#### 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. In modern conditions, one of the key priorities of the state policy in the field of healthcare and pharmaceutical industry of the Republic of Kazakhstan is the development of domestic pharmaceutical manufacturing, reduction of dependence on imported medicines, and the implementation of innovative technologies in the development of effective and safe pharmaceutical products. According to the National Development Plan of the Republic of Kazakhstan until 2029, one of the central strategic priorities is the prevention of non-communicable diseases and the advancement of the national pharmaceutical sector. The full and rational utilization of Kazakhstan's unique flora, the establishment of competitive and highly efficient production of herbal medicinal products (HMPs), reduction of import dependency, as well as expansion of domestic manufacturing capacities, raw material supply, and scientific-technological potential, are essential directions for achieving national objectives related to economic modernization and improving the quality of life of the population. Currently, the share of domestically produced pharmaceuticals on the national market is only 14.4%, indicating a high degree of import dependency-86.6% in monetary terms and 73.8% in physical volume. This situation underscores the need for active development and introduction of domestic medicines, including those derived from medicinal plant materials.

In this context, the study of the therapeutic potential of Kazakhstan's natural resources becomes especially relevant. One of the promising sources is *Cetraria islandica*, a species from the Parmeliaceae family, which is widely distributed in the mountainous and subalpine regions of the country and possesses pronounced pharmacological activity. According to scientific literature, lichen acids contained in *Cetraria islandica* exhibit antiseptic, anti-inflammatory, immunomodulatory, and antioxidant properties, justifying its potential use in the treatment of inflammatory conditions of the upper respiratory tract and a range of other pathologies.

Global and domestic experience in the development of plant-based pharmaceuticals demonstrates a consistently high interest in the creation of standardized extracts and innovative dosage forms based on them. In light of the urgent need for import substitution and ensuring national drug security, the development of an effective and safe medicine based on *Cetraria islandica*—in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, the regulations of the Eurasian Economic Union (EAEU), and international quality standards—holds particular importance.

The relevance of this dissertation research is determined by the necessity for comprehensive pharmacognostic investigation of *Cetraria islandica* thalli, development of an optimal extraction technology, standardization of biologically

active substances, as well as assessment of pharmacological activity and toxicological safety. Furthermore, the development of a spray dosage form represents a modern pharmaceutical approach that provides localized therapeutic action, high bioavailability, and ease of administration, which aligns with current global trends in the treatment of respiratory tract diseases.

Thus, the pharmaceutical development of a medicinal product based on *Cetraria islandica* extract is aligned with the strategic priorities of Kazakhstan's public health system. It promotes the rational use of natural resources, supports the advancement of national pharmaceutical science and industry, and creates new opportunities for the integration of Kazakhstani innovations into the global pharmaceutical arena.

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

#### **Research Tasks:**

1. To collect *Cetraria islandica* (L.) Ach. raw material in accordance with GACP requirements, to develop a harvesting technology, and to conduct a comprehensive pharmacognostic study of the thallus, including morphological-anatomical, physicochemical, and phytochemical analyses.

2. To develop an optimal extraction technology for obtaining *Cetraria islandica* (L.) *Ach.* extract, to justify the choice of extraction method, to standardize the extract, and to assess its stability.

3. To study the biological activity and toxicological safety of *Cetraria islandica* (*L*.) *Ach*. extract within the framework of preclinical research.

4. To develop the manufacturing technology for a spray dosage form based on *Cetraria islandica* (*L.*) *Ach.* extract, and to conduct quality control and stability testing of the finished dosage form.

5. To carry out a techno-economic justification for the production and prospects of introducing the developed medicinal product into the pharmaceutical market of the Republic of Kazakhstan.

**Objects of the Study**: the objects of the study include the herbal raw material of *Cetraria islandica* (L.) *Ach.*, extracts obtained from this raw material, and the medicinal spray formulation developed on their basis for local application.

**Subject of the Study:** the subject encompasses the pharmacognostic investigation of *Cetraria islandica* (*L.*) *Ach.*, herbal raw material, development of extraction and spray formulation technologies, their standardization, evaluation of biological activity, toxicological safety, quality, and stability, as well as the techno-economic justification of the production of the *Cetraria islandica* (*L.*) *Ach.* - based spray.

# Key Provisions of the Dissertation Submitted for Defense

• Scientifically substantiated results of the development and optimization of the extraction technology for *Cetraria islandica*, demonstrating process efficiency and reproducibility.

- Comprehensive data on the biological activity and toxicological safety of the *Cetraria islandica* extract, confirming its potential application in clinical practice.
- Results of the pharmaceutical development of a spray dosage form based on *Cetraria islandica* extract, including justification of the dosage form choice and its pharmacotechnical characteristics.

## Summary of Key Research Findings:

This dissertation focuses on the pharmaceutical development of medicinal products derived from the herbal raw material *Cetraria islandica* (L.) Ach. A comprehensive pharmacognostic study was carried out, an optimal extraction technology was developed, and the biological activity of the obtained extracts was evaluated. The data obtained confirm the high pharmacological potential of *Cetraria islandica* as a renewable source of biologically active compounds.

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

Morphological and anatomical analysis demonstrated that the thallus of *Cetraria islandica* is heteromerous and foliose, measuring 12–15 cm in height, with well-defined cortical and photobiont layers. Histochemical tests confirmed the presence of usnic and fumarprotocetraric acids, supporting the pharmacognostic significance of the species.

Phytochemical screening revealed the presence of polysaccharides and phenolic compounds; the usnic acid content amounted to 2.98% relative to the dry mass. Quality control results for physicochemical, microbiological, and toxicological parameters met pharmacopeial requirements, qualifying the raw material as a standardized pharmaceutical substance.

Seventy percent ethanol was identified as the optimal extractant for producing a thick extract, yielding the highest concentration of biologically active substances. The technological process was validated at the production facility of LLP "PLP ZhanaPharm," confirming process stability and reproducibility, thus ensuring consistent extract quality.

Stability studies confirmed that the extract retained its physicochemical and microbiological characteristics for 24 months when stored at  $25\pm2$  °C and  $60\pm5\%$  relative humidity, indicating a high level of formulation stability and uniformity.

The biological activity of *Cetraria islandica* extract was confirmed by pharmacological investigations. The extract demonstrated a pronounced antiinflammatory effect—reducing edema by 69.90% at a dose of 500 mg/kg comparable to the effect of ibuprofen. In addition, it showed antimicrobial and antifungal activity against several clinically significant microorganisms, including *Staphylococcus aureus*, as well as antimalarial activity against *Plasmodium falciparum*. Economic modeling of the spray formulation's production demonstrated high profitability. At an annual output of 36,000 bottles, the unit cost per bottle was 895 KZT, while the wholesale price was set at 2,000 KZT. The projected annual net profit amounts to 30.9 million KZT, with a payback period of less than one year.

# Justification of Scientific Novelty:

For the first time:

- A technology for obtaining *Cetraria islandica* extract was developed and optimized, including scientific justification of the extraction method, its standardization, and stability assessment.
- A comprehensive preclinical study of the biological activity and safety profile of the extract was conducted, including antimicrobial and anti-inflammatory properties, as well as toxicological and allergenic evaluations.
- A pharmaceutical technology for producing a spray dosage form based on *Cetraria islandica* extract was developed and scientifically substantiated, including formulation optimization, preparation method, stability and quality assessment, and a techno-economic feasibility analysis.

# **Practical Significance of the Results**

A quality specification for the herbal raw material *Cetraria islandica* (L.) Ach. was developed.

An optimized method for obtaining thick extract from *Cetraria islandica* thalli was tested at the LLP "PLP ZhanaPharm" facility, enabling higher yields of active substances and reduced extraction time.

A quality specification for the thick extract of *Cetraria islandica* (L.) Ach. was developed.

The production technology and quality specification of the spray dosage form based on the thick extract were successfully validated at the pharmaceutical production facility of "IMR" LLC, Saint Petersburg, Russian Federation.

# Personal Contribution of the PhD Candidate

- The doctoral candidate independently conducted an extensive review and analysis of national and international scientific literature relevant to the dissertation topic. This analytical work formed the theoretical foundation of the study and helped identify promising directions for future scientific and applied research. All experimental procedures were performed using modern equipment and pharmacopoeia-grade materials, ensuring the reliability of the results obtained, which were validated under both laboratory and production conditions.
- The reliability and scientific validity of the findings were supported by the topical relevance of the research problem, adherence to scientific methodology, and the implementation of all experimental stages at a modern research facility. Within the framework of this dissertation, regulatory documentation was developed for the *Cetraria islandica* extract and the spray dosage form, which underscores the practical significance of the findings and their applicability to real-world pharmaceutical production.

## Conclusions

This dissertation is dedicated to the pharmaceutical development of medicinal products based on the herbal raw material *Cetraria islandica* (L.) Ach. The research involved a comprehensive pharmacognostic study, development of an optimized extraction technology, and assessment of the biological activity of the extracts. The results confirm the value of *Cetraria islandica* as a renewable source of herbal raw material with pronounced pharmacological potential.

The first section of the work outlines the collection and procurement process for *Cetraria islandica*, which was based on the principles of sustainable use and aligned with the Good Agricultural and Collection Practice (GACP) guidelines. The harvesting process was carried out in accordance with the Law of the Republic of Kazakhstan "On the Plant World," ensuring rational use of biological resources. Samples were collected in ecologically stable areas, confirming the health of local populations and the possibility of partial harvesting without ecological harm.

Morphological and anatomical studies confirmed that *Cetraria islandica* forms a heteromerous foliose thallus 12–15 cm in height, with distinct cortical and photobiont layers. Histochemical tests verified the presence of usnic and fumarprotocetraric acids, highlighting the pharmacognostic importance of the raw material.

Phytochemical analysis revealed a wide spectrum of biologically active constituents, including polysaccharides and phenolic compounds, with a usnic acid content of 2.98% on a dry weight basis. Quality control confirmed compliance with regulatory requirements for physicochemical, microbiological, and toxicological parameters, allowing the raw material to be classified as a standardized pharmaceutical substance.

The extraction technology section established that 70% ethanol was the most effective solvent, yielding the maximum amount of active constituents, including usnic acid. Validation of the technological process at LLP "PLP ZhanaPharm" confirmed both its stability and manageability, ensuring the high quality of the extract.

Long-term stability studies demonstrated that the *Cetraria islandica* extract retained its physicochemical and microbiological properties over 24 months at a temperature of  $25\pm2$  °C and relative humidity of  $60\pm5\%$ , confirming the formulation's uniformity and quality.

Experimental studies on the anti-inflammatory activity of the extract showed a significant reduction in edema volume—69.90% at a dose of 500 mg/kg— comparable to the effect of ibuprofen. Antimicrobial and antifungal activities were confirmed against ten clinically relevant microorganisms, including *Staphylococcus aureus*, and the extract also demonstrated antimalarial activity against *Plasmodium falciparum*.

The financial feasibility analysis for the production of a spray based on *Cetraria islandica* extract demonstrated high profitability and rapid return on investment. With a projected annual output of 36,000 bottles, the total cost per unit is approximately 895 KZT, while the maximum retail price is 2,000 KZT. The anticipated annual net profit is approximately 30.9 million KZT, corresponding to

43% of gross revenue. The payback period is estimated at approximately one year, supporting the project's economic viability.

Thus, the project for manufacturing a spray formulation based on *Cetraria islandica* extract demonstrates considerable potential for successful implementation in the Kazakhstani pharmaceutical market and confirms the rationale for its adoption. It also opens up opportunities for 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 peer-reviewed journal indexed in the Scopus database;
- Three articles in journals recommended by the Committee for Quality Assurance in the Field of Education and Science under the Ministry of Science and Higher Education of the Republic of Kazakhstan;
- One utility model patent granted by the RSE "National Institute of Intellectual Property."

# Volume and Structure of the Dissertation:

The dissertation comprises 212 pages of typed text, 42 tables, 45 figures, and 144 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.