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:

– Development of a methodology for the creation of herbal pharmaceutical substances based on the technology of cultivation, collection, processing and storage of plants of the genus *Adonis* L. in accordance with the requirements of GACP

– Pharmacognostic, pharmaceutical-technological and chemical study of plant raw materials from *A. tianschanica* and *A. aestivalis*

– Development of standardization criteria and stability study of herbal pharmaceutical substance from *A. tianschanica*

– Determination of safety and pharmacologic activity profile of herbal pharmaceutical substance from *A. tianschanica*

– Development of cultivation technology and feasibility study of production of herbal pharmaceutical substance from *A. tianschanica*

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

Subject of the study: methodology and development of herbal pharmaceutical substance; study of chemical composition, safety and pharmacological activity profile, standardization, stability study and feasibility study of production.

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:

- based on the concept of GxP and ICH (Q9, Q10, Q11) the methodology for the creation of herbal pharmaceutical substance from plants of the genus *Adonis* L. was developed, the Certificate of inclusion of information in the state register of rights to copyrighted objects No. 53681 dated January 21, 2025 was obtained. (Annex A).

- technology of cultivation of plant raw materials of *A. tianschanica* in accordance with the GACP requirements was developed. Patent No. 7727 "Method of phytointroduction of plants of the genus *Adonis* L." was obtained. (Appendix B).

- pharmacognostic analysis and standardization of medicinal plant raw materials of *A. tianschanica* was carried out, morphological, anatomo-diagnostic features of plant materials were established. The results of phytochemical investigation by HPLC-ESI-qTOF-MS/MS (high-performance liquid chromatography with electron spray ionization and tandem mass spectrometry based on a quadrupole-time-of-flight analyzer) revealed 27 compounds. They were detected: flavanoids (isoquercitrin, kaempferol and its derivatives, adonivertin, sinapoylsaponarin, isoorientin, isovitexin, orientin, orientin glucoside, vitexin, luteon glycoside), organic alcohols (adonitol), cardiac glycosides (strophanthidin, cimarin) and other compounds, and two unknown flavonoid glycosides containing hexose and tetrose residues were also identified.

- pharmacognostic analysis and standardization of plant materials of *A. aestivalis* was carried out, morphological, anatomo-diagnostic signs of plant materials were established. Phytochemical analysis of *A. aestivalis* by HPLC-ESI-qTOF-MS/MS method showed the presence of 21 compounds including: flavanoids (kaempferol and its derivatives, isoorientin, isovitexin, orientin, orientin glucoside, vitexin, isoquercetin, luteon glycoside), organic alcohols (adonitol), cardiac glycosides (strophanthidin, cymarin), organic acids (maleic acid, citric acid), fatty acids (hydroxypalmitic acid, conjugated linoleic acid) and other compounds.

- safety as well as absence of cytotoxic effect of extracts of medicinal plant raw materials was established (Appendix B).

- The anti-inflammatory effect of *A. tianschanica* rich in flavonoid compounds was established. Isoquercitrin was isolated from the compounds by HSCCC (High-Speed Counter-Current Chromatography), the identification was confirmed by analyzing ^{1}H-NMR and ^{13}C-NMR spectra. It was found that this compound reduces nitric oxide (NO) production under the action of lipopolysaccharides (LPS) and exhibits anti-inflammatory activity, reducing the level of proinflammatory cytokines IL-6, TNF-α and IL-1β.

- The antioxidant activity of *A. aestivalis* was studied by DPPH (IC50 = 14.07 ± 0.10 μg/mL), ABTS (IC50 = 10.75 ± 0.11 μg/mL) and CUPRAC (A0.5 = 45.00 ± 0.88 μg/mL) methods. The results of antioxidant activity of *A. aestivalis* are comparable to the antioxidant activity of standard compounds such as α-tocopherol (12.75 μg/mL) and BHT (16.77 μg/mL).

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

- results of phytochemical analysis of *A. tianschanica* and *A. aestivalis*, stability studies, standardization, terms and conditions of storage of herbal pharmaceutical substances;

- results of safety study and establishment of pharmacological activity profile of herbal pharmaceutical substances of *A. tianschanica* and *A. aestivalis*;

- methodology of herbal pharmaceutical substance development based on cultivation, collection, processing and storage technology from plants of *Adonis* L. genus within the framework of modern quality concept (GxP and ICH (Q9, Q10, Q11)) and feasibility study of production.

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 (Annex 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 (Annex 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 129 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.