# ANNOTATION

of dissertation work by **Zhumashova Gulsim Tokanovna** on the topic **«Pharmacognostic study and technological aspects of developing new drugs from herbal plant *Rheum cordatum* Losinsk.»**, submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D110400 -" Pharmacy "

**Relevance of the research topic**

The State program for the development of health care of the Republic of Kazakhstan "Densaulyk" for 2016-2019 provides for the implementation of the National Medicines Policy aimed at ensuring the availability and rational use of medicines.

In accordance with the Strategic Plan of the Ministry of Health of the Republic of Kazakhstan for 2017-2021 the intensive development of the domestic pharmaceutical industry provides for an increase in the competitiveness and export orientation of products through the introduction of good manufacturing practices (GMP) and harmonized quality requirements for medicines, including those produced from medicinal plant materials in the domestic growing area. The solution of the problem requires scientific research to identify new types of medicinal and medicinal plant raw materials, the development of safe, effective and high-quality drugs from them, their introduction into medical practice.

One of the most common pathological conditions is diseases of the gastrointestinal tract. Improper nutrition and violation of its regime, an increase in the causes of stress, low physical activity and bad habits are decisive factors in the development of diseases of the gastrointestinal tract. A significant part of the population (up to 70 %) with varying frequency face a violation of the gastrointestinal tract, which manifests itself in a difficult or rare emptying of the intestines (constipation). Laxatives are used to treat these functional disorders of the digestive system. Laxative properties are especially pronounced in a number of medicinal plants, including plants of the genus Rhubarb (*Rheum* L.).

Among the plants of this genus, growing in Kazakhstan *Rheum cordatum* Losinsk. is of significant scientific and practical interest. It is a valuable source of biologically active substances and is used in folk medicine for diseases of the gastrointestinal tract.

In this regard, a comprehensive study of medicinal plants and raw materials from them, their standardization, the development of a technology for obtaining a plant pharmaceutical substance, the development of composition and technologies for obtaining medicinal products is an urgent scientific problem.

**Purpose of the study:** study of anatomical and diagnostic features, phytochemical research and standardization of raw materials from rhubarb and the creation of safe and effective drugs on its basis.

**Research objectives:**

- to analyze the domestic pharmaceutical market for laxative drugs;
- to develop an appropriate technology for the collection and procurement of raw materials;

- to establish the main morphological and anatomical features of raw materials from *Rheum cordatum* and carry out a comparative analysis with the features of the official species - Tangut rhubarb (*Rheum palmatum* L. var. *tanguticum* Regel.);

- to carry out phytochemical analysis and determine the quantitative content of the main groups of biologically active substances in various organs of the medicinal plant (roots, leaves, stems and seeds);

- to develop an optimal technology for obtaining an extract from the roots of *Rheum cordatum* and to carry out standardization;

- to study the acute and sub-acute toxicity, antioxidant and anti-inflammatory activities of the extract;

- to carry out pharmaceutical development of coated tablets based on an extract from the roots of *Rheum cordatum*;

- feasibility study for the production of coated tablets.

**Objects of research:** *Rheum cordatum* and its parts (roots, leaves, petioles and seeds), thick extract from *Rheum cordatum* roots, coated tablets.

**Research methods:** physical, physico-chemical, chemical, pharmacognostic, pharmaceutical-technological, pharmacological, microbiological, statistical methods.

**Scientific novelty**

The morphological and anatomical diagnostic features of raw rhubarb are determined in comparison with the features of the official species.

As a result of phytochemical studies of organs (roots, leaves, stems and seeds) of *Rheum cordatum* L., 22 compounds (anthracene derivatives, tannins, catechins and their gallates, flavonoids and their glycosides) have been identified for the first time. All identified compounds were previously found in other plant species of the genus Rhubarb (*Rheum* L.); however, most of the presented components are described for the first time in extracts from *Rheum cordatum*.

New data were obtained on the composition and quantitative content of biologically active substances (anthracene derivatives, polysaccharides, tannins, flavonoids, organic acids, amino acids, chlorophylls and carotenoids), the content of 19 macro- and microelements, 18 amino acids in the raw material of *Rheum cordatum* was established.

As a result of a comparative analysis of the extracts, it was found that the most enriched with metabolites (anthracene derivatives and other phenolic compounds) organ of *Rheum cordatum* is its roots. According to the results of the study, the standardization of the root of *Rheum cordatum* was carried out in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan.

A new method has been developed for obtaining a thick extract from the roots of *Rheum cordatum*, which makes it possible to increase the yield of biologically active substances and reduce the extraction time in comparison with the traditional method of percolation. The novelty of the developed method is confirmed by patents for utility model No. 4555 "Method for obtaining an extract from crushed plant materials" and No. 4553 "Method for obtaining an extract from rhubarb root for use in pharmaceutical and food

products", registered in the State Register of Utility Models of the Republic of Kazakhstan on December 19, 2019.

For the first time, the safety, high antioxidant and anti-inflammatory activity of aqueous and alcoholic extracts from the roots of *Rheum cordatum* has been determined.

For the first time, the composition and technology for producing coated tablets based on a thick extract from the roots of *Rheum cordatum* were developed. The novelty of the developed method is confirmed by the utility model patent No. 4554 "Pharmaceutical composition from plant raw materials with a laxative effect", registered in the State Register of Utility Models of the Republic of Kazakhstan on December 19, 2019.

**The main provisions of the dissertation research submitted for defense:**

- the analysis of the pharmaceutical market of the segment of laxative drugs for the period from 2016-2019 in terms of sales in physical and monetary terms allows identifying stable growth;

- pharmacognostic study of raw rhubarb, a comprehensive phytochemical study of various organs (roots, leaves, stems and seeds) of *Rheum cordatum* made it possible to identify the raw material and belonging to the producing plant species (*Rheum cordatum* L.), as well as to determine the organ (roots) with the highest content of biologically active substances (anthracene derivatives);

- development of an appropriate technology for the collection and procurement of raw materials from *Rheum cordatum*, obtaining a thick extract from its roots make it possible to isolate a plant pharmaceutical substance of pharmacopoeial quality with established safety and pharmacological activity;

- rational composition, optimal technology of coated tablets based on a thick extract of the roots of *Rheum cordatum*, compliance of their quality indicators with pharmacopoeial requirements and feasibility study results allow us to recommend this project to domestic manufacturers for implementation in production.

**The practical significance of the study**

The technology of collection, harvesting and storage of the roots of *Rheum cordatum* was introduced by FitOleum LLP, Esik, Republic of Kazakhstan.

A quality specification for "*Rheum cordatum* roots" has been developed.

An optimal method for obtaining a dense extract from the roots of *Rheum cordatum* with the use of various fractions of raw materials crushed by rolling and intensification of the process by evacuating the receiver has been tested, which allows increasing the yield of extractive substances and reducing the extraction time on the basis of the Department of Industrial Pharmacy of the National University of Pharmacy, Kharkov, Ukraine (Approbation Act 05.12. 2018).

A quality specification has been developed for "Thick extract of the roots of *Rheum cordatum*".

The production technology and quality control methods of coated tablets based on a thick extract from the roots of *Rheum cordatum* have been successfully tested in the pharmaceutical development department of Viva Pharm LLP, Almaty, Republic of Kazakhstan (Approbation Act).

A quality specification has been developed for “Film-coated tablets, based on the thick extract from the roots of *Rheum cordatum*”.

**Personal contribution**

All the results of the dissertation research were obtained by the author independently, which testifies to the personal contribution of the applicant to the science in the field of pharmacy. The reliability of the results, the main provisions for the defense, the findings and conclusions formulated in the dissertation work are justified by a significant amount of experimental material, fully confirmed by the results of our own research carried out in laboratory and industrial conditions, using modern instruments and accurate measurement methods, as well as comparison with literary data.

**Approbation of work**

The main provisions of the dissertation work were reported and published in the materials of the following scientific forums: International Scientific and Practical Conference "Science and Education in the Modern World" (Karaganda, Kazakhstan, 2018); V International Scientific and Practical Conference of Students and Young Scientists "Science and Medicine: Modern View of Youth" (Almaty, Kazakhstan, 2018); International scientific-practical conference "Modern methods of correction of acne and other skin problems in the practice of a cosmetologist" (Kharkov, Ukraine, 2018); International scientific and practical conference "IV Global Science and Innovation 2019: Central Asia" (Astana, Kazakhstan, 2019); VII All-Russian scientific and practical conference with international participation "Innovations in the health of the nation" (St. Petersburg, Russia, 2019); International scientific and practical conference "Medical science of the XXI century - a look into the future" (Dushanbe, Tajikistan, 2019); International Scientific and Practical Internet Conference "Modern achievements of pharmaceutical science in the creation and standardization of medicines and dietary supplements that contain components of natural origin" (Kharkov, Ukraine, 2020); International online conference “Modern science. Management and standards of scientific research "(Prague, Czech Republic, 2020).

**Information about publications**

According to the research results, 21 scientific works were published, including:

- an article in an international peer-reviewed scientific journal included in the Scopus and Web of Science Core Collection databases - 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 - 6;
- abstracts and articles at international scientific and practical conferences (Russia, Ukraine, Czech Republic, Tajikistan, Kazakhstan) - 8;
- articles in international journals (Russia, Tajikistan, Kyrgyzstan) - 3;
- utility model patents - 3.

**The volume and structure of the thesis**

The thesis is presented on 132 pages of typewritten text in a computer set. It contains 35 tables, 63 figures, a list of references, including 188 sources, as well as 23 applications.

The work consists of an introduction, a review of the literature, a section devoted to materials and research methods, three sections of our own research, findings and conclusion.

**Conclusions**

1. Analysis of literature data revealed that official plant species of the genus Rhubarb (*Rheum* L.) do not grow in Kazakhstan. An assessment of the prospects for studying domestic plant species of this genus showed that *Rheum cordatum* Losinsk. is of scientific and practical interest as a renewable source of plant raw materials for the production of medicines.

2. The results of the analysis of the domestic pharmaceutical market in the segment of laxative drugs for 2012-2016 showed a pronounced dependence on imports, with the share of domestic producers averaging 30,2 %. The study of the sales volume of 4 herbal laxative medicines in the form of tablets showed an increase in both natural and monetary terms, which confirms the increase in consumer demand and, accordingly, the prospects for the development and feasibility of producing domestic herbal laxative medicines.

3. In accordance with the modern requirements of the GACP standard, for the first time, a technology for collection, preparation and storage of raw materials from *Rheum cordatum* has been developed. As a result of studying the dynamics of accumulation of the main groups of biologically active substances (anthracene derivatives and tannins), the optimal period for collecting roots was established - after insemination and at the beginning of the growing season.

4. The morphological and anatomical diagnostic features of raw rhubarb are determined in comparison with the features of the official species.

For the first time, a phytochemical study of raw materials (roots, leaves, stems and seeds) of *Rheum cordatum* was carried out and 22 phenolic compounds (anthracene derivatives, catechins and their gallates, tannins, flavonoids (quercetin, apigenin, kaempferol) and their glycosides were identified. The content of emodin (1,29 %), epicatechin gallate (5,00 %) in the root extract based on 50 % *ethanol* and rutoside (4,87 %) in an aqueous extract from leaves was noted. They can be considered marker compounds that determine the species belonging of *Rheum cordatum*.

5. The quantitative content of the main groups of biologically active substances (anthracene derivatives, polysaccharides, flavonoids, tannins, organic acids, amino acids, chlorophylls and carotenoids) was established, the content of 19 macro- and microelements, 18 amino acids in the raw rhubarb was determined.

As a result of a comparative analysis of the extracts, it was found that the most enriched in metabolites organ (anthracene derivatives and other phenolic compounds) is the roots of *Rheum cordatum*. According to the results of the study, the standardization of the root of *Rheum cordatum* was carried out in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan.

A comprehensive pharmacognostic study of various organs (roots, leaves, stems, seeds) of *Rheum cordatum* made it possible to identify the raw material, establish the quality and belonging of the species *Rheum cordatum* L. to the producing plant, and also

identify the organ (roots) with the highest content of biologically active substances (anthracene derivatives).

Indicators of the quality of *Rheum cordatum* roots and their acceptance criteria have been established, and a quality specification has been developed. The quality specification for "*Rheum cordatum* roots" has been developed.

As a result of long-term stability tests of raw materials, a shelf life of 24 months (temperature 25 ± 2 ° C and relative humidity 60 ± 5%) was established.

6. To select the optimal conditions for the extraction process, the pharmaceutical and technological parameters of raw materials from the roots of *Rheum cordatum* with various particle were studied. The optimal conditions and technological parameters of the extraction process (extractant *30 % ethanol*) were selected. Evaluation of the process efficiency criteria confirmed vacuum filtration extraction as the best method compared to traditional percolation. An optimal method has been developed for obtaining a thick extract from *Rheum cordatum* roots using various fractions of raw materials crushed by rolling and intensification of the process by evacuating the receiver, which allows increasing the yield of extractive substances and shortening the extraction time. At the Department of Industrial Pharmacy of the National University of Pharmacy (Kharkiv, Ukraine), the technology of obtaining a thick extract from the roots of *Rheum cordatum* has been tested (Approbation act dated 05.12.2018). The novelty is confirmed by patents for useful model No. 4555 "Method for obtaining an extract from crushed plant materials" and No. 4553 "Method for obtaining an extract from rhubarb root for use in pharmaceutical and food products", registered in the State Register of Utility Models of the Republic of Kazakhstan on December 19, 2019. Indicators of quality of the thick extract from the roots of *Rheum cordatum* and their acceptance criteria have been established, a quality specification has been developed.

The stability test, the determination of the shelf life and the recommended storage conditions of the thick extract from the roots of *Rheum cordatum* were carried out by the main quality indicators.

7. As a result of a safety study, it was found that the thick extract from the roots of *Rheum cordatum* belongs to relatively harmless substances (VI class of toxicity) and does not have an allergenic effect. Specific pharmacological studies have shown that the thick extract from rhubarb roots at a concentration of 100 mg / kg has anti-inflammatory activity comparable to the activity of the reference drug Ibuprofen in the form of a suspension.

By inhibiting free radicals DPPH, a high antioxidant activity of extracts from the roots of *Rheum cordatum* was determined – 92,3 % (extractant 96 % *ethanol* ) and 91,59 % (aqueous extract). The maximum "Total Phenolic Index" (GAE 751,8 mg/l) was determined in an aqueous extract from the roots of *Rheum cordatum*.

8. Based on the principles of the “Quality by Design” concept, a rational composition and an optimal technology for producing coated tablets based on a thick extract of the roots of *Rheum cordatum* have been theoretically and experimentally substantiated and developed. The novelty is confirmed by the utility model patent No. 4554 "Pharmaceutical composition from plant raw materials with a laxative effect", registered in the State

Register of Utility Models of the Republic of Kazakhstan on 19.12.2019. The technology for producing coated tablets was tested in the pharmaceutical development department of Viva Pharm LLP, Almaty, the Republic of Kazakhstan. The quality indicators of coated tablets and their acceptance criteria have been established. A quality specification for coated tablets has been developed.

Within 18 months stability tests, the establishing of shelf life and recommended storage conditions for coated tablets were carried out. Long-term stability tests are ongoing.

The rational composition, optimal technology of coated tablets based on the thick extract from the roots of *Rheum cordatum*, compliance of their quality indicators with pharmacopoeial requirements and the results of a feasibility study allow us to recommend this project to domestic manufacturers for implementation in production.

**Assessment of the completeness of the tasks solution.** The dissertation work has an internal unity and demonstrates the full completion of the set tasks:

to analyze the domestic pharmaceutical market of laxative drugs in order to determine the prospects for the development and feasibility of producing domestic herbal laxative drugs;

to develop appropriate technology for the collection and procurement of raw materials;

to establish the main morphological and anatomical features of raw materials of *Rheum cordatum* and conduct a comparative analysis with the features of the official species - Tangut rhubarb (*Rheum palmatum* L. var. *tanguticum Regel*.);

to conduct the phytochemical analysis and quantification of the main groups of biologically active substances in various organs of the medicinal plant (roots, leaves, stems and seeds);

to develop an optimal technology for obtaining an extract from the roots of *Rheum cordatum* and conduct standardization;

to study acute and subacute toxicity, antioxidant and anti-inflammatory activity of the extract;

to implement pharmaceutical development of coated tablets based on an extract from the roots of *Rheum cordatum*;

feasibility study for the production of coated tablets.

**Recommendations and baseline data for the specific use of the results.** The results of evaluating the prospects of studying *Rheum cordatum* show that it is of scientific and practical interest, and determine this medicinal plant as a renewable source of plant raw materials for the production of drugs.

The developed technology for the collection, preparation and storage of raw materials from *Rheum cordatum* according to the GACP requirements was tested in the production conditions of FitOleum LLP and can be used in pilot and industrial conditions for the production of plant substances in a full cycle.

The developed new effective method for obtaining a thick extract from the roots of *Rheum cordatum* by vacuum filtration extraction can be recommended to domestic

manufacturers producing plant substances.

The results of the pharmaceutical development of coated tablets, carried out on the principles of the Quality by Design concept, can be recommended to the domestic manufacturer Viva Pharm LLP for the transfer of technology to a pilot industrial scale and further for proper research within the framework of creation of new medicines.

**Assessment of the technical and economic efficiency of implementation.** The results of the dissertation research have a high technical and economic efficiency, since the introduction into production of new plant substances "*Rheum cordatum* roots" and "Thick extract from the roots of *Rheum cordatum*" will expand the nomenclature of active pharmaceutical ingredients and will allow using them for creation new medicines with ATC classification A06A (laxatives).

**Assessment of the scientific level of the work performed in comparison with the best achievements in the field.** The obtained results of the work performed are innovative in nature, confirmed by 3 patents for a useful model and reflected in 6 articles published in journals recommended by the Committee for quality assurance in the field of education and science of the Ministry of Education and Science of the Republic of Kazakhstan; in the article “Phytochemical and Antioxidant Studies on a Rare *Rheum cordatum* Losinsk. Species from Kazakhstan”, published in the journal included in the Scopus and Web of Science Core Collection (Impact factor 4,868). The main results of the study were also included in the materials of international scientific and practical conferences (Russia, Ukraine, Czech Republic, Tajikistan, and Kazakhstan).

On the whole, the scientific and methodological level of the dissertation work meets the modern requirements of this category of work.