ANNOTATION

Dissertation on the topic **"Theoretical and practical aspects of creating phytosubstances from some species of the genus *Adonis* L."**
for the degree of Doctor of Philosophy (PhD) in the specialty
6D074800 - "Pharmaceutical production technology"

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Relevance of the research topic

Currently, one of the priority directions of the state policy of the Republic of Kazakhstan in the field of health care and medicines is the strengthening of public health. According to the Comprehensive Plan for the Development of the Pharmaceutical and Medical Industry for 2020 - 2025 (On Amendments and Additions to the Order of the Prime Minister of the Republic of Kazakhstan dated October 6, 2020 № 132-r, paragraph 30, section IV) one of the most important tasks is to organize the production of drugs using medicinal plants growing in the territory of the Republic of Kazakhstan.

In order to achieve the goals and implement the objectives of the development of the pharmaceutical industry and to cover the needs of Kazakhstanis in medicines, it is necessary to conduct comprehensive scientific and practical research on the creation of high-quality, safe medicines, including those of plant origin.

In order to ensure a systematic reduction in the dependence of the domestic pharmaceutical market on imported medicines, it is envisaged to make fuller use of own raw material resources, create new pharmaceutical substances and medicines from plant raw materials in accordance with good GxP practices. Cultivation of medicinal plants is aimed at sustainable utilization of natural resources, reducing dependence on wild-growing raw materials and increasing the reproducibility of phytopreparations. In addition, the cultivated raw materials can be adapted to obtain the highest content of targeted bioactive substances (BAS), which increases the efficiency of the production process.

Expansion of the nomenclature of plant raw materials and the development of a full cycle of substances for the creation of drugs is a promising area of pharmaceutical research. In this regard, *Adonis tianschanica (*Adolf.) Lipsch.) and *Adonis aestivalis* L. growing in Kazakhstan are of special interest. Despite the fact that the above species are valuable sources of biologically active substances and are widely used in folk medicine as anti-inflammatory, cardiotonic, sedative, diuretic and anticonvulsant agents, to date they remain insufficiently studied.

In order to create a sustainable raw material base for the production of medicinal products from these plants and to ensure the stable quality of their plant raw materials, it is necessary to introduce these medicinal plants into culture in compliance with the requirements of "Good Practices for the cultivation, collection, processing and storage of raw materials of plant origin" GACP. Cultivation of medicinal plant raw materials will not only allow to control the growing conditions of plants for obtaining quality raw materials, but also minimize the influence of natural factors such as climatic and seasonal changes on their

chemical composition, and gives the opportunity to adapt plants to obtain the highest content of target BAS.

The development of a pharmaceutical substance from plant raw materials is a multi-step process that requires a scientifically sound concept and a properly designed methodology based on the application of standard procedures with a risk- based approach to ensure the quality, safety and reproducibility of the target product in accordance with GxP requirements.

Purpose of research: experimental and theoretical substantiation of the technology and methodology of obtaining herbal pharmaceutical substances from
A. tianschanica and *A. aestivalis,* establishing the profile of pharmacological activity and safety.

Research objectives:

– Comparative pharmacognostic analysis of the herbal raw materials of *Adonis tianschanica* and *Adonis aestivalis*;

– Standardization and stability assessment of herbal pharmaceutical substances derived from *Adonis tianschanica* and *Adonis aestivalis*;

– Determination of safety profile and pharmacological activity of the obtained herbal pharmaceutical substances;

– Development of a full-cycle methodology for the production of a herbal pharmaceutical substance based on representatives of the genus *Adonis* L.;

– Development of a cultivation technology for *Adonis tianschanica* and substantiation of the techno-economic feasibility of producing a herbal pharmaceutical substance.

Objects of research: *Adonis tianschanica* (Adolf.) Lipsch. and *Adonis aestivalis* L. herbs.

Subject of the study: Development of herbal pharmaceutical substances: a comprehensive study of the morphological and anatomical diagnostic features and chemical composition of the plant raw materials, standardization, investigation of stability, safety, and pharmacological activity profiles, development of a full-cycle methodology for the production of herbal pharmaceutical substances, and techno-economic justification of their manufacture.

Research methods: information-analytical, standard pharmacopoeial and non-pharmacopoeial methods (physical, physico-chemical, pharmaceutical-technological, pharmacological, biological, statistical) and agronomic methods.

Scientific novelty.

For the first time:

- morphological and anatomical diagnostic features were identified within the framework of the pharmacognostic analysis of the medicinal plant raw materials of *Adonis tianschanica* and *Adonis aestivalis*.

- a detailed phytochemical analysis of extracts from *Adonis tianschanica* and *Adonis aestivalis* was conducted using high-performance liquid chromatography coupled with electrospray ionization and quadrupole time-of-flight tandem mass spectrometry (HPLC/ESI-QTOF-MS/MS), characterized by high sensitivity and accuracy in molecular mass measurement. The application of high-resolution mass

spectrometry in both positive and negative ionization modes, as well as at various fragmentation levels and collision energies, enabled the acquisition of high-quality chromatographic profiles and informative fragmentation spectra.

The identification of extract components was predominantly carried out in negative ionization mode, due to the high content and diversity of polyphenolic compounds, which ionize most effectively under these conditions. Positive ionization mode enabled the detection of trace amounts of cardiac glycosides.

As a result of the analysis, 27 compounds were identified in the *Adonis tianschanica* extract, including flavonoids (isorhamnetin, kaempferol and its derivatives, adonivernite, synapoylsaponarin, isoorientin, isovitexin, orientin, orientin glucoside, vitexin, luteolin glycoside), an organic alcohol (adonitol), cardiac glycosides (strophanthidin, cymarin), and two unidentified flavonoid glycosides containing hexose and tetrose residues. The analysis of the *Adonis aestivalis* extract revealed the presence of 21 compounds, including flavonoids (isorhamnetin, kaempferol and its derivatives, isoorientin, isovitexin, orientin, orientin glucoside, vitexin, luteolin glycoside), an organic alcohol (adonitol), cardiac glycosides (strophanthidin, cymarin), organic acids (maleic and citric acids), fatty acids (hydroxypalmitic acid and conjugated linoleic acid), as well as other biologically active components.

It should be noted that the content of cardiac glycosides in the extracts of both species was negligible, detected only at trace levels. At the same time, the marker compound adonitol, which holds diagnostic significance within the chemotaxonomic systematics of the genus *Adonis* L., was reliably identified in both samples.

- the safety of the herbal pharmaceutical substances of *Adonis tianschanica* and *Adonis aestivalis* was established, along with the absence of their cytotoxic effects (Appendix A).

- an anti-inflammatory effect was demonstrated for *Adonis tianschanica*, which is rich in flavonoid compounds. Using high-speed counter-current chromatography (HSCCC), isorhamnetin was isolated, and its identification was confirmed by analysis of ^1H-NMR and ^13C-NMR spectra. It was found that this compound reduces nitric oxide (NO) production induced by lipopolysaccharides (LPS) and exhibits anti-inflammatory activity by decreasing the levels of pro-inflammatory cytokines IL-6, TNF-α, and IL-1β.

- The antioxidant activity of *Adonis aestivalis* was studied using DPPH (IC_{50} = 14.07 ± 0.10 μg/mL), ABTS (IC_{50} = 10.75 ± 0.11 μg/mL), and CUPRAC ($A_{0.5}$ = 45.00 ± 0.88 μg/mL) assays. The antioxidant activity of *Adonis aestivalis* was found to be comparable to that of standard compounds such as α-tocopherol (12.75 μg/mL) and BHT (16.77 μg/mL).

- based on the GxP and ICH (Q9, Q10, Q11) guidelines, a full-cycle methodology for the development of pharmaceutical substances from plants of the genus Adonis L. was established. A certificate of registration in the State Register of Copyright-Protected Objects No. 53681 dated January 21, 2025, was obtained (Appendix B).

- A cultivation technology for Adonis tianschanica was developed in accordance with GACP requirements. Patent No. 7727, titled "Method for Phytointroduction of Plants of the Genus Adonis L.," was granted (Appendix C).

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

- results of a comprehensive pharmacognostic study of the medicinal plant raw materials of *Adonis tianschanica* and *Adonis aestivalis*: phytochemical and morphological-anatomical analyses, and testing for the development of standardization approaches;
- results of the experimental and theoretical justification for the development of a technology for producing herbal pharmaceutical substances based on *Adonis tianschanica* and *Adonis aestivalis* raw materials with anti-inflammatory and antioxidant activities;
- results of *Adonis tianschanica* cultivation and techno-economic justification for the production of the herbal pharmaceutical substance.

Practical relevance of the study:

The technology of cultivation, collection, harvesting and storage of medicinal plant raw materials *A. tianschanica* at the enterprise "FitOleum" LLP, Act of introduction at "FitOleum" LLP №1 from 20.05.2022 (Appendix D).

Standard Operating Procedure (SOP) "Cultivation, harvesting, drying and storage of *Adonis tianshanica*" within the framework of modern quality concept (GxP and ICH (Q9, Q10, Q11)) was developed (Appendix D).

Expansion of the nomenclature of pharmacopoeial species of *Adonis* by including in the project "List of pharmacopoeial species of medicinal plants of the Republic of Kazakhstan" species *A. tianschanica* and *A. aestivalis*.

Draft regulatory and technical documents were developed: "Technological regulations for the production of herbal pharmaceutical substance "Adonis of Tien Shan herb", quality specification of "Adonis of Tien Shan herb", quality specification of " summer Adonis herb" (Appendices G, I, K).

At the Department of Pharmaceutical and Toxicological Chemistry, Pharmacognosy and Botany NJSC "KazNMU named after S.D.Asfendiyarov" introduced in the educational process "Comparative analysis of pharmacopoeial requirements for plants containing cardiac glycosides and flavanoids", act of introduction № 2 from 01.02.2023 and "Methodology for the creation of plant pharmaceutical substance from plants of the genus *Adonis* L.)", act of implementation № 3 from 27.10.2023 (Appendices L, M).

On the basis of the Department of Microbiology, Lublin Medical University, the results of phytochemical analysis and establishment of the profile of pharmacological activity were introduced into the educational process (Annex H).

A feasibility study for the production of plant pharmaceutical substance by cultivation at the enterprise "Fitoleum" LLP, Esik, Republic of Kazakhstan was developed.

Author's personal contribution.

The author independently analyzed and systematized the data of domestic and foreign scientific literature, as well as conducted a full cycle of experimental research within the framework of the thesis work. The reliability of the results and

the main provisions put forward for defense is confirmed by a significant amount of experimental data obtained in the course of research, carried out in laboratory and production conditions using modern equipment and innovative techniques.

Approbation of the work

The main provisions of the dissertation work reported and published in the materials: VI All-Russian scientific-practical conference with international participation "Innovations in the health of the nation" (St. Petersburg, Russia, 2018); VII scientific-practical conference with international participation "Priorities of pharmacy and dentistry: from theory to practice" (Almaty, 2018); VI International scientific conference of young scientists and students "Perspectives of development of biology, medicine and pharmacy" (Shymkent, 2018); IV International scientific-practical conference "Global science and dentistry: from theory to practice" (Almaty, 2018).); VI International Scientific Conference of Young Scientists and Students "Prospects of Development of Biology, Medicine and Pharmacy" (Shymkent, 2018); IV International Scientific Practical Conference "Global science and innovations 2019: Central Asia" (Astana, 2019); XIV International Scientific and Practical Conference of Young Scientists and Students devoted to "Years of Rural, Tourism and Folk Crafts (2019-2021)" "Scientific Discussion: Current Issues, Achievements and Innovations in Medicine" (Dushanbe, Tajikistan, 2019); IV International Scientific Conference "Scientific Discoveries" (Karlovy Vary, Czech Republic - Moscow, Russia, 2019); International Scientific and Practical Conference of Students, Young Scientists and Teachers "Akanov Readings: The Role of PHC in Achieving Universal Health Coverage" (Almaty, 2019).

Information on publications

According to the results of the research 13 scientific papers were published, including:

- article in an international peer-reviewed scientific journal included in the Scopus and Web of Science Core Collection databases (Q2 quartile) - 1 (Appendix M);
- articles in journals recommended by the Committee for Quality Assurance in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan - 4;
- abstracts at international scientific and practical conferences (Czech Republic, Russia, Tajikistan, Kazakhstan) - 6;
- patents of the Republic of Kazakhstan for utility model - 1;
- certificate of inclusion of information in the state register of rights to copyrighted objects - 1.

Relation of research objectives to the plan of scientific programs

The dissertation work was carried out within the framework of implementation of the Complex Plan of Development of Pharmaceutical and Medical Industry for 2020 - 2025 years, initiative project "Ethnopharmaceutical study of flora of Kazakhstan" № 0219RKI0150 and grant project of young scientists "Zhas galym" IRN № AP22686038.

Scope and structure of the dissertation

The dissertation is set out on 130 pages of typewritten text in computer typesetting, contains 27 tables, 29 figures, a list of literature, including 135 sources, as well as appendices. The work consists of an introduction, literature review, a section on materials and methods of research, three sections of own research, conclusions and conclusion.