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Antiradical activity of dry extracts of *Dracocephalum nutans* and *Dracocephalum ruyschiana* herb growing on the territory of the Central Kazakhstan

This article presents the results of our studies on the assessment of the antiradical activity of dry extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. herb in relation to the diphenylpicrylhydrazyl radical (DPPH-radical, DPPH), obtained with the use of ultrasonic extraction. This antiradical activity research method is widely used to model hyperproduction of reactive oxygen species (ROS) *in vitro* and is one of the methods that most significantly correlated with the total antioxidant activity. To obtain dry extracts, the method of ultrasonic extraction of *D. nutans* and *D.ruyschiana* aerial parts was used. The use of ultrasound has significant advantages over traditional technologies for raw materials processing. In particular, it provided the deeper penetration of the solvent into the material with a cellular structure, reduced the processing time, provided a higher product yield and reproducibility, reduced solvent consumption, increased the speed of the process, and allowed the extraction of thermolabile substances. The equipment does not require large maintenance costs; less energy is consumed for processing; as a result, the processes become more environmentally friendly and economically viable.

Keywords: Dracocephalum nutans, Dracocephalum ruyschiana, diphenylpicrylhydrazyl, DPPH, antiradical activity, ultrasonic extraction, reactive oxygen species.

Introduction

Activation of free radical processes in living organisms leads to the formation of a number of pathological conditions, such as cancer, atherosclerosis, coronary heart disease, etc. Substances with antiradical activity are used to treat and prevent free radical pathologies [1]. It is a common fact that the leading molecular-cellular mechanism of the pathogenesis of many diseases is the induction of free radical oxidation processes induced by an excess of free radicals [2, 3]. In this regard, the search for new drugs with high antioxidant potential is an urgent task.

A large number of plants, including medicinal ones, grow on the territory of the Republic of Kazakhstan. From the point of view of practical use in medicine species from genus (family *Lamiaceae* Lindl.) are of a great interest [4]. The genus *Dracocephalum* L. belongs to the family *Lamiaceae* (*Labiatae*) [5], whose plants are of interest as sources for drugs with potential biological activities. Several species from genus *Dracocephalum* is used in folk medicine. For example, *Dracocephalum moldavica* L. is used for treatment respiratory diseases, as an antipyretic, for asthenia, as apotency increasing agent. Studies showed that *Dracocephalum* species have antibacterial, antiradical, antioxidant, anticancer, antitussive, antidiarrheal, anti-inflammatory, antidiabetic, and soothing properties [6].

Dracocephalum nutans L. contains a complex of biologically active substances, and is used in the folk medicine of Southeast Asia and Tibet for kidney inflammation treatment and gastrointestinal diseases, such as hepatitis, gastritis, etc. *D. nutans* is a valuable medicinal and ornamental plant. *D. nutans* herb contains a high content of various biologically active compounds: saponins, coumarins, essential oils, traces of flavonoids, and alkaloids [7, 8].

Dracocephalum ruyschiana L. is an essential oil plant, is also used in folk medicine. This plant contains in aerial parts such active compounds, as cardenolides, tannins, coumarins, alkaloids and flavonoids. This plant is used against diseases of the respiratory tract, as an antipyretic, for asthenia, as a potency increasing agent. *D. ruyschiana* nectar has activity against gram-positive and gram-negative bacteria [9, 10].

The aim of this research is a comparative study of the antiradical activity of extracts from the aerial parts of *D. nutans* and *D. ruyschiana*.

Experimental

The objects of the study were aerial parts of *D. ruyschiana* and *D. nutans*, collected in the flowering phase. Place of raw material collection was Karkaraly mountains (Karaganda region); 1-3 decades of May, 2021 (Fig. 1). Dried plants were identified by specialists of Botany Department of Karaganda University of the name of academician E.A. Buketov. Herbarium samples are stored in herbarium fund of Karaganda Medical University.

The raw materials were dried to an air-dry state, crushed to a particle size of 2 mm, packed in kraft-paper bags and stored in a cold dry place [11].

A B

Figure 1. *Dracocephalum ruyschiana* (A) and *Dracocephalum nutans* (B) in the flowering phase

To obtain dry extracts, the method of ultrasonic extraction of *D. nutans* and *D.ruyschiana* aerial parts was used. Ultrasound destroyed the biological cellular structure, which caused the formation of cellular pores and increased the surface area of the extraction. This extraction method can be used at various stages (for raw materials and final products) and in various industries (food, biotechnology or agricultural technology). The choice of the ultrasonic method was due to the fact that in a short time (15-30 minutes) biologically active substances can be extracted from plant raw materials; by using classical methods of extraction it would take 8–24 hours [12].

The Stegler 3DT ultrasonic bath (3 L, 20-80 °C, 120W, frequency 40 kHz) was used for analysis. The raw material was extracted with a mixture of water and ethyl alcohol (in the ratio 1:1) and ethyl alcohol in the ratio of raw material-extractant — 1:10.

Ultrasonic extraction was carried out using distilled water and ethyl alcohol (at the ratio 1:1) as an extractant. The raw material was pre-soaked for 10 minutes, and then subjected to ultrasonic treatment for 30 min at room temperature (22 ºC). The extraction of raw material samples was repeated three times under the same conditions; the filtrates were combined, cooled to room temperature, and distilled off on a rotary evaporator. As a result, 8.75 g of *D. nutans* dry extract and 9 g of *D.ruyschiana* dry extract were obtained. Ultrasonic extraction was carried out at a frequency of 40 kHz.

The antiradical activity of the obtained samples was studied in relation to the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) radical [13]. An ethanol solution of DPPH (100 μM) was used to assess the antiradical activity of the studied samples in the test with DPPH radical.

To select samples with pronounced antiradical activity, 2 ml of a 100 μM ethanol solution of DPPH were 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 mg/ml. 10 minutes after adding the test sample solution to the DPPH-radical solution, the decrease in optical density was measured at 515 nm. For the test samples, interaction with DPPH-radical at final concentrations of test extracts was from 5 to 100 mg/ml. After that, the concentration of the test extract was determined, which was capable for reducing the optical density of a 100 μM DPPH-IC_{50} (DPPH) solution by 50 %.

The value of DPPH radical inhibition (in %) was calculated by the formula:

$$I = \frac{Ao - Ax}{Ao} \times 100$$

where A_0 — reference optical density of a solution that contained all reagents except the test sample; A_x — optical density of the sample.

Results and Discussion

Both extracts of studied plants showed antiradical activity. Extract of *D. ruyschiana* was more active than extract of *D. nutans*. Table 1 presents the results of screening of potential extracts for the antiradical activity.

Table 1

Optical density values for a solution of 100 µM DPPH radical after 10 minutes incubation with test extracts at a final concentration of 100 mg/ml

Sample number	Test extract	Absorbance, OD
1	Extract of *Dracocephalum nutans*	0.062
2	Extract of *Dracocephalum ruyschiana*	0.603
3	Control (DPPH solution without test sample)	0.995

Results show that the *D. nutans* plant extract is promising for further studies, as it reduced the optical density of the DPPH radical solution by more than 50 %. *D. ruyschiana* extract did not show pronounced antiradical activity under the conditions of this test system.

In the second series of the experiment, we studied the ability of *D. nutans* extract to interact with the DPPH radical at various concentrations (from 5 to 100 mg/ml).

Using the constructed calibration curves, the IC_{50} (DPPH) was determined for the test extract of *Dracocephalum nutans* L. (Fig. 2). IC_{50} (DPPH) values for *D. nutans* extract are shown in Table 2.

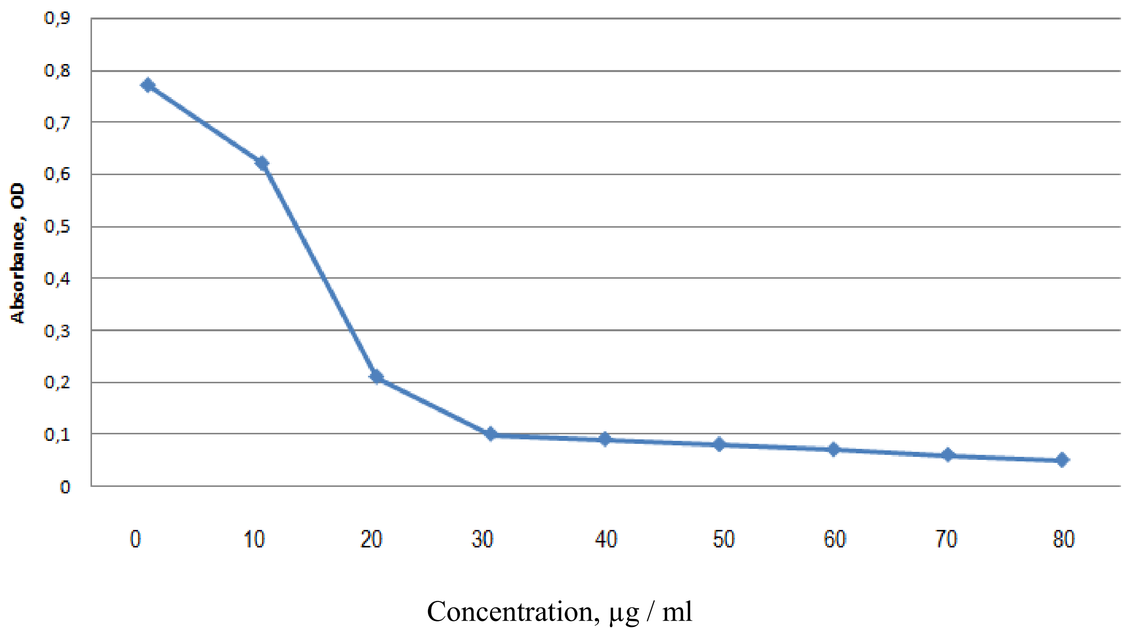

Figure 2. Calibration curve for extract of *Dracocephalum nutans*

Table 2

IC_{50} (DPPH) values for test extract of *Dracocephalum nutans*

Test extract	IC_{50} (DPPH), mg/ml
Extract of *Dracocephalum nutans*	9.8

Conclusions

The antiradical effect of the presented samples was assessed in relation to the DPPH radical. Under the conditions of this test system, the most pronounced antiradical activity was shown by the *D. nutans* extract, for which the concentration was determined that can reduce the optical density of a 100 μM solution of the DPPH radical by 50 %. For the extract of *D. nutans* IC_{50} (DPPH) was found to be 9.8 mg/ml.

Under the conditions of this test system, *D. ruyschiana* L. extract did not show pronounced antiradical activity. The data obtained indicated that the dry extract of *D. nutans* has the ability to restore biological substrates and that it exhibits a pronounced antiradical effect.

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Орталық Қазақстан аумағында өсетін *Dracocephalum nutans* және *Dracocephalum ruyschiana* шөптерінен алынған құрғақ экстрактілердің радикалғақарсы белсенділігі

Мақалада дифенилпикрилгидразил радикалына (DPPH-радикал, DPPH) қатысты ультрадыбыстық экстракцияны қолдану арқылы алынған *Dracocephalum nutans* L. және *Dracocephalum ruyschiana* L. шөптерінен құрғақ сығындылардың антирадикалдық белсенділігін бағалау бойынша зерттеулердің нәтижелері берілген. Радикалға қарсы белсенділікті зерттеудің бұл әдісі *in vitro* оттегінің белсенді түрлері (ОБТ) гиперөндірісін модельдеу үшін кеңінен қолданылады және жалпы антиоксиданттық белсенділікті бағалаумен ең маңызды корреляциялық әдістердің бірі болып табылады. Құрғақ сығындыларды алу үшін *D. nutans* және *D. ruyschiana* жерүсті бөліктерін ультрадыбыстық экстракциялау әдісі қолданылды. Ультрадыбысты қолдану шикізатты өңдеудің дәстүрлі технологияларына қарағанда айтарлықтай артықшылықтарға ие. Атап айтқанда, ол еріткіштің жасушалық құрылымы бар материалға тереңірек енуін қамтамасыз етті, өңдеу уақытын қысқартады, өнімнің жоғары өнімділігі мен қайталануын қамтамасыз етеді, еріткіштің шығынын азайтады, процестің жылдамдығын арттырады және термолабильді заттардың экстракциясына мүмкіндік береді. Жабдық көп техникалық қызмет көрсетуді қажет етпейді; қайта өңдеуге аз энергия жұмсалады; нәтижесінде бұл процестер экологиялық таза және экономикалық тұрғыдан тиімді болады.

Кілт сөздер: Dracocephalum nutans, Dracocephalum ruyschiana, дифенилпикрилгидразил, DPPH, радикалғақарсы белсенділік, ультрадыбыстық экстракция, оттегінің белсенді түрлері.

А. Сабиева, Г.А. Атажанова, М.К. Смагулов, Г.К. Курмантаева, Б.Б. Аширбекова

Антирадикальная активность сухих экстрактов из трав *Dracocephalum nutans* и *Dracocephalum ruyschiana,* произрастающих на территории Центрального Казахстана

В статье представлены результаты исследований по оценке антирадикальной активности сухих экстрактов из трав *Dracocephalum nutans* L. и *Dracocephalum ruyschiana* L., полученных с применением ультразвуковой экстракции, в отношении дифенилпикрилгидразильного радикала (DPPH-радикал, DPPH). Этот метод исследования антирадикальной активности широко используется для моделирования гиперпродукции активных форм кислорода (АФК) *in vitro* и является одним из методов, наиболее значимо коррелирующих с оценкой общей антиоксидантной активности. Для получения сухих экстрактов использовали способ ультразвуковой экстракции надземных частей *D. nutans* и *D.ruyschiana*. Использование ультразвука имеет значительные преимущества перед традиционными технологиями обработки сырья. В частности, оно обеспечивало более глубокое проникновение растворителя в материал с ячеистой структурой, сокращало время обработки, обеспечивало более высокий выход продукта и воспроизводимость, снижало расход растворителя, увеличивало скорость процесса и допускало экстракцию термолабильных веществ. Оборудование не требует больших затрат на обслуживание; расходуется меньше энергии на переработку; в результате эти процессы становятся более экологичными и экономически жизнеспособными.

Ключевые слова: Dracocephalum nutans, Dracocephalum ruyschiana, дифенилпикрилгидразил, DPPH, антирадикальная активность, ультразвуковая экстракция, активные формы кислорода.

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