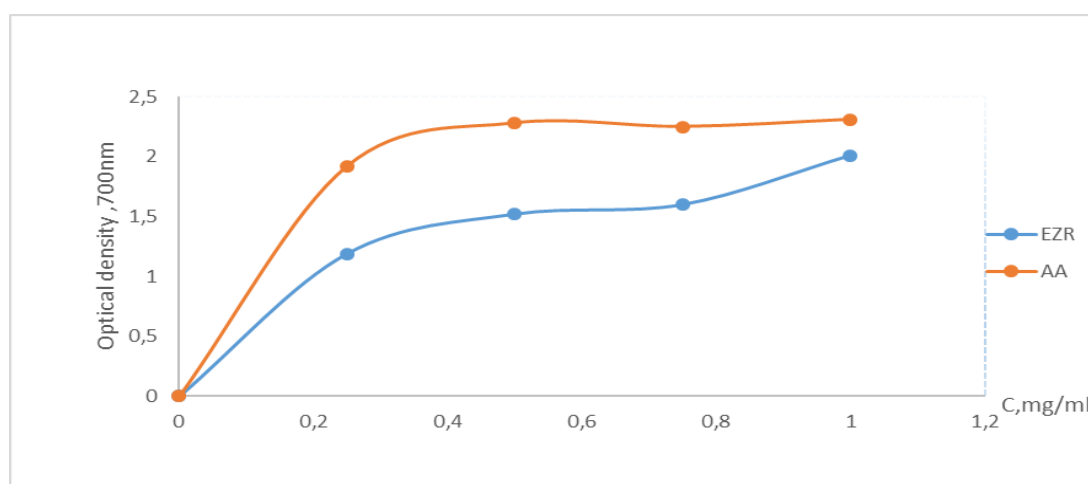


Figure 2. Dependence of the optical density on the concentration of *Dracocephalum ruyschiana* extract

The presented data shows that the ultrasonic extract (EZR) has a relatively high antioxidant activity, as evidenced by a significant increase in the optical density of the solution as its concentration increases.

Table 2 presents the results of screening the given extracts for antiradical activity.

Table 2

Optical density values of a 100 μ M DPPH radical solution after 10 min of incubation with *Dracocephalum ruyschiana* extract at a final concentration of 100 μ g/mL

Nº	Test extract	Absorbance, OD
1	extract <i>Dracocephalum ruyschiana</i> L.	0,062
2	Control (DPPH solution without the test sample)	0,995

Table 2 shows that the plant extract *Dracocephalum ruyschiana* L. is promising for further research, as it reduces the optical density of the DPPH radical solution by more than 50 %. In the second series of experiments, the ability of the *Dracocephalum ruyschiana* L. extract at various concentrations (from 5 to 100 mg/mL) to interact with the DPPH radical was studied.

Using the constructed calibration curves, the IC_{50} (DPPH) value for the tested extract of *Dracocephalum ruyschiana* was determined (Fig. 3). The IC_{50} (DPPH) values for the extract of *Dracocephalum nutans* are presented in Table 3.

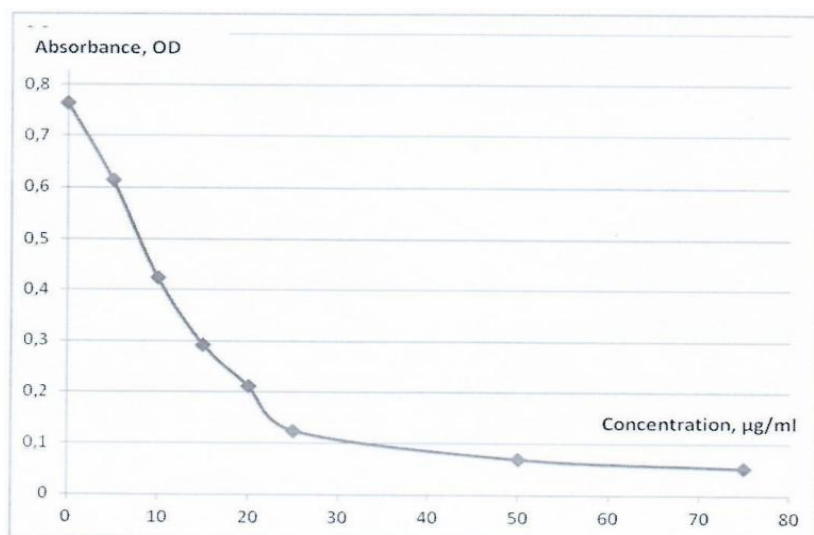


Figure 3. Dose–response curve of DPPH radical scavenging activity of the extract of *Dracocephalum ruyschiana*

Table 3

IC_{50} (DPPH) values for the tested extract of *Dracocephalum ruyschiana*

Nº	Test extract	IC_{50} (DPPH), mg/mL
1	extract <i>Dracocephalum ruyschiana</i>	9,8

The most pronounced antiradical activity under the conditions of this test system was exhibited by the extract of *Dracocephalum ruyschiana* L., for which the concentration capable of reducing the optical density of a 100 μ M DPPH radical solution by 50 % was determined. For the *Dracocephalum ruyschiana* L. extract, the IC_{50} (DPPH) value was found to be 9.8 mg/mL.

In the next series of experiments, the antimicrobial activity of the *Dracocephalum ruyschiana* extract was investigated (Tab. 4). Currently, a large number of microorganisms, particularly nosocomial strains, pose a serious threat to human life and health due to the widespread emergence of multidrug resistance and the resulting difficulties in selecting effective chemotherapy. One of the key factors contributing to resistance development is the extensive use of antimicrobial agents, which in some cases leads to adverse effects such as dysbiosis, anaphylactic shock, and the formation of cross-resistance. Therefore, there is an ongoing search for new antimicrobial agents that, on the one hand, exhibit antimicrobial activity via mechanisms distinct from those of conventional antibiotics and, on the other hand, lack significant side effects [28].

Table 4

Antimicrobial activity of the *Dracocephalum ruyschiana* soft extract (inhibition zone diameter, mm)

mm Sample code	Staphylococcus aureus ATCC 6538	Bacillus subtilis ATCC 6633	Escherichia coli ATCC 25922	Pseudomonas aeruginosa ATCC 27853	Candida albicans ATCC 10231
EZR (70 %)	22± 0.5	25±1	10±0.5	9±1	22± 0.6
Benzylpenicillin sodium salt	16 ± 0.1	14 ± 0.1	15 ± 0.1	12±1	-
Ethyl alcohol (70 %)	10 ± 0.1	10± 0.1	10 ± 0.1	10 ± 0.1	10 ± 0.1
Nystatin	-	-	-	-	21 ± 0.2
Note. n = 3, P ≤ 0.05					

Conclusion

A comprehensive study of the antiradical, antioxidant, and antimicrobial activities of the ultrasonic extract of *Dracocephalum ruyschiana* was conducted for the first time. It was established that the thick extract obtained using ultrasonic cavitation exhibits pronounced antiradical and antioxidant activity. The results of antimicrobial assays demonstrated that the *Dracocephalum ruyschiana* extract shows high activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), moderate activity against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and pronounced activity against the yeast *Candida albicans*. These findings indicate the significant pharmacological potential of the extract and highlight its prospects for use in the development of pharmaceutical products.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Sabiyeva A.** — conceptualization, data analysis, investigation; **Atazhanova G.A.** — plant material collecting, writing draft, data curation; **Ashirbekova B.** — obtaining ultrasonic extracts; **Vlassova L.** — investigation of biological activity and methodology; **Sabyrova S.** — statistical processing of results and collection of raw materials.

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***Dracocephalum ruyschiana* L. қою сығындысының антиоксидантты, радикалға қарсы және микробқа қарсы белсенділігін зерттеу**

Мақалада Орталық Қазақстан жағдайында өсетін өсімдіктің жерүсті бөлігінен ультрадыбыстық экстракция әдісі арқылы алынған *Dracocephalum ruyschiana* L. қою экстрактының антиоксиданттық, антирадикалдық және антимикробтық белсенділігін зерттеу нәтижелері ұсынылған. Зерттеудің мақсаты — ультрадыбыстық экстракттардың *in vitro* жағдайында биологиялық белсенділігін кешенді түрде зерттеу және олардың болашақ фармацевтикалық қолдану мүмкіндіктерін бағалау. Зерттеу нәтижесінде *Dracocephalum ruyschiana* қою экстракты айқын антирадикалдық белсенділікке ие екендігі анықталды, бұл оның бос радикалдарды тиімді бейтараптандыру және тотығу процестерінің қарқындылығын төмендету қабілетін көрсетеді. Экстрактың антиоксиданттық қасиеттері фенолдық табиғаттағы биологиялық белсенді қосылыстарды қамтитын экстрактивті заттардың жоғары мөлшерімен түсіндіріледі. Зерттеуде ультрадыбыстық кавитацияны қолдануға ерекше назар аударылған, себебі ол дәстүрлі экстракция әдістерімен салыстырғанда белсенді компоненттердің тиімдірек бөлініп алынуын қамтамасыз етеді. Зерттеудің өзектілігі бос радикалдық процестердің көптеген аурулардың, соның ішінде атеросклероз, жүректің ишемиялық ауруы, онкологиялық және қабыну ауруларының патогенезіндегі маңызды рөлімен байланысты. Антиоксиданттық және антирадикалдық белсенділігі бар заттар осы патологиялардың алдын алу мен кешенді терапиясында кеңінен қолданылады. Алынған нәтижелер *Dracocephalum ruyschiana* өсімдігінің фармакологиялық әлеуетінің жоғары екенін көрсетіп, оны табиғи биологиялық белсенді қосылыстардың перспективасы көзі ретінде әрі қарай зерттеудің маңыздылығын дәлелдейді.

Кілт сөздер: антиоксиданттық белсенділік, радикалға қарсы белсенділік, жерүсті бөлігі, 2,2-дифенил-1-пикрилгидразил, бутилгидроксанизол, оптикалық тығыздық, *Dracocephalum ruyschiana*, ультрадыбыстық кавитация.

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Исследование антиоксидантной, антирадикальной и антимикробной активности густого экстракта *Dracocephalum Ruyschiana* L.

В статье представлены результаты исследования антиоксидантной, антирадикальной и антимикробной активности густого экстракта *Dracocephalum ruyschiana* L., полученного методом ультразвуковой экстракции из надземной части растения, произрастающего в условиях Центрального Казахстана. Целью исследования являлось комплексное изучение биологической активности ультразвуковых экстрактов *in vitro* и оценка их перспективности для дальнейшего фармацевтического применения. Установлено, что густой экстракт *Dracocephalum ruyschiana* обладает выраженной антирадикальной активностью, что свидетельствует о его способности эффективно нейтрализовать свободные радикалы и снижать интенсивность окислительных процессов. Антиоксидантные свойства экстракта обусловлены высоким содержанием экстрактивных веществ, включая биологически активные соединения фенольной природы. Особое внимание уделено применению ультразвуковой кавитации, которая обеспечивает более эффективное извлечение активных компонентов по сравнению с традиционными методами экстракции. Актуальность исследования обусловлена важной ролью свободнорадикальных процессов в патогенезе многих заболеваний, включая атеросклероз, ишемическую болезнь сердца, онкологические и воспалительные заболевания. Вещества с антиоксидантной и антирадикальной активностью широко применяются в профилактике и комплексной терапии данных патологий. Полученные результаты подтверждают значительный фармакологический потенциал *Dracocephalum ruyschiana* и обосновывают перспективность его дальнейшего изучения как источника природных биологически активных соединений.

Ключевые слова: антиоксидантная активность, антирадикальная активность, надземная часть, 2,2-дифенил-1-пикрилгидразил, бутилгидроксанизол, оптическая плотность, *Dracocephalum ruyschiana*, ультразвуковая кавитация.

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