

ANNOTATION

of the dissertation titled

“Pharmaceutical development of medicinal products based on herbal raw material of *Cetraria islandica* (L.) Ach.”

submitted for the degree of Doctor of Philosophy (PhD)

in the specialty 8D10102 – “Pharmacy”

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Relevance of the Research Topic. Under current conditions, one of the priority areas of state policy in the healthcare and pharmaceutical sectors of the Republic of Kazakhstan is the development of domestic pharmaceutical production, the reduction of dependence on imported drugs, and the introduction of innovative technologies for the creation of effective and safe medicinal products. In accordance with the National Development Plan of the Republic of Kazakhstan until 2029, one of the key priorities in the implementation of strategic directions is the prevention of non-communicable diseases and the advancement of local pharmaceutical manufacturing.

The full and rational utilization of unique representatives of Kazakhstan’s native flora, the establishment of competitive and high-performance production of phytopharmaceuticals, the reduction of reliance on imported medicinal products, and the enhancement of the country’s pharmaceutical production capacity, raw material base, and scientific and technological potential are essential components of economic modernization and improving the population’s quality of life. Currently, domestically produced medicinal products account for only 14.4% of the national pharmaceutical market, indicating a high level of import dependency (86.6% in monetary terms and 73.8% in physical volume). This situation highlights the urgent need for the active development and implementation of domestically manufactured medicinal products, including those based on medicinal plant materials [1–3].

In this context, the exploration of the therapeutic potential of Kazakhstan’s natural resources becomes especially relevant. One of the promising species is *Cetraria islandica* (L.) Ach., a member of the Parmeliaceae family, which is widely distributed in the mountainous and foothill regions of the country and exhibits pronounced pharmacological activity. According to scientific literature, the lichen acids present in *Cetraria islandica* (L.) Ach. possess antiseptic, anti-inflammatory, immunomodulatory, and antioxidant properties, supporting the rationale for its use in the treatment of inflammatory diseases of the upper respiratory tract and a number of other conditions [4].

Global and domestic practices in the development of herbal medicines consistently demonstrate a strong interest in the creation of standardized extracts and innovative dosage forms based on them. In light of the need for import substitution and ensuring pharmaceutical security, the development of an effective and safe medicinal product based on *Cetraria islandica* (L.) Ach. that complies with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, the standards of the Eurasian Economic Union (EAEU), and international quality norms is particularly significant [5].

The relevance of this dissertation research lies in the necessity of conducting a comprehensive pharmacognostic study of *Cetraria islandica* (L.) Ach. thalli, developing an optimal extraction technology, standardizing biologically active substances, and evaluating both pharmacological activity and toxicological safety. Furthermore, the development of a pharmaceutical form in the form of a spray represents a modern therapeutic approach, ensuring localized therapeutic action, high bioavailability, and ease of use, which aligns with current global trends in the treatment of respiratory diseases.

Thus, the pharmaceutical development of a medicinal product based on *Cetraria islandica* extract corresponds to the priority areas of the national healthcare strategy, promotes the rational use of natural resources, contributes to the development of domestic pharmaceutical science and industry, and opens up opportunities for integrating Kazakhstani innovations into the global pharmaceutical landscape.

Research Objective. The aim of the dissertation research is to provide scientific justification and develop approaches for the creation of a medicinal product based on standardized plant raw material of *Cetraria islandica* (L.) Ach.

Research Tasks:

1. To procure raw material of *Cetraria islandica* (L.) Ach. in accordance with GACP requirements, develop a harvesting protocol, and conduct a comprehensive pharmacognostic study of the thalli, including morphological, anatomical, and phytochemical analyses.
2. To develop an optimal extraction technology for *Cetraria islandica* (L.) Ach., justify the choice of extraction method, standardize the extract, and evaluate its stability.
3. To assess the toxicological safety and biological activity of the *Cetraria islandica* (L.) Ach. extract within the framework of preclinical studies.
4. To develop a production technology for a spray dosage form based on *Cetraria islandica* extract, perform quality control, and evaluate the stability of the finished medicinal product.
5. To carry out a techno-economic feasibility study for the production and potential introduction of the developed medicinal product into the pharmaceutical market of the Republic of Kazakhstan.

Objects of the Study: The objects of the study are the medicinal plant raw material *Cetraria islandica* (L.) Ach., the extracts obtained from this raw material, and the medicinal product developed on their basis—a locally applied spray.

Subject of the Study: The subject of the study includes the pharmacognostic investigation of *Cetraria islandica* (L.) Ach. raw material, the development of the extraction technology and spray dosage form, their standardization, evaluation of toxicological safety, biological activity, quality, and stability, as well as the techno-economic justification for the production of the spray containing *Cetraria islandica* extract.

Key Provisions of the Dissertation Submitted for Defense

- A comprehensive pharmacognostic study of *Cetraria islandica* (L.) Ach. thalli collected in the Karaganda region of the Republic of Kazakhstan, including

morphological and anatomical analysis using light, fluorescence, and scanning electron microscopy, as well as energy-dispersive X-ray spectroscopy (EDS);

- Scientifically substantiated results of the development and optimization of the extraction technology for *Cetraria islandica*, demonstrating the efficiency and reproducibility of the process (Appendix B);
- Comprehensive data on the toxicological safety and biological activity of *Cetraria islandica* extract, confirming its potential for use in medical practice;
- The results of the pharmaceutical development of a spray dosage form based on *Cetraria islandica* extract, including the rationale for the choice of dosage form and its characteristics.

Summary of Key Research Findings:

The dissertation is devoted to the pharmaceutical development of medicinal products based on the medicinal plant raw material *Cetraria islandica* (L.) Ach. The study involved a comprehensive pharmacognostic evaluation, the development of an optimal extraction technology, and the assessment of the biological activity of the resulting extracts. The obtained data confirm the high pharmacological value of *Cetraria islandica* (L.) Ach. as a renewable source of biologically active compounds.

A harvesting and procurement protocol was developed and tested in compliance with the principles of sustainable resource use and the requirements of Good Agricultural and Collection Practice (GACP). The collection was carried out in accordance with the Law of the Republic of Kazakhstan "On the Plant World," ensuring the rational use of natural resources and the preservation of ecosystems.

A morphological and anatomical study revealed that the thallus of *Cetraria islandica* (L.) Ach. is heteromerous and foliose, with a height of 12–15 cm, characterized by distinct cortical and photobiont layers. Histochemical reactions confirmed the presence of usnic and fumarprotocetraric acids, highlighting the pharmacognostic significance of the species.

Phytochemical analysis identified the presence of polysaccharides and phenolic compounds; the content of usnic acid was determined to be 2.98% relative to dry weight. Quality control results—including physicochemical, microbiological, and toxicological parameters—met regulatory requirements, allowing the raw material to be classified as a standardized pharmaceutical substance.

Seventy percent ethanol was identified as the optimal extractant for obtaining a thick extract, providing the highest yield of biologically active compounds. Validation of the technological process conducted at the production facility of LLP "PLP Zhanafarm" confirmed the stability and controllability of the process, ensuring the reproducibility of extract quality.

Stability studies demonstrated the preservation of physicochemical and microbiological characteristics over a 24-month period at a temperature of (25±2) °C and relative humidity of 60±5%, indicating high stability and homogeneity of the product.

The biological activity of the *Cetraria islandica* (L.) Ach. extract was confirmed through pharmacological studies. The extract exhibited a pronounced anti-inflammatory effect—reducing edema by 69.90% at a dose of 500 mg/kg, which is comparable to the effect of ibuprofen. Additionally, it showed antimicrobial and

antifungal activity against a range of clinically significant microorganisms, including *Staphylococcus aureus*, as well as antimalarial activity against *Plasmodium falciparum*.

Economic modeling of the spray dosage form production demonstrated high profitability. With an annual output of 36,000 units, the cost price per bottle is 895 KZT, while the wholesale price is 2,000 KZT. The projected annual net profit reaches 30.9 million KZT, with a payback period of less than one year.

Justification of Scientific Novelty:

For the first time:

- A comprehensive pharmacognostic study of *Cetraria islandica* (L.) Ach. thalli collected in the Karaganda region has been conducted, including morphological and anatomical analysis using light, fluorescence, and scanning electron microscopy, as well as energy-dispersive X-ray spectroscopy (EDS);
- A technology for obtaining *Cetraria islandica* extract has been developed and optimized, incorporating a scientifically justified extraction method, its standardization, and stability evaluation;
- A comprehensive investigation of the safety parameters and biological activity of *Cetraria islandica* extract has been performed within the framework of preclinical studies, including antimicrobial and anti-inflammatory activity, as well as toxicological and allergenic properties (Appendix A);
- A scientifically substantiated production technology for a spray dosage form containing *Cetraria islandica* extract has been developed, including formulation optimization, manufacturing methodology, stability and quality assessment, as well as a techno-economic feasibility analysis for production.

Practical Significance of the Results

- A harvesting and procurement technology for the medicinal plant raw material *Cetraria islandica* (L.) Ach. has been recommended. The botanical identification was confirmed by the RSE on the REM "Institute of Botany and Phytointroduction," Almaty, Republic of Kazakhstan. Certificate No.: 01-05/252 (Appendix B);
- A phytosanitary inspection of the medicinal plant raw material was conducted to check for the presence of harmful quarantine organisms by the State Institution "Territorial Inspection of the Committee for State Inspection in the Agro-Industrial Complex for the City of Almaty of the Ministry of Agriculture of the Republic of Kazakhstan." Phytosanitary certificate No.: O702/202109151456744 (Appendix C);
- A quality specification for "*Cetraria islandica* (L.) Ach. medicinal plant raw material" has been developed;
- A method for obtaining a thick extract of *Cetraria islandica* using maceration with ultrasonic treatment has been developed. A quality specification for the "Thick extract of *Cetraria islandica*" was compiled and introduced into the educational process at the Department of Pharmaceutical Technology of Asfendiyarov Kazakh National Medical University (Appendices D, E, F);
- The extraction technology and quality specification for the spray dosage form based on the thick extract of *Cetraria islandica* have been successfully tested at the

pharmaceutical manufacturing facility of LLC "IMR," Saint Petersburg, Russian Federation (Appendix I);

- The results of the dissertation have been implemented into the educational and research processes of the Department of Industrial Drug Technology at the St. Petersburg State Chemical-Pharmaceutical University of the Ministry of Health of the Russian Federation (Appendix K);

- A specimen of *Cetraria islandica* (L.) Ach., collected in the Karaganda region of the Republic of Kazakhstan, within the territory of Karkaraly National Park, was assigned the number NCNPR #24269 and included in the Botanical Repository of the National Center for Natural Products Research at the University of Mississippi (USA) (Appendix L);

- The *Cetraria islandica* (L.) Ach. specimen was identified by Professor Bruce McCune, Distinguished Professor Emeritus of the Department of Botany and Plant Pathology at Oregon State University (Corvallis, Oregon, USA), and registered in the Oregon State University Herbarium (USA) under the code OSC-M-050921 (Appendices M and N).

Personal Contribution of the PhD Candidate

As part of the research, an independent analysis and an extensive review of domestic and international scientific literature related to the dissertation topic were conducted. This formed a solid theoretical foundation for the study and identified promising directions for further scientific and applied developments. All experimental work was carried out using modern equipment and pharmacopeial-grade materials, ensuring the reliability of the results obtained, which were confirmed through both laboratory and production-scale studies.

The validity and justification of the results are supported by the relevance of the research problem, adherence to scientific methodology, and the implementation of all study stages within a modern research center. Within the framework of the dissertation, regulatory documentation was developed for the *Cetraria islandica* (L.) Ach. extract and the spray dosage form, which confirms the practical significance of the findings and their applicability to real-world pharmaceutical manufacturing.

Conclusions

The dissertation is devoted to the pharmaceutical development of medicinal products based on the medicinal plant raw material *Cetraria islandica* (L.) Ach. The study included a comprehensive pharmacognostic evaluation, the development of an optimal extraction technology, and the assessment of biological activity, which confirmed the value of *Cetraria islandica* (L.) Ach. as a renewable source of plant material with significant pharmacological potential.

The first part of the study describes the harvesting and procurement technology of *Cetraria islandica* (L.) Ach., based on the principles of sustainable resource management and the recommendations of Good Agricultural and Collection Practice (GACP). The collection was carried out in accordance with the Law of the Republic of Kazakhstan "On the Plant World," ensuring the rational use of biological resources. Samples were collected under ecologically stable conditions, confirming the health of the population and the possibility of partial harvesting without damage to the ecosystem.

Morphological and anatomical analysis revealed that *Cetraria islandica* (L.) Ach. possesses a heteromerous, foliose thallus 12–15 cm in height with distinct anatomical structures, including cortical and photobiont layers. Histochemical reactions confirmed the presence of usnic and fumarprotocetraric acids, underscoring the pharmacognostic importance of this raw material.

Phytochemical investigation revealed a broad spectrum of biologically active compounds, including polysaccharides and phenolic compounds, with the usnic acid content reaching 2.98% on a dry weight basis. Quality control confirmed compliance with regulatory requirements for physicochemical, microbiological, and toxicological parameters, allowing *Cetraria islandica* (L.) Ach. to be classified as a standardized pharmaceutical substance.

In the section dedicated to extraction technology, 70% ethanol was determined to be the most effective extractant, providing the highest yield of active compounds such as usnic acid. Validation of the technological process at the production site of LLP “PLP Zhanafarm” confirmed process stability and controllability, ensuring high and reproducible extract quality.

Long-term stability studies of the *Cetraria islandica* (L.) Ach. extract demonstrated that its physicochemical and microbiological properties were maintained over 24 months at a temperature of 25 ± 2 °C and relative humidity of $60 \pm 5\%$, confirming high uniformity and product quality.

Experimental studies on the anti-inflammatory activity of the extract showed a significant reduction in edema volume at a dose of 500 mg/kg, reaching an effect of 69.90%, which is comparable to that of ibuprofen. The extract also exhibited antimicrobial and antifungal activity against ten clinically significant microorganisms, including *Staphylococcus aureus*, as well as antimalarial activity against *Plasmodium falciparum*.

The financial analysis of the spray production project based on *Cetraria islandica* (L.) Ach. extract demonstrates high profitability and a short payback period. With a planned annual output of 36,000 spray bottles, the total cost per unit is approximately 895 KZT, and the maximum wholesale price is 2,000 KZT. The projected net annual profit is approximately 30.9 million KZT, accounting for 43% of the total revenue. The payback period is estimated at around one year, confirming the economic feasibility of the project.

Thus, the spray production project based on *Cetraria islandica* (L.) Ach. extract holds significant potential for successful implementation in the pharmaceutical market of Kazakhstan, confirming its practical relevance and opening prospects for the further use of this extract in the development of new phytopharmaceutical products.

Dissertation Results Dissemination

The key findings of the dissertation research were presented at international scientific forums and published in a number of academic publications:

- International Council for Small Business (ICSB) Congress, Oxford, Mississippi State, USA, March 2022.

- Poster presentation: *Ultrastructural and Energy-Dispersive X-ray Spectroscopy Characterization of Cetraria islandica (L.) Ach.*, Oxford, Mississippi State, USA, April 2022.
- Poster presentation: *Chemical Profiling of Cetraria islandica Lichen Using LC-DAD-QToF*, Oxford, Mississippi State, USA, April 2022.

Publications

The results of the dissertation research were published in four scientific works, including one article in an international journal indexed in the Scopus database; three articles in journals recommended by the Committee for Quality Assurance in the Sphere of Education and Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan; and one utility model patent registered with the RSE "National Institute of Intellectual Property."

Volume and Structure of the Dissertation:

The dissertation comprises 176 pages of typed text, 42 tables, 55 figures, and 143 references to national and international sources, along with appendices. The structure of the dissertation includes an introduction, a literature review, a section on materials and methods, four experimental research chapters, conclusions for each chapter, and a general conclusion.