**ANNOTATION**

of the dissertation work on the topic

**"Pharmacognostic analysis of medicinal plant raw material *Onosma gmelinii* and pharmaceutical development of a drug based on it"**

for the degree of Doctor of Philosophy (PhD) in the specialty

6D074800 – "Technology of Pharmaceutical Production" of

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**Relevance of the research topic**

According to the decree of the Government of the Republic of Kazakhstan dated November 24, 2022, No. 945 "On Approval of the Concept of Healthcare Development of the Republic of Kazakhstan until 2026", the share of domestically produced medicines in the pharmaceutical market of Kazakhstan was 23.4 % in 2021, and is represented mainly by generic drugs, while ensuring national drug safety should be at least 30 %. The issue of a shortage of domestically produced medicines and the need to develop its own pharmaceutical industry has become especially relevant in the context of the recent coronavirus infection (COVID-19) pandemic, when all countries imposed bans on the export of medicines. The key role in the import substitution of medicines in the domestic market is played by pharmaceutical enterprises based on the development of new drugs from domestic raw materials.

The Republic of Kazakhstan has rich natural resources, including plant diversity, which is a valuable source of biologically active compounds. The use of plant raw materials for the development of new drugs is one of the key directions in the development of the pharmaceutical industry in the Republic of Kazakhstan.

Natural compounds and medicinal preparations from them have a number of undeniable advantages over synthetic analogues, as they are less toxic and more effective in treating various diseases, and they also have a wider range of biological effects on the human body.

Plants of the genus *Onosma* comprise a group of perennial herbaceous plants, subshrubs, and prostrate subshrubs belonging to the family *Boraginaceae*. Almost all species of the genus *Onosma* are widely used as medicinal plants for treating various diseases. The beneficial properties of these plants are attributed to the presence of components with pharmacological effects in their composition, such as naphthoquinones, flavonoids, alkaloids, saponins, and higher fatty acids. *Onosma* is actively used in both official and traditional medicines, providing sedative, hypotensive, and diuretic effects, as well as having antiseptic, anti-inflammatory, and antimicrobial properties. Of particular interest in this regard is the wild plant *Onosma gmelinii*, which grows in Kazakhstan and whose chemical composition and properties are poorly studied. The roots of the plant, which contain biologically active substances such as naphthoquinones, are of practical scientific interest. Derivatives of these compounds are associated with many pharmacological properties of the plant, including antioxidant, antitumor, antimicrobial, antiviral, and anti-inflammatory effects.

In this regard, a comprehensive study of the component composition and pharmacological properties of *Onosma gmelinii*, as well as the development of a drug

containing an extract from the plant, represents a timely, promising, and relevant direction.

**The aim of the dissertation research:** pharmacognostic analysis of the medicinal plant raw materials from *Onosma gmelinii*, obtaining and researching *Onosma gmelinii* extract for the pharmaceutical development of a drug based on it.

**Research objectives:**

- carry out the collection and pharmacognostic analysis of medicinal plant raw materials from *Onosma gmelinii*;
- obtain and screen extracts from the medicinal plant materials of *Onosma gmelinii*;
- standardize the extract from the medicinal plant raw materials of *Onosma gmelinii*;
- conduct pharmaceutical development of an ointment with the dry extract of *Onosma gmelinii*;
- study the safety and pharmacological efficacy of the dry extract of *Onosma gmelinii* and the ointment with the extract *in vivo* studies.

**Research methods:** pharmaceutical-technological, pharmacognostic, physical, physicochemical, pharmacological, statistical.

**Objects of research:** medicinal plant raw material – above-ground and below-ground parts of *Onosma gmelinii*; pharmaceutical substance of plant origin – extracts obtained from plant raw materials of *Onosma gmelinii*; medicinal plant preparation – ointment with the extract from plant raw materials of *Onosma gmelinii*.

**Subject of the research:** analysis of the growing area and determination of pharmacognostic characteristics of plant raw materials from *Onosma gmelinii*, and its standardization; development of technology for obtaining extracts, studying their phytochemical composition and biological activity; development of technology for obtaining ointment with the extract from the roots of *Onosma gmelinii*; study of the safety and pharmacological properties of the extract obtained by ultrasound-assisted extraction and the ointment with the dry extract.

**The main provisions of the dissertation research submitted for defense:**

1) results of the pharmacognostic study of medicinal plant raw materials from *Onosma gmelinii*;

2) results of research on the development of technology for obtaining extracts from the root of *Onosma gmelinii*;

3) results of pharmaceutical development of ointment with the dry extract from the root of *Onosma gmelinii*;

4) results of the safety and pharmacological efficacy of the dry extract from the root of *Onosma gmelinii* and ointment with the dry extract.

**Scientific novelty:**

- for the first time in Kazakhstan, a pharmacognostic study was conducted in a poorly studied plant of the species *Onosma gmelinii*, which belongs to the genus *Onosma* of the family *Boraginaceae*;
- dry extracts were obtained from medicinal plant raw materials of *Onosma gmelinii* by percolation and ultrasound-assisted extraction, as well as a thick extract by supercritical carbon dioxide extraction;

- the chemical composition, safety, and effectiveness were studied *in vitro*. The extract obtained by ultrasound-assisted extraction, which has a wider spectrum of biologically active compounds and has demonstrated antimicrobial and antiviral properties, was selected as the optimal one;

- the optimal technology for an ointment with dry extract from the root of *Onosma gmelinii* was developed;

- safety and effectiveness studies of the dry extract from the root of *Onosma gmelinii* and ointment with dry extract were conducted, proving the expressed antimicrobial and wound-healing properties of the ointment *in vivo* studies.

The scientific novelty of the research is confirmed by the patent for utility model under registration number № 5972 dated April 09, 2021 "Method for obtaining dry extract from plant raw material *Onosma gmelinii*".

**Practical significance of the research:**

- the technology for collecting and harvesting medicinal plant raw material, *Onosma gmelinii*, is recommended. Identification was confirmed by the Department of Biodiversity and Bioresources of the Faculty of Biology and Biotechnology, al-Farabi KazNU, Almaty, RK. Reference number: № 12-23/619;

- a phytosanitary examination of medicinal plant raw materials for the presence of harmful quarantine organisms was carried out at the state institution "Territorial Inspection of the Committee of State Inspection in the Agroindustrial Complex in Almaty city of the Ministry of Agriculture of the Republic of Kazakhstan". Phytosanitary certificate number: O702/2019102800586977;

- a draft Regulatory Document (RD) for medicinal plant raw materials – root of *Onosma gmelinii* was developed;

- a method of obtaining an extract by percolation from *Onosma gmelinii* was developed and implemented at the Department of Pharmaceutical Technology of NJSC "Asfendiyarov Kazakh National Medical University";

- a method of obtaining an extract by ultrasound-assisted extraction from *Onosma gmelinii* was developed and implemented into the pilot production at JSC "Scientific Center for Anti-infectious Drugs";

- a draft Regulatory Document (RD) for the pharmaceutical substance of plant origin – dry extract from the root of *Onosma gmelinii* obtained by ultrasound-assisted extraction was developed;

- a draft technological instruction in the production of dry extract from the root of *Onosma gmelinii* obtained by ultrasound-assisted extraction was developed;

- for the first time, the optimal composition and technological scheme for the production of ointment with dry extract from the root of *Onosma gmelinii* was developed and implemented at the Department of Pharmaceutical Technology, NJSC "Asfendiyarov Kazakh National Medical University";

- for the first time, the optimal composition and technological scheme for the production of an ointment with dry extract from the root of *Onosma gmelinii* was developed and implemented into the pilot production at JSC "Scientific Center for Anti-infective Drugs";

- a draft Regulatory Document (RD) for a medicinal herbal preparation – ointment with dry extract from the root of *Onosma gmelinii* was developed;

- a draft technological instruction for an ointment with dry extract from the root of *Onosma gmelinii* was developed;

- the results of the dissertation work were implemented in the educational and research process of the Department of Pharmacognosy with the course of botany and elements of phytotherapy at the federal state budgetary educational institution of higher education “Bashkir State Medical University”.

**Doctoral personal contribution**

The dissertation author independently conducted an extensive review and analysis of both domestic and foreign literature and performed experimental work corresponding to the objectives. This is confirmed by research results obtained in both laboratory and production conditions using modern equipment and literature.

The reliability and validity of the obtained results are confirmed by the focus on solving current problems in pharmaceutical science and practice, particularly in the development of new domestically produced drugs, as well as by conducting research in a modern research center and the development of draft regulatory documents.

**Conclusion**

*Onosma gmelinii* was collected and harvested in accordance with the requirements of “Good Agricultural and Collection Practices (GACP)”. Pharmacognostic analysis of both the root and above-ground parts of the *Onosma gmelinii* plant was carried out. Pharmaceutical and technological parameters of the medicinal plant raw materials were determined to select the optimal extraction technology for maximum extraction of biologically active substances (BAS). The qualitative and quantitative analyses of BAS from *Onosma gmelinii* plant raw material revealed the presence of flavonoids, polysaccharides, tannins, alkaloids, saponins, naphthoquinones, essential oils, esters, organic, and phenolic compounds in both the root and the above-ground parts of the plant. The mineral and amino acid compositions of the medicinal plant raw materials were studied. Based on the results obtained, particularly the total quantitative content of biologically active substances and the quantitative content of specific BAS from plants of the genus *Onosma* – naphthaoquinones (in the root – 1.579 %; in the aboveground part – 0.075 %), it was decided to use the roots of *Onosma gmelinii* as the medicinal plant raw material. Quality parameters were determined, and a quality specification for *Onosma gmelinii* root was developed. The results of a long-term study of the stability of *Onosma gmelinii* at a temperature not exceeding (25 ± 2) °C and relative humidity not exceeding (60 ± 5) % established its shelf life to be 36 months.

Technologies were developed, and technological schemes for obtaining extracts from the roots of *Onosma gmelinii* by percolation, ultrasound-assisted, and carbon dioxide extraction methods were presented. The component composition and biological activity of the obtained extracts were studied. According to the results obtained, the extract produced by ultrasound-assisted extraction, with antiviral, antibacterial, and antifungal activities, was selected for the production of the ointment. The quality specification of the dry extract from the root of *Onosma gmelinii* obtained by ultrasound-assisted extraction was developed, including: description, identification of naphthoquinones, solubility, loss in mass during drying, heavy metals, microbiological purity, quantitative determination of naphthoquinones,

packaging, labeling, transportation, storage, shelf life, and main pharmacological effect. The shelf life was established under long-term test conditions at a temperature not exceeding (25 ± 2) °C and relative humidity not exceeding (60 ± 5) % over three series – 2 years. Validation of the methodology for the quantitative determination of shikonin was carried out according to the validation characteristics: linearity, specificity, accuracy, and reproducibility.

Pharmaceutical development of an ointment with dry extract from the root of *Onosma gmelinii* was carried out. The optimal composition and technology for ointment production were selected, which includes: dry extract of *Onosma gmelinii* root obtained by ultrasonic extraction (0.3 g), base – vaseline (90.0 g), emulsifier – Tween 80 (3.0 g), and solvent – purified water (6.7 ml). The quality specification of the ointment with dry extract of *Onosma gmelinii* root has been developed. The norms of naphthoquinone content in the ointment were established at a level from 0.0088 % to 0.0132 % (0.0110 % with an acceptable interval of ±20 %). During long-term studies of ointment stability (temperature (25 ± 2) °C, relative humidity (60 ± 5) %), qualitative and quantitative indicators, pH of aqueous extract, and microbiological purity were within the established permissible limits. The shelf life is set at 18 months.

The safety and effectiveness of *Onosma gmelinii* dry extract and ointment with extract were tested *in vivo* studies. According to the results of an acute toxicity study of the extract on white mongrel bisexual mice, the extract belongs to the 4th class of low-toxic substances. Tests of an acute toxicity of the ointment with dry extract of *Onosma gmelinii* root did not reveal significant deviations in the behavior of animals, indicating the low toxicity of the study drug. When studying the allergenic effect of *Onosma gmelinii* root extract and ointment with dry extract on rabbits, no allergic reaction was observed at the application sites. The developed ointment exhibits high antimicrobial and wound-healing activity *in vivo* experiments, reducing the time of wound healing by 1.5 and 2 times compared to the use of comparison drugs ointment “Levomecol” and cream “Terfalin 1%”, respectively.

**Approbation of the dissertation results**

The main results of the dissertation research were published and reported in the materials of various conferences including the VII scientific and practical with international participation “Priorities of pharmacy and dentistry: from theory to practice” (Almaty, 2018); the IV international scientific and practical conference “GLOBAL SCIENCE AND INNOVATIONS 2019: CENTRAL ASIA”. (Astana, 2019); the International scientific and practical conference of students, young scientists and teachers “Akanov Readings: the role of primary health care in achieving universal coverage of services” (Almaty, 2019) and the III international scientific and practical conference dedicated to the memory of Professor R.D. Dilbarkhanov “Formation and prospects of development of the scientific school of pharmacy: continuity of generations” (Almaty, 2020).

**Publications**

The results of the dissertation research were published in 12 scientific papers, including:

- an article in an international journal included in the Scopus database – 1;

- articles in journals recommended by the Committee for Quality Assurance in the Field of Science and Higher Education– 4;

- abstracts in the materials of international scientific and practical conferences – 6;

- patent for utility model – 1.

**Scope and structure of the dissertation**

The dissertation includes 169 pages of typewritten text in a computer set, 47 tables, 53 figures, 129 domestic and foreign literature, as well as applications. The work consists of an introduction, literature review, materials and methods, 4 sections of the experimental part, conclusions by section and conclusion.