

ABSTRACT

of the dissertation on the topic «**Methodological approaches to obtaining phytosubstances from certain species of *Inula* and the development of dosage forms based on them**» for the degree of Doctor of Philosophy (PhD) in the speciality 6D074800 – «Pharmaceutical Production Technology»

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Relevance of the research topic

The development of the pharmaceutical industry in the Republic of Kazakhstan is one of the strategic priorities of state policy. In his policy document ‘Kazakhstan in the Age of Artificial Intelligence: Current Challenges and Solutions through Comprehensive Digital Transformation’, Head of State Kassym-Jomart Tokayev emphasised the need to develop the pharmaceutical industry, highlighting the strategic importance of expanding the range and increasing the volume of domestically produced medicines.

The pandemic has highlighted the risks associated with dependence on external supplies and confirmed that the development of domestic production is a key prerequisite for ensuring national security and the economic resilience of the healthcare system.

Achieving these objectives requires the scientifically grounded development of pharmaceutical manufacturing, the translation of research findings into practice, and the enhancement of the sector’s technological capacity. The key mechanisms for this area are set out in strategic documents such as the ‘Concept for the Development of Healthcare Infrastructure for 2024–2030’ and the ‘Comprehensive Plan for the Development of the Pharmaceutical and Medical Industry for 2020–2025’. These documents identify the improvement of production infrastructure, the implementation of economic and technological support measures, and the development of pharmaceutical production based on medicinal plants growing in Kazakhstan as priority areas.

The flora of Kazakhstan represents a significant source of biologically active compounds and possesses considerable scientific potential. At the same time, the morphological and diagnostic characteristics, chemical composition and pharmacological activity of a number of plant species remain insufficiently systematised and studied.

This circumstance highlights the relevance of conducting comprehensive research into promising medicinal plants, scientifically substantiating their quality indicators and developing standardisation methods as a priority scientific task.

In this context, species of the genus *Inula* L., belonging to the family *Asteraceae*, are of particular scientific interest. Although *Inula helenium* is listed in the pharmacopoeias of a number of countries, the species *Inula britannica* and *Inula caspica*, which are widespread in Kazakhstan, have not yet been subjected to comprehensive pharmacognostic and phytochemical studies. According to the literature, these plants are characterised by high levels of sesquiterpene lactones,

flavonoids and phenolic compounds, which justifies their pharmacological potential and the advisability of further in-depth study.

According to the literature, these plants are characterised by high levels of sesquiterpene lactones, flavonoids and phenolic compounds, which accounts for their pharmacological potential and justifies further in-depth research. The expected results will contribute to the development of pharmaceutical production based on the principle of a complete technological cycle, reduce dependence on imported substances, and increase the competitiveness of the national pharmaceutical industry. The expected outcomes will contribute to the development of pharmaceutical manufacturing based on the principle of a full production cycle, reduce dependence on imported active pharmaceutical ingredients, and enhance the competitiveness of the national pharmaceutical industry.

The relevance of the research topic is therefore directly linked to government strategic initiatives, sectoral development programmes and the need for the scientifically sound development of the country's raw material base.

Aim of the dissertation research:

The aim of the study was to conduct a comprehensive pharmacognostic investigation of *Inula britannica* and *Inula caspica* species distributed in the flora of Kazakhstan, as well as to establish scientific and methodological foundations for obtaining standardized substances based on these plants and developing effective and safe dosage forms.

Research objectives:

- To conduct comparative morphological, anatomical, and phytochemical studies of the plant raw materials of *Inula britannica* and *Inula caspica*.

- To determine the quality indicators of the investigated plant raw materials in accordance with the requirements of regulatory documentation and to develop quality specifications.

- To develop a technology for obtaining extracts from the raw material of *Inula britannica*, as well as to perform its validation, standardization, and stability assessment.

- To investigate the safety and biological activity profile of the obtained extracts.

- To evaluate the technical and economic feasibility of implementing the developed extract production technology in industrial manufacturing.

Research methods: To accomplish the stated objectives, a комплекс of pharmacognostic methods (macro- and microscopic analysis, determination of numerical indicators in accordance with the State Pharmacopoeia of the Republic of Kazakhstan), as well as physicochemical methods (GC-MS, HPLC-ESI-QTOF-MS, RP-HPLC-DAD, spectrophotometry, and atomic absorption spectroscopy (AAS)) were employed.

Technological studies included modeling and validation of extraction and granulation processes with the calculation of reproducibility indices. Preclinical safety assessment (acute and chronic toxicity in vivo) and evaluation of specific biological activity (antimicrobial activity in vitro) were conducted using certified

cell cultures and laboratory animals. Statistical data analysis was performed using the variation method with the application of the «STATISTICA» software package.

Object of the study: The objects of the study were the aerial parts (herb) of *Inula britannica* L. and *Inula caspica* F.K. Blum ex Ledeb. collected in the territory of Kazakhstan, as well as dosage forms obtained on their basis, including liquid and dry extracts.

Subject of the study: The subject of the study was the investigation of the complex of biologically active compounds in the aerial parts of *Inula britannica* and *Inula caspica*, determination of the patterns of their efficient extraction, development of technologies for obtaining liquid and dry extracts, standardization of the quality indicators of the obtained substances, as well as evaluation of their safety and biological activity.

The main provisions of the dissertation research put forward for defense:

- Results of the determination of morphological, anatomical, and phytochemical characteristics of the plant raw materials of *Inula britannica* L. and *Inula caspica* F.K. Blum ex Ledeb. in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, as well as their standardization based on quality indicators.

- Results of the selection of an effective method for obtaining extracts from *Inula britannica* and investigation of their phytochemical composition using modern chromatographic methods.

- Results of the evaluation of the safety and biological activity profile of plant pharmaceutical substances.

- Results of the evaluation of quality indicators of the obtained plant pharmaceutical substances as dosage forms and their standardization in accordance with pharmacopoeial requirements.

Description of the main results of the study: As a result of a comprehensive pharmacognostic study of two species, *Inula britannica* L. and *Inula caspica* F.K. Blum ex Ledeb., and the development of a plant substance and dosage form based on them, the following scientific and practical data were obtained.

During the expedition, two types of raw materials were collected: *Inula britannica* L. (Aktobe Region, Kargaly District) and *Inula caspica* F.K. Blum ex Ledeb. (Almaty Region, Kegen District). Macroscopic and microscopic analysis revealed diagnostic features. *Inula britannica* is characterised by dense pubescence of the stem and leaves, the presence of specific multicellular hairs and essential oil glands. The anatomical structure of the stem includes well-developed collenchyma and parenchyma, which provides mechanical strength to the mesophyte. *Inula caspica* is distinguished by adaptive features of a xerophyte-halophyte (inhabitant of saline dry soils). The stems are stiffer, with a more compact tissue structure and less pronounced pubescence compared to *I. britannica*.

Comparative analysis of chemical composition using GC-MS and HPLC-MS methods revealed fundamental differences between species. *Inula britannica* is a rich source of sesquiterpene lactones, among which britannilactone, 1,6-O,O-diacetylbritannilactone and 1-O-acetylbritannilactone have been identified. It also has a high content of phenolic compounds: chlorogenic acid, quercetin, luteolin and

cynarin. This combination determines the high pharmacological value of this species. *Inula caspica* raw materials contain virtually no sesquiterpene lactones typical of the *Inula* genus. Instead, a high content of lipid components (ethyl esters of linoleic and linolenic acids) and specific terpenoids has been identified. This discovery justified the choice of *I. britannica* as the main source for the development of a medicinal product with anti-inflammatory and antimicrobial activity.

A technology for obtaining a dry extract from *Inula britannica* herb using ultrasonic extraction has been developed and validated, and the optimal parameters have been determined: extractant - 70% ethyl alcohol, hydromodule 1:10, ultrasonic treatment (35–40 kHz) for 30 minutes (3 cycles) at a temperature of 23–25 °C. The technology reduces the process time from 48 hours (traditional percolation) to 90 minutes and increases the yield of extractive substances by 10-15%. The process has been confirmed in three pilot industrial series, and the reproducibility indicators (Cp, Cpk > 1.33) testify to the stability of the technology. A patent for the method of production has been obtained for utility model RK No. 10800.3.4.

A comprehensive assessment of the safety and activity of the dry extract of *I. britannica* showed that the extract belongs to toxicity class V according to the OECD classification. No animal deaths were recorded after a single administration to mice at a dose of 5000 mg/kg. With prolonged administration, no pathological changes in the structure of internal organs (liver, kidneys, heart) or allergenic effects were detected. The extract has been found to be highly active against *Helicobacter pylori* (MIC 0.125–0.25 mg/ml), which opens up prospects for its use in gastroenterology. The extract showed a high ability to neutralise free radicals, comparable to standards, which correlates with its high polyphenol content.

Justification of scientific novelty

1. Microscopic diagnostic features of the vegetative organs of *I. britannica* (developed collenchyma, essential oil glands) and *I. caspica* (adaptive xeromorphic structures) have been identified and systematised for the first time, allowing reliable identification of raw materials of these species in the Kazakhstani flora.

2. For the first time, fundamental differences in metabolome profiles have been established: it has been proven that *I. britannica* is a source of valuable sesquiterpene lactones (britannilactone) and flavonoids (cynarine), while *I. caspica* is characterised by the absence of typical lactones and the accumulation of specific lipids and terpenoids.

3. A scientifically proven and experimentally developed resource-saving technology for obtaining dry extract from *I. britannica* by ultrasonic maceration has been developed. The novelty of the proposed method is confirmed by utility model patent No. 10800 (19 March 2025) of the Republic of Kazakhstan.

4. For the first time, pronounced specific activity of dry extract *I. Britannica* against *Helicobacter pylori* bacteria has been identified, which, combined with proven safety (toxicity class V), opens up prospects for the creation of a new domestic gastroprotective agent.

5. For the first time, the technology for producing a dosage form based on dry extract of *I. britannica* has been scientifically substantiated, and its stability and economic efficiency in industrial production have been proven.

Practical significance of the results obtained

The results of the dissertation have been widely applied in the pharmaceutical industry and educational process, as confirmed by relevant implementation acts.

The technology for collecting, primary processing and storage of medicinal plant raw materials *I. britannica* and *I. caspica* has been introduced into the production cycle of Fitoleum LLP (Act of Introduction dated 1 October 2025).

The method for obtaining dry extract from *I. britannica* herb has been implemented in the technological process of the Scientific Centre for Anti-Infective Drugs JSC.

The innovative extraction method developed is protected by a security document, Utility Model Patent No. 10800 of the Republic of Kazakhstan (registered on 19 March 2025).

The developed methodology has been implemented in the educational process of the Department of Engineering Disciplines and Best Practices as part of the discipline 'Processes and Equipment of Chemical and Pharmaceutical Production' and integrated into the educational, research and industrial practice of students, actively applied in the educational activities of students of the School of Pharmacy of the S.D. Asfendiyarov KazNMU NAO.

Data on the antimicrobial and antioxidant activity of Inula extracts are included in the scientific and educational programme of the Department of Pharmaceutical Microbiology at the Medical University of Lublin (Poland).

A draft quality specification for the finished substance (dry extract of *I. britannica*) has been developed.

Personal Contribution of the Doctoral Candidate.

The author took a direct and leading part in all stages of the research: She independently conducted a patent information search, justified the methodology of the experiment, and carried out a critical analysis of the data obtained.

Personally prepared raw materials in expedition conditions and conducted macro- and microscopic analysis. Independently carried out the entire cycle of phytochemical research using complex analytical equipment (GC-MS, HPLC-MS), conducted technological experiments to select extraction parameters and develop the composition of the extract.

I conducted statistical analysis of the results and formulated scientific conclusions and propositions. I prepared 9 scientific publications, including in highly ranked international journals, and filed a patent application.

Conclusions:

1. During macro- and microscopic analysis, reliable diagnostic features for species identification were established: *Inula britannica* is characterised by developed collenchyma, numerous essential oil glands and dense pubescence (mesophyte), while *Inula caspica* is distinguished by a compact tissue structure and a stiff stem (halophyte). Comparative phytochemical screening (GC-MS, HPLC) revealed fundamental chemotaxonomic differences: *I. britannica* is a rich source of sesquiterpene lactones (britannilactone) and flavonoids (cinnarin), while *I. caspica* lacks lactones but is rich in lipids and specific terpenoids.

2. Quality indicators for the medicinal plant raw materials *Inula britannica* and *Inula caspica* have been developed and experimentally substantiated. Pharmacognostic indicators have been determined: moisture content not exceeding 13%, total ash not exceeding 12%, extractive substances not less than 15%, which meets the requirements of the State Pharmacopoeia of the Republic of Kazakhstan and the Eurasian Economic Union and allows for reliable assessment of the quality of domestic raw materials.

3. A resource-saving technology for obtaining dry extract from *Inula britannica* herb by ultrasonic maceration (extractant – 70% ethanol, hydromodule 1:10, temperature 23–25 °C, 3 cycles of 30 minutes each) has been developed and validated. The method ensures a high yield of biologically active substances and reduces the process time. Validation tests confirmed the reproducibility and stability of the process (Cp, Cpk indices > 1.33). A quality specification for the dry extract has been developed and its stability during storage for 24 months has been confirmed. The novelty of the method is confirmed by Patent of the Republic of Kazakhstan No. 10800.

4. A comprehensive pharmacotoxicological assessment showed that dry extract of *Inula britannica* belongs to toxicity class V and is safe for long-term use. The extract has been found to have high specific antimicrobial activity against *Helicobacter pylori* (MIC 0.125–0.25 mg/ml) and pronounced antioxidant activity, which justifies its therapeutic potential.

5. Technical and economic calculations confirmed the economic feasibility and profitability of industrial production of dry extract of *Inula britannica* L., with a unit cost of 10,622.8 tenge, a minimum selling price of 13,809.60 tenge at 30% profitability, and a projected payback period of approximately 3.33 years.

Approbation of the Dissertation Results

The main results of the dissertation have been presented and discussed at international scientific conferences:

-«Modern scientific, practical and educational approaches in the context of the modernisation of pharmacy in Kazakhstan» (Almaty, 2018)

- International Scientific and Practical Conference ‘Contemporary Aspects of Medicine and Pharmacy: Education, Science and Practice’, dedicated to the 40th anniversary of the South Kazakhstan Medical Academy (Shymkent, 2019)

-IV International Scientific and Practical Conference ‘Innovative Technologies in Pharmacy’ (Prague, 2021).

-XI International Youth Scientific Medical Forum WHITE FLOWERS (Kazan, 2024)

Publications

Nine scientific papers have been published on the topic of the dissertation, including one article in peer-reviewed journals indexed in the Scopus and Web of Science databases, three in the list of scientific publications recommended by the authorised body in the field of science and higher education, and materials from international conferences.

Scope and structure of the dissertation

The dissertation comprises 139 typewritten pages and includes an introduction, a literature review, a description of materials and methods, the author's own research results, a conclusion, a bibliography (187 entries) and appendices. The work contains 46 tables and 34 figures.