ANNOTATION

Dissertation work on the topic «Development of a conceptual project for the production of therapeutic and prophylactic products based on fruit *Morus Alba* L.» for the degree of Doctor of Philosophy (PhD) in the specialty 8D07201 – «Technology of pharmaceutical production»

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Relevance of the research topic

One of the key areas of the National Development Plan of the Republic of Kazakhstan until 2029 is improving the quality of life and the healthcare system. The plan considers the prevention of non-communicable diseases, reducing mortality among the population, and developing domestic pharmaceutical production as its main priorities. In order to expand the production of medicines and increase their availability, it is planned to strengthen cooperation with global pharmaceutical corporations, attract investment, introduce advanced technologies and new developments, and locate production facilities in the regions.

The development of healthcare infrastructure is an important component of improving the well-being of the population. This direction is clearly outlined in the Concept for the Development of Healthcare Infrastructure for 2024–2030 (Decree of the Government of the Republic of Kazakhstan No. 454 of 12 June 2024). In addition, in accordance with the Comprehensive Plan for the Development of the Pharmaceutical and Medical Industry for 2020–2025, the development of drug production based on local medicinal plant raw materials is one of the strategically important areas.

It was noted above that government programmes for the pharmaceutical industry place particular emphasis on reducing dependence on imported products and making effective use of plant resources grown within the republic. However, at present, domestically produced medicines account for only 14.9% of the market. This indicates the need for more efficient use of local medicinal raw materials and expansion of pharmaceutical production. The rational use of local natural resources and the introduction of innovative technologies are important factors in ensuring the sustainable development of the pharmaceutical industry in Kazakhstan. The study is being conducted in accordance with the Sustainable Development Goals, namely: SDG 2 – Zero Hunger and Good Health and Well-being, SDG 3 – Good Health and Well-being, and SDG 12 – Responsible Consumption and Production.

In this regard, the development of biologically active supplements and medicines from local plants is a strategically important area. Of particular interest is the white mulberry (*Morus alba* L.), which contains biologically active substances (BAS) with antioxidant, anti-inflammatory and immunomodulatory properties.

Morus alba L. is a perennial woody plant belonging to the Moraceae family, growing in many countries of Asia and Europe, as well as in Northern India, Afghanistan, Iran, the Caucasus, Turkey, China, Korea, Southern Europe, America and a number of regions in Africa. The leaves and fruits of white mulberry are rich

in biologically active compounds such as anthocyanins, flavonoids, vitamins and minerals, which help maintain overall health.

White mulberry, in addition to its antioxidant and anti-inflammatory properties, helps regulate blood sugar levels, prevent cardiovascular disease and strengthen the immune system. However, in Kazakhstan, the level of processing of medicinal plant raw materials and their use in pharmaceutical production is lower than in foreign countries, which indicates the need to increase the country's pharmaceutical independence. The development of therapeutic and prophylactic products based on *Morus alba* L. increases the competitiveness of the pharmaceutical industry in Kazakhstan and contributes to improving the health of the population.

Therefore, this dissertation research is aimed at a comprehensive study of the pharmacognostic, chemical, and pharmaceutical-technological properties of the *Morus alba* L. plant with the goal of developing therapeutic and prophylactic products and creating a concept for their production. The results of the study are of both scientific and practical importance and serve as a basis for the introduction of innovative technologies in the pharmaceutical industry.

Research objective:

To study the *Morus Alba* L. plant as a source of raw materials, develop a therapeutic and prophylactic product based on an extract obtained using modern methods, and create a concept for its production.

Research tasks:

1) Conduct pharmacognostic and phytochemical analysis of the *Morus Alba* L. plant;

2) Select an effective technology for obtaining extract from the fruits of *Morus Alba* L. and standardise it;

3) Evaluate the chemical composition and safety of the extract from the fruits of *Morus Alba* L.;

4) Select and standardise the technology for obtaining a therapeutic and prophylactic product (capsules) based on *Morus alba* L. extract;

5) Develop a conceptual design and technical and economic justification for the production of a therapeutic and prophylactic product.

Research objects:

Morus Alba L. fruit; *Morus Alba* L. fruit extract; therapeutic and prophylactic product (in capsule form).

Research methods:

Pharmacopoeial and non-pharmacopoeial methods (physical, physicochemical, pharmaceutical-technological), information-analytical, statistical methods, as well as marketing analysis methods.

Subject of research:

Optimal technology for obtaining extract from Morus Alba L. fruit, research into the component composition, safety and stability of the extract, as well as the development of a conceptual design and technical and economic justification.

Scientific novelty of the research:

During the research, the following fundamental and applied new results were obtained for the first time, aimed at solving urgent problems in the field of pharmaceuticals and phytochemistry:

- comprehensive standardisation and quality assessment of local raw materials: for the first time, a comprehensive pharmacognostic quality profile of white mulberry (Morus alba L.) raw materials collected from the flora of the Turkestan region was determined and standardised. This result scientifically substantiated the compliance of the raw materials with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan;

- based on the results of phytochemical analysis, the qualitative and quantitative indicators of the main biologically active compounds of Morus alba L. fruit raw materials — flavonoids, anthocyanins and phenolic compounds — were established for the first time, and their pharmacological activity was confirmed;

- Development and patenting of the optimal extraction technology: an optimal technology for obtaining extract from Morus alba L. fruit has been created, based on maceration with ultrasonic exposure, which allows for the most efficient extraction of biologically active substances (BAS). The component composition of the extract has been determined and its toxicological safety has been assessed. This technological solution is confirmed by the Republic of Kazakhstan patent for utility model No. 7396 ‘Method for obtaining extract from white mulberry (*Morus alba* L.) fruits by maceration with ultrasonic exposure’ (registered on 26 August 2022);

- Technological justification of a domestic therapeutic and prophylactic product: based on the Morus alba L. extract obtained, the optimal composition and technology for the production of therapeutic and prophylactic capsules have been developed for the first time, and the quality indicators of the finished product and the results of stability studies determining its shelf life have been substantiated. Conceptual design documentation and a technical and economic feasibility study (TEFS) for the organization of production of this product in Kazakhstan have been created, which contributes to the solution of tasks to replace imports and ensure the country's drug safety through the production of domestic pharmaceutical products.

Issues to be defended:

- Results of pharmacognostic analysis and standardisation of Morus alba L. fruit raw materials grown in the Turkestan region;

- Optimal technology for obtaining extract from Morus alba L. fruit, determination of its component composition and safety assessment;

- Development of a technology for obtaining capsules for therapeutic and prophylactic use based on Morus alba L. extract, their standardisation and determination of stability;

- Conceptual design for the production of a therapeutic and prophylactic product and a technical and economic feasibility study.

Practical significance of the work and implementation of research results in practice

Practical significance of the work and implementation of research results: scientific results of studying Morus alba L. extract and the therapeutic and

prophylactic product based on it, as well as the concept of its production, were implemented in the educational process of the Kazakh-Russian University of Medicine, ARDO - Fito LLP and the Kazakh National Medical University named after S.D.Asfendiyarov.

Personal contribution of the doctoral student

The doctoral student independently carried out all stages of the research work, selected the necessary methodological approaches, analysed and processed the data obtained, and published articles based on scientific conclusions. In addition, in the process of writing his dissertation, he systematised the research results and formulated scientific conclusions.

Approval of the work

The main provisions of the dissertation were presented and published in the materials of international scientific conferences:

– International Scientific and Practical Conference 'Current Issues and Trends in the Development of Pharmacognosy', dedicated to Professor D. A. Muravyova, Pyatigorsk, RIA-KMV Publishing House, 2021;

– III International Scientific and Practical Conference 'Formation of a Scientific School in the Field of Pharmacy, Prospects for Development and Continuity of Generations', dedicated to Professor R. Dilbarhanov, Almaty, 2020;

– International Scientific Conference for Young Scientists and Students 'Current Issues and Prospects for Development in the Field of Biology, Medicine and Pharmacy', organised jointly by the South Kazakhstan Medical Academy and the Nazarbayev Foundation, 10–11 December 2020 (in video conference format).

Information about publications

A total of 13 scientific papers have been published on the topic of the dissertation, including:

– 2 articles in international peer-reviewed scientific journals included in the Scopus and Web of Science Core Collection databases;

– 6 articles in journals recommended by the Committee for Quality Assurance in Education and Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan;

– 3 abstracts at international scientific and practical conferences in Kazakhstan and Russia;

– 1 patent for a utility model has been obtained.

Scope and structure of the dissertation

The dissertation consists of 150 pages and contains 61 tables, 27 figures and 145 domestic and foreign literature sources. In addition, 18 appendices are attached to the work. The dissertation includes an introduction, a literature review, a section on research materials and methods, four chapters devoted to individual studies, conclusions based on the results obtained, and a conclusion.

Purpose of research: Pharmacognostic analysis of plant raw materials of *Stachys sylvatica* L. and development on its basis of technology for obtaining extracts of pharmacopoeial quality.

Research objectives:

- Pharmacognostic analysis of *Stachys sylvatica* L. and standardisation of plant raw materials;

- Preparation of extracts from plant raw materials of *Stachys sylvatica* L. and evaluation of quality parameters;

- Safety assessment of *Stachys sylvatica* L. extract and study of biological activity profile;

- Transfer of technology for obtaining *Stachys sylvatica* L. extract and development of feasibility study.

Objects of research: plant material of *Stachys sylvatica* L. and the extract obtained from standardised raw materials.

Methods of research: pharmacopoeial, pharmacognostic, pharmaceutical-technological, pharmacological, biological and statistical methods.

Scientific novelty

For the first time:

– morphological and anatomo-diagnostic identification features of the above-ground part of the plant raw material of *Stachys sylvatica* L. growing in Almaty region;

- extract from plant raw material of *Stachys sylvatica* L. by ultrasonic maceration method, which is confirmed by the patent for utility model №7763 from 06.10.2022 (Appendix A);

- acute and subacute toxicity of *Stachys sylvatica* L. extract was studied, and its antimicrobial, anti-inflammatory, antiviral, antitumour and antihelminthic properties were revealed, indicating high biological activity of the extract;

- the study of *Stachys sylvatica* L. extract by GC-MS method revealed that the main volatile substances are esters of diterpenoids and fatty acids. According to the results of RP-HPLC/PDA analysis, 10 compounds were detected in the extract, the main ones being chlorogenic acid and verbascoside;

– extract obtained by ultrasonic maceration from plant material of *Stachys sylvatica* L., 17 compounds were detected by HPLC-ESI-QTOF-MS/MS. Flavonoids and their glycosides (chlorogenic acid and verbascoside) constitute the main group of compounds of plant raw materials of *Stachys sylvatica* L., their content is not less than 2.0%. Quality indicators and criteria of their suitability were established for the raw material of *Stachys sylvatica* L., and the extract was standardised;

– The extract of *Stachys sylvatica* L. used in the study showed high antimicrobial activity against Gram-positive bacteria, especially *Bacillus cereus*, with a minimum inhibitory concentration (MIC) in the range of 0.5-2 mg/ml. The bactericidal effect of the extract was most pronounced against *B. cereus*. The extract of *S. sylvatica* L. showed low cytotoxicity on VERO cells (CC_{50} 0.810±0.013 mg/ml) and medium cytotoxicity on MRC-5 cells (CC_{50} 0.0891±0.014 mg/ml). On MRC-5 cells, the *S. sylvatica* L. extract reduced the viral load of HCoV-229E virus by 1.56 log, showing no cytopathic effect, whereas on VERO cells it showed dose-dependent efficacy against HHV-1 virus, significantly reducing the cytopathic effect and reducing the viral load by 1.11 log. The extract was tested for antitumour activity against FaDu, H1HeLa and RKO

cell lines. It showed weak cytotoxicity to FaDu and RKO cells and moderate cytotoxicity to H1HeLa cells. When tested for antihelminthic activity, the extract showed activity similar to albendazole at the concentrations tested.

The main provisions of the dissertation research submitted for defense:

The results of determination of quality indicators of plant raw materials of *Stachys sylvatica* L. in accordance with pharmacopoeial requirements of the Republic of Kazakhstan and EAEU;

The results of the choice of technology for obtaining an extract from the plant raw material of *Stachys sylvatica* L. and determination of its chemical composition by gas chromatography-mass spectrometry and HPLC;

The results of studies on the evaluation of some preclinical parameters and biological activity profile of the extract obtained from the raw material of *Stachys sylvatica* L., based on the conducted studies.

Practical significance of the research:

- Identification of the species of the plant of *Stachys sylvatica* L. is confirmed by certificate №01-05/309 from 23.09.2021, issued by the Institute of Botany and phytointroduction of the Committee of Forestry and Wildlife of the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan, Almaty (Appendix B);

- The technology of collection, harvesting and storage of plant raw materials of *Stachys sylvatica* L. confirmed by the act of introduction of LLP «Fitoleum», Esik, Kazakhstan (Appendix C), and introduced a regulatory document «*Stachys sylvatica* L.» from 03.09.2022 (Appendix D);

- The technology of obtaining the extract from plant material of *Stachys sylvatica* L. is confirmed by the act of introduction of LLP «Fitoleum» (Appendix E);

- The production process of pilot series of extracts obtained from plant material of *Stachys sylvatica* L. is confirmed by the technological act at the pharmaceutical company LLP «Fitoleum» (Appendix F);

- A normative document on the quality of extract obtained from *Stachys sylvatica* L. (Appendix G);

- Studies of biological activity of dry extract of *Stachys sylvatica* L. introduced in the educational process at the departments of «Pharmaceutical Microbiology» Lublin Medical University and the departments of «Pharmacognosy with a course of botany» Pharmacy School of the Kazakh National Medical University named after S.D. Asfendiyarov (Appendices H, I);

- Certificate of inclusion of information in the state register of rights to copyrighted objects №47427 12 June 2024 on the topic «Pharmacognostic studies of the herb *Stachys sylvatica* L. and development of the technology of extracts of pharmacopoeial quality» was received (Appendix J).

Personal contribution of the author. In the process of carrying out the thesis work, the author effectively used domestic and foreign sources of information, conducting comprehensive research and analyses on the topic of the study. All experimental work was carried out in full in accordance with the set tasks. The reliability of the obtained results was confirmed by new sources

obtained using modern methods of analysis and equipment in scientific centres and laboratories, as well as reflected in scientific articles.

Reliability and validity of the obtained results, as well as the focus of the research on solving topical problems are confirmed by draft regulatory documents performed in the world's leading research centres. The analysis of the research results is based on a significant amount of experimental material and was carried out using modern certified equipment and validated methods, which emphasises the author's personal contribution to science in the field of pharmaceutical production technologies.

Approbation of the work

The main data of the dissertation work were presented and published in the materials of international conferences:

- At the international scientific-practical conference «Modern Pharmacy: New Approaches and Current Research» organised within the framework of «Days of the University» named after S.D. Asfendiyarov KazNMU (Almaty, October 2021);
- Awarded II degree diploma in the IV international publication «Best Young Scientist – 2021», presented by the countries of the Commonwealth of Independent States (Nur-Sultan, 2021);
- At the conference «Innovative Technologies in Pharmacy» (Czech Republic, Prague, April 2021);
- At the XI International Scientific and Practical Conference «Priorities of pharmacy and dentistry: from theory to practice»;
- 1st place in the section «Pharmacy» at the I International Forum Asfen.forum. «New Generation – 2023» (Almaty, June 2023).

Information about publications:

The results of the dissertation work have been published in 11 scientific papers. In the international scientific journal, included in the databases Scopus and Web of Science Core Collection - 1, in publications submitted by the Ministry of Science and Higher Education of the Republic of Kazakhstan, the Committee for Quality Assurance in the field of science and higher education - 3, in the materials of international scientific conferences - 5, patent for utility model - 1, register of rights to copyrighted objects - 1.

Scope and structure of the dissertation:

The thesis is presented on 142 pages of printed text, includes 39 tables, 52 figures, a list of literature from 181 sources, as well as 14 appendices. The work consists of an introduction, literature review, a section devoted to research materials and methods, three sections of own research, conclusions and findings.