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.

Aim of the Dissertation 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 *su*;

–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.

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

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.

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.

Description of Main Research Findings

The synthesis of data from the literature review enabled the systematic classification of morphological, anatomical, chemical, and pharmacological characteristics of the genus *Adonis* L. Based on this, the selection of the studied species was substantiated as promising sources for the development of plant-derived pharmaceutical substances.

Distinct differences in morphological and anatomical structure were established, including stem shape and pubescence, leaf dissection, flower coloration, as well as the thickness of the epidermis and palisade mesophyll. These features ensured the reliable identification of the investigated raw plant materials.

Experimental data demonstrated that the concentration of cardiac glycosides in the herb of *A. tianschanica* (0.0002%) and *A. aestivalis* (0.00017%) is significantly lower than that of the pharmacopoeial species *A. vernalis*, thereby excluding them as viable sources of cardiotonic glycosides. However, extracts from both species were found to contain substantial amounts of biologically active compounds: the total phenolic content in *A. tianschanica* was 6.272%, including 3.992% flavonoids, whereas in *A. aestivalis* it amounted to 4.033% and 2.625%, respectively. A 50% ethanol solution was determined to be the most effective solvent for extracting polar metabolites. Chromatographic analysis using HPLC/ESI-QTOF-MS/MS identified 27 compounds in *A. tianschanica*, including flavonoids (isoquercitrin, kaempferol, quercetin, among others), adonitol, trace amounts of cardiac glycosides, and an alkaloid. In *A. aestivalis*, 21 compounds of similar nature were detected. Particular attention is drawn to the high concentration of the pentahydroxy alcohol adonitol found in both species. This compound is considered a chemotaxonomic marker of the genus *Adonis* L. and is also known for its pronounced diuretic and dehydrating effects, as supported by scientific literature.

Isoquercitrin, identified as the predominant flavonoid in *A. tianschanica*, was isolated using high-speed counter-current chromatography (HSCCC) and characterized by NMR and mass spectrometry. The study of biological activity revealed pronounced anti-inflammatory and antioxidant properties of the extracts: isoquercitrin significantly reduced the production of pro-inflammatory cytokines IL-6 and IL-1β, while the extract of *A. aestivalis* exhibited high antioxidant activity (IC_{50} ~10–14 μg/mL). Both in vitro and in vivo assays confirmed the low toxicity and favorable biocompatibility of the investigated extracts.

Additional evidence supporting the pharmacological potential of the studied species was obtained through the analysis of their amino acid, lipid, and mineral composition. The amino acid profile of *A. tianschanica* is characterized by a high content of serine, asparagine, glutamic acid, tryptophan, and valine, with a total concentration of 8.777 mg/g, indicating the presence of plastic and functional components essential for metabolic processes. Unsaturated fatty acids predominated in the lipid fraction: both species contained substantial amounts of linoleic and linolenic acids, with the ω-3 linolenic acid in the polar lipids of *A. aestivalis* reaching up to 55.23%. Such a lipid profile suggests potential cardioprotective and anti-inflammatory activity. Mineral analysis confirmed the presence of 17 micro- and macroelements, including potassium, calcium, magnesium, iron, manganese, zinc, and selenium. *A. tianschanica* was found to have a more balanced mineral composition, with calcium content reaching 1200 mg/kg, iron – 40 mg/kg, and manganese – 43 mg/kg. These parameters support the consideration of this species as a promising raw material for the development of substances aimed at maintaining electrolyte balance and normalizing metabolic functions.

Based on comprehensive pharmacognostic studies confirming the potential of the investigated plant material as a source of biologically active compounds, the obtained plant-derived pharmaceutical substances were standardized.

The stability of these substances was assessed over a 24-month period under

long-term storage conditions (25 ± 2 °C, 60 ± 5% relative humidity) across three production batches, stored in both primary and secondary packaging. All monitored quality parameters remained within the established specifications throughout the study period, confirming the stability of the plant-derived pharmaceutical substances and supporting a shelf life of no less than 24 months.

To evaluate the safety and pharmacological profile of the plant-derived pharmaceutical substances, toxicological and pharmacological studies were conducted. The results of *in vivo* studies on the acute and subacute toxicity of aqueous-ethanolic extracts of *A. tianschanica* and *A. aestivalis* demonstrated low toxicity following oral administration, with LD_{50} values of 2853.7 mg/kg and 5012.8 mg/kg, respectively. These values correspond to hazard classes IV and V according to the OECD classification. Histomorphological analysis revealed no pathological changes in the liver, kidney, or heart tissues.

In vitro studies on the macrophage cell line RAW 264.7 confirmed the absence of cytotoxic effects of the *A. tianschanica* extract (at concentrations up to 50 μg/mL) and its major flavonoid, isoquercitrin (up to 100 μL), with cell viability maintained above 85%. In an LPS-induced macrophage inflammation model, a significant reduction in nitric oxide (NO) production and pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) was observed, indicating the anti-inflammatory activity of these compounds. Additionally, the *A. aestivalis* extract exhibited pronounced antioxidant activity, with IC_{50} values of 14.07 μg/mL in the DPPH assay and 10.75 μg/mL in the ABTS assay. This activity is attributed to the presence of polyphenolic compounds, including isoquercitrin.

As part of the practical implementation of the obtained results, special attention was given to the cultivation and harvesting of plant raw materials. An agrobiological study of *A. tianschanica* was carried out on pilot-industrial plots of LLP 'Fitoleum', during which the main ontogenetic stages were examined and agrotechnical conditions were optimized (stratification, substrate composition, fertilization, and irrigation regime) in accordance with GACP requirements. The use of vermicompost (biohumus) ensured the highest seedling survival rate, reaching 86%.

The developed cultivation technology, along with protocols for harvesting, drying, and storage of raw materials, was formalized in the form of a Standard Operating Procedure (SOP) and a technological regulation. The optimal drying temperature was determined to be 50–60 °C, under which flavonoid losses did not exceed 4%, compared to up to 18% during air-shade drying. The highest flavonoid content was recorded during the flowering and early fruiting stages. These findings confirm the effectiveness of the implemented technology in establishing a stable raw material supply chain.

The conducted techno-economic assessment of the production of a plant-based pharmaceutical substance derived from *A. tianschanica* confirmed its high production efficiency and commercial feasibility. The use of local raw materials and adapted technologies ensures a high level of competitiveness of the product in both domestic and international markets. The results obtained served as a basis for introducing a domestically produced plant-based substance to the pharmaceutical market, intended for the manufacture of medicinal products, with confirmed potential for industrial implementation.

Justification of Scientific Novelty

For the first time:

- Morphological and anatomical diagnostic characteristics were identified as part of the pharmacognostic analysis of the medicinal plant raw materials *A. tianschanica* and *A. aestivalis*.

- A detailed phytochemical analysis of *A. tianschanica* and *A. aestivalis* extracts was performed using high-performance liquid chromatography coupled with electrospray ionization and quadrupole time-of-flight tandem mass spectrometry (HPLC/ESI-QTOF-MS/MS), a technique known for its high sensitivity and precision in molecular mass determination.

- The safety of the plant-derived pharmaceutical substances from *A. tianschanica* and *A. aestivalis*, as well as the absence of cytotoxic effects, was confirmed.

- The anti-inflammatory activity of the *A. tianschanica* substance and the antioxidant activity of the *A. aestivalis* substance were established. In in vitro studies, the extract of *A. tianschanica* and its major flavonoid, isoquercitrin, reduced the production of nitric oxide (NO) and pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) in LPS-induced macrophages. The extract of *A. aestivalis* demonstrated pronounced antioxidant activity, comparable to standard antioxidants, attributed to the presence of polyphenolic compounds including isoquercitrin.

- A methodology for the full-cycle development of a pharmaceutical substance derived from Adonis L. species was developed. A certificate of registration in the State Register of Copyright-Protected Works was obtained (No. 53681, dated January 21, 2025; Appendix B).

- A cultivation technology for *A. tianschanica* was developed in accordance with GACP guidelines. A national patent was granted for the invention: Patent No. 7727 “Method of Phytointroduction of Plants of the Genus *Adonis* L.”.

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.

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.

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 tianshanica* herb", quality specification of " *Adonis aestivalis* L. herb".

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.

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

Personal Contribution of the Doctoral Candidate.

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.

Conclusions:

1. The results of the literature review enabled the systematization of morphological, chemical, pharmacological, and agrobiological characteristics of plants from the genus *Adonis* L. It was found that A. aestivalis had been only sparsely described, while A. tianschanica remained virtually unexplored in contemporary scientific literature. This highlights the need for in-depth investigation of these species.

2. The development of a plant-derived pharmaceutical substance represents a scientifically grounded, multi-stage process that complies with pharmacopoeial and regulatory requirements for quality, safety, and efficacy. In this study, a step-by-step methodology for the standardization of substances derived from Adonis L. species was developed, taking into account the potential for adaptation to domestic pharmaceutical production. The methodology was registered as an intellectual property object under Certificate No. 53681 dated January 21, 2025.

3. Clear differences in macro- and microscopic structures were identified between *A. tianschanica* and *A. aestivalis*. The concentrations of cardiac glycosides in both species (*A. tianschanica* — 0.0002 ± 0.001%, *A. aestivalis* — 0.00017 ± 0.002%) were significantly lower than in the pharmacopoeial species *A. vernalis*, thus ruling them out as potential sources of cardiotonic glycosides. Comparative extraction using water, 50% ethanol, and 96% ethanol demonstrated that 50% ethanol was the most effective solvent for flavonoid extraction. The chemical composition of the extracts was determined by HPLC/ESI-QTOF-MS/MS: 27 compounds were identified in *A. tianschanica* and 21 in *A. aestivalis*. The predominant components in both cases were flavonoids — kaempferol, quercetin, and isoquercitrin. Trace amounts of strophanthidin and cymarin (cardiac glycosides), as well as the alkaloid embelin, were also detected. A considerable content of adonitol — a chemotaxonomic marker of the *Adonis* genus - was confirmed in both samples.

4. In the standardization of pharmaceutical substances derived from *A. tianschanica* and *A. aestivalis*, the quantification of biologically active compounds (BACs) was based on the total content of flavonoids and adonitol. Flavonoid content was determined using UV-visible spectrophotometry, recalculated as isoquercitrin equivalents. The validated spectra confirmed complete alignment between the flavonoid complex and standard isoquercitrin (~λ = 410 nm), supporting the accuracy and selectivity of the method. Stability testing of the substances was conducted over a 24-month period under standard long-term storage conditions, using paper packaging (50 g) in three independent batches.

5. Water-ethanol extracts of *A. tianschanica* and *A. aestivalis* demonstrated low oral toxicity in acute toxicity tests: LD_{50} for *A. tianschanica* was 2853.7 mg/kg and for

A. aestivalis - 5012.8 mg/kg, corresponding to toxicity classes IV and V according to OECD guidelines. Histomorphological studies revealed no pathological changes in liver, kidney, or heart tissues. In in vitro experiments on RAW 264.7 macrophage cells, no cytotoxic effect was observed for the *A. tianschanica* extract (up to 50 μg/mL) or for isolated isoquercitrin (up to 100 μM), with cell viability exceeding 85% in all cases, confirming good biocompatibility. A significant reduction in the production of nitric oxide (NO), IL-6, TNF-α, and IL-1β was established upon treatment: the *A. tianschanica* extract reduced NO by 24.5% and IL-6 by 60%; isoquercitrin reduced NO by 34.3% and IL-1β by 52.7%. The *A. aestivalis* extract exhibited strong antioxidant activity: IC_{50} values were 14.07 ± 0.10 μg/mL (DPPH), 10.75 ± 0.11 μg/mL (ABTS), and $A_{0.5}$ = 45.00 ± 0.88 μg/mL (CUPRAC), which is attributed to the presence of polyphenolic compounds, including isoquercitrin.

6. The experimental data obtained from the pilot-industrial sites of the pharmaceutical company LLP "Fitoleum" enabled a comprehensive justification of the biological and agrotechnical conditions required for the cultivation of *A. tianschanica* under controlled conditions. The use of biohumus ensured optimal seedling survival and growth rates (86%), confirming the effectiveness of implementing this cultivation technology for the development of a stable raw material base. Based on the research findings, the company developed and approved a Standard Operating Procedure (SOP) entitled “Cultivation, Harvesting, Drying, and Storage of *A. tianschanica*”, as well as a technological regulation for the production of a plant-based pharmaceutical substance from *Adonis tianschanica* herb. The conducted techno-economic assessment substantiates the feasibility of using domestically sourced plant raw materials and adapted technological solutions. Considering the high cost of imported phytosubstances, the integration of local resources enhances the accessibility of the final product and ensures its sustainable competitiveness in both domestic and international pharmaceutical markets.

Approbation of the Dissertation Results

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).

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;
- 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.

Scope and structure of the dissertation

The dissertation is set out on 131 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.