

## ANNOTATION

Ramazanova Assel's dissertation work on the topic «**Development of new antimicrobial medicines based on *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L.**», submitted for the degree of Doctor of Philosophy (PhD) in the specialty 8D07201 – «Pharmaceutical production technology»

### **Relevance of the research topic**

One of the current priority areas of pharmaceutical activity is to ensure the population of the Republic of Kazakhstan with relatively safe, high-quality, effective, and affordable domestic pharmaceuticals. To implement this direction, research is needed on the effective use of domestic natural raw materials in the pharmaceutical industry. In accordance with the People's Address of the President of the Republic of Kazakhstan “Kazakhstan in a new reality: time for action” and the Order of the Prime Minister of the Republic of Kazakhstan on the “Comprehensive Plan for the Development of the Pharmaceutical and Medical Industry for 2020-2025”, as well as the Development Resolutions of the Republic of Kazakhstan on the “Concept for the Development of the Manufacturing Industry for 2023-2029” increasing innovation Acquaintance with the creation of domestic medicines using medicinal plants produced on the territory of the Republic of Kazakhstan, as well as the development of the pharmaceutical cluster by increasing the competitiveness of domestic products and entering foreign markets.

Currently, the arsenal of the pharmaceutical market has significantly expanded with highly effective herbal medicinal products. More than 1000 essential oil plants grow in the flora of Kazakhstan. Some species from the Lamiaceae family are of great interest, as they have previously been poorly studied or have only limited information available about their chemical composition and biological properties. In this regard, the Lamiaceae L. family is of particular interest, being one of the leading families in the flora of Kazakhstan. In fact, 233 species of this family are found in the territory of the Republic.

Plants of the genus *Dracocephalum* are the most well-known and are a rich and widespread source of essential oils. According to The Plant List database (as of August 2020), the genus consists of 74 species, with 20 species growing in Kazakhstan. Studies have shown that some species of *Dracocephalum* have antibacterial, antitussive, antidiarrheal, antioxidant, anticancer, anti-inflammatory, antidiabetic, and soothing properties.

The essential oil of *Dracocephalum moldavica* L. possesses pronounced antimicrobial properties, and its chemical composition consists of limonene, caryophyllene oxide, 1,8-cineole, verbenone, perillyl alcohol, nerol, and geranyl acetate.

Previously, the Kazakhstani species of *Dracocephalum* had not been studied for their content of biologically active compounds. Therefore, the targeted search for new and effective antimicrobial drugs based on *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L., which grow in Central Kazakhstan, is both scientifically and practically relevant and represents a promising direction for development.

In folk medicine, due to the high content of various biologically active compounds (essential oils, flavonoids, saponins, coumarins, polyphenolic acids), extracts of *Dracocephalum nutans* L. are widely used in the treatment of diseases such as kidney inflammation, hepatitis, gastritis, and others. In Tibetan traditional medicine, a decoction of the aerial parts of the herb is used for kidney inflammation, hepatitis, gastritis, while in Mongolian traditional medicine it is used for gastrointestinal diseases.

*Dracocephalum ruyschiana* L. is an aromatic plant used in folk medicine. It is employed for respiratory tract diseases and as a fever-reducing remedy. It is reported that extracts of *Dracocephalum ruyschiana* are used as a hemostatic, anti-inflammatory, analgesic, and diuretic agent.

Therefore, the search and development of new antimicrobial drugs based on *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. represent a relevant and promising direction.

**The purpose of the dissertation research:** Development of domestic antimicrobial drugs based on the plant material of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L., along with studying their biological activity profile and safety.

**Research objectives:**

1. Development of harvesting technology, preparation of raw materials, and pharmacognostic research on the aerial parts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L.
2. Determination of quality specifications and shelf life of the plant material of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L.
3. Development of an optimal technology for obtaining extracts from the plant material of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L., studying their chemical composition, and screening samples for biological activity.
4. Extraction of essential oils from the herb of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L., studying their chemical composition using GC-MS, developing quality specifications, and determining shelf life.
5. Development of the composition and technology for obtaining a gel based on the essential oil of *Dracocephalum nutans* L.
6. Investigation of the acute toxicity of the essential oil of *Dracocephalum nutans* L.
7. Screening samples of the essential oil of *Dracocephalum nutans* L. and the gel based on it for antimicrobial activity.

**Research methods:** Pharmacopoeial, pharmacognostic, pharmacological, biological, statistical.

**Objects of research:** Plant material: *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. collected in the territory of Central Kazakhstan; concentrated extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L., essential oil of *Dracocephalum nutans* L. and a gel based on it.

**The subject of the study:** Resource science data, biomorphological characteristics, diagnostic features, numerical indicators of the raw material, and chemical composition of *Dracocephalum nutans* L. (Nodding Dragonhead) and

*Dracocephalum ruyschiana* L. (Ruysch's Dragonhead); concentrated extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. obtained by ultrasonic extraction; chemical composition and biological properties of concentrated extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. obtained by ultrasonic method; essential oil of *Dracocephalum nutans* L. and gel based on it; safety assessment of the essential oil; investigation of the biological activity of the essential oil and the gel based on it; development of regulatory documents; compiling final materials determining the theoretical and practical value of the dissertation.

#### **The main provisions submitted for protection:**

1. Results of pharmacognostic analysis of the aerial parts of the plant material of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. and standardization results;

2. Component composition and biological activity of concentrated extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L.;

3. Component composition, acute toxicity, antimicrobial activity of the essential oil of *Dracocephalum nutans* L. and standardization results;

4. Antimicrobial activity and technology of obtaining a gel based on the essential oil of *Dracocephalum nutans* L. and standardization results..

#### **Scientific novelty of the research:**

As a result of scientific research for the first time:

- the chemical composition of the essential oil of *Dracocephalum nutans* L., obtained by hydrodistillation, has been determined, with the main components being: 1,8-cineole - 34%, 2-bornanone - 9%, endobornol - 2.5%, camphene - 2.6%,  $\alpha$ -pinene - 2.6%;

- the antimicrobial, antifungal activity, and acute toxicity of the essential oil of *Dracocephalum nutans* L. have been studied;

- a technology for obtaining an antimicrobial gel based on the essential oil of *Dracocephalum nutans* L. has been developed;

- the antimicrobial activity of the gel based on the essential oil of *Dracocephalum nutans* L. has been determined.

The scientific novelty of the research is confirmed by a utility model patent under registration number №8038 dated May 5, 2023: "Application of the essential oil of *Dracocephalum nutans* L. as an antimicrobial agent."

#### **Practical significance of the results obtained**

As a result of the conducted research, the raw material reserves of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. in the territory of Central Kazakhstan have been determined.

Based on the results obtained during the dissertation research, the medicinal plant material of two species is recommended: the herb of *Dracocephalum nutans* L. and the herb of *Dracocephalum ruyschiana* L.

Moreover, processing technology and quality specifications have been developed, along with draft regulatory documents for the medicinal plant materials "Herb of *Dracocephalum nutans* L." and "Herb of *Dracocephalum ruyschiana* L."

By hydrodistillation, essential oil from *Dracocephalum nutans* L. has been obtained and its chemical composition has been determined using the GC-MS method.

Based on the pharmaceutical substance (essential oil of *Dracocephalum nutans* L.), a medicinal product in the form of an antimicrobial gel has been developed. According to the results of non-clinical trials, the pharmaceutical substance does not possess toxic properties and is recommended as an antimicrobial agent. A laboratory regulation has been developed, and draft regulatory documents for the production of the antimicrobial gel have been prepared.

The results of the scientific research on the pharmacognostic study of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. have been implemented in the educational process of the School of Pharmacy of the North Kazakhstan region at the Medical University of Karaganda.

#### **Doctoral student's personal contribution:**

The author conducted all the results of the dissertation research independently and is a personal contribution of the doctoral student to the technology of pharmaceutical production.

#### **Conclusions:**

As a result of the dissertation research, the following conclusions were made:

1.1 The distribution and resources of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. have been studied. The research conclusions indicate that the herbs *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. are widely distributed in Kazakhstan, including the Karaganda region. According to the research results, the exploitable reserves of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. are 14.5 tons and 9.5 tons, respectively, with a potential annual harvest volume of 7.3 tons and 5.7 tons, respectively.

1.2 Pharmacognostic features of the plant materials *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. have been determined: macroscopic and microscopic analysis of the raw materials have been conducted. The histochemical composition has been identified.

Identification parameters have been determined based on the results of histochemical and phytochemical analysis.

For the first time, an examination of the aerial organs of *D. ruyschiana* L. and *D. nutans* L. has been conducted using light microscopy in combination with histochemical tests. As a result of the conducted histochemical tests on transverse sections of leaves, stems, and superficial flower sections of *D. ruyschiana* L. and *D. nutans* L., essential oils, phenolic acids, flavonoids have been found, and their localization has been established.

- Essential oils are located in the oil glands and capitate hairs.

- Phenolic acids are found in the sclerenchyma and chlorenchyma of the leaves, the cortex and conducting zone of the stem, the conducting zone, and mechanical tissue of the petiole.

- Flavonoids are present in the leaf mesophyll, corner collenchyma, epidermis, chlorenchyma, and vascular bundles of the stem, as well as in the chlorenchyma and vascular bundles of the petiole.

1.3 A technology for the collection and preparation of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. has been developed.

2.1 Quality standards for *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. have been developed, and shelf life has been determined. Over the 24-month stability study period of the plant materials *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. in their primary packaging, the quality stability indicators remained within the regulated limits. The obtained data has been included in the draft regulatory documentation for the raw materials.

3.1 Parameters of the extracts obtained using the ultrasonic method for *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. have been determined: The highest product yield is achieved by subjecting air-dried raw materials, ground to a particle size of 3-5 mm, to dual ultrasound-assisted extraction with 70% ethanol at a frequency of 40 kHz for 30 minutes. A relatively high yield of dense extract obtained from ultrasonic extraction for *Dracocephalum nutans* L. was 4.5 g, and for *Dracocephalum ruyschiana* L. it was 4.2 g. Percolation method yielded 2.0 g and 2.5 g for *Dracocephalum ruyschiana* L. and *Dracocephalum nutans* L., respectively.

3.2 The chemical composition of the extracts was determined using an HPLC method. The extracts of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. show similarities in the qualitative composition of phenolic compounds, but there are significant differences in the quantitative composition of phenolic acids and flavonoids. In the extracts of *Dracocephalum ruyschiana* L. and *Dracocephalum nutans* L., the dominant phenolic compounds are rosmarinic acid (44.76 and 19.54 mg/g, respectively). Additionally, in the extract of *Dracocephalum nutans* L., the dominant component is quercetin-3'-glucoside (isoquercetin) at 47.96 mg/g.

3.3 In the screening of extracts from *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. obtained using ultrasound-assisted extraction for biological activity, it was established that the extracts from both species exhibit weak antimicrobial activity against the gram-positive bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, the gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, and the yeast *Candida albicans*.

However, due to the lack of pronounced biological activity, these extracts were deemed unpromising for further development of a technology for antimicrobial agents based on them.

4.1 Essential oils of *Dracocephalum nutans* L. and *Dracocephalum ruyschiana* L. were obtained by hydrodistillation:

As a result, the yield of essential oil of *Dracocephalum nutans* L. was 0.6%, and only traces of essential oil were found in the herb *Dracocephalum ruyschiana*, therefore, further study of its chemical composition, screening for biological activity and the development of a drug based on it was impossible.

4.2 The chemical composition of the essential oil of *Dracocephalum nutans* L. was studied, where the main components are: 1,8-cineol-34%, 2-bornanone – 9%, endo-borneol - 2.5%, camphene – 2.6%,  $\alpha$ -pinene-2.6%.

4.3 The quality specification of the essential oil of *Dracocephalum nutans* has been developed and the shelf life has been determined.

5. The optimal composition and technology for obtaining an antimicrobial gel based on essential oil of *Dracocephalum nutans* L. has been developed.

Ingredients:

• \* \* \* active substance - essential oil of *Dracocephalum nutans* L.-5 ml, excipients: sodium carboxymethylcellulose (20.0 g) - gel educator, glycerin (10 ml) - plasticizer, twin 80-emulsifier (1 ml) and purified water.

The gel quality specification has been developed and the shelf life has been determined.

A feasibility study was carried out for the production of essential oil of *Dracocephalum nutans* and a gel based on it. The total cost per unit of production was 1084.6 tenge, the retail price was 1409.9 tenge. The payback period for the production of a gel based on the essential oil of *Dracocephalum nutans* L. with a profitability of 30% was 3 years and 4 months.

6. During the analysis of the results of observations of experimental mice for 7 and 14 days after acute administration of the essential oil substance in doses of 500, 1000, 1500 mg / kg, there were no negative effects on the appearance, general condition, body weight and behavior of animals, as well as negative effects on the biochemical parameters of blood and basic physiological the functions of the body. It has been established that the essential oils of *D. nutans* belong to the group "Practically non-toxic" (class V - low-toxic).

7. As a result of a study on antimicrobial activity, it was found that the essential oil of *Dracocephalum nutans* L. and a gel based on it exhibit pronounced antimicrobial activity against strains of Gram-positive bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and weak activity against Gram-negative bacteria *E. coli*, *Pseudomonas aeruginosa* and weak activity against gram-negative bacteria *E. coli*, *Pseudomonas aeruginosa* yeast fungus *Candida albicans*.

#### **Approbation of the dissertation results**

The results and main positions of the scientific work were presented at:

1. International conference dedicated to the 100th anniversary of the Department of Analytical Chemistry "Modern Aspects of Drug Development" (Kharkiv, Ukraine, 2021);

2. Student Scientific and Practical Conference "Pharmacy Blossom!" (Karaganda, 2023);

3. XI International Scientific and Practical Conference "Priorities of Pharmacy and Dentistry: From Theory to Practice" (Almaty, November 4, 2022).

#### **4. Publications:**

Based on the materials of the dissertation, 7 printed works have been published, including: 3 articles in an international scientific publication included in the Scopus international database (percentile - 58%); 1 in a publication

recommended by the Committee on Control of Medical and Pharmaceutical Activities of the Ministry of Health of the Republic of Kazakhstan; abstracts of 3 reports in the materials of international conferences. A patent for a utility model has been obtained.

The volume and structure of the dissertation: The dissertation is presented in 136 pages of computer text, including 160 literature sources. The dissertation is illustrated by 45 tables and 23 figures and includes 13 appendices.