

ABSTRACT

of the dissertation work on the topic "Pharmaceutical justification for obtaining a phytosubstance from plant materials of *Lavatera thuringiaca* 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

The national project "Healthy Nation" high-quality and affordable healthcare for every citizen" is aimed at developing the domestic production of medicines and medical devices according to the development potential of the pharmaceutical industry in Kazakhstan. One of the main objectives of the project is a breakthrough in the production of domestic medicines and medical devices, ensuring their sufficient supply and expanding the range. The Kazakh people should have free access to quality medical products.

In addition, a comprehensive plan for the development of the pharmaceutical and medical industry for 2020-2025 has come into force today. The President of the Republic of Kazakhstan in his message for 2022 "The unity of the people and systemic reforms is a powerful basis for the country's prosperity" noted the need to increase the share of medicines and medical products of domestic production by 2025 to 17-50%. One of the main tasks of the comprehensive plan is to study the development of biologically active substances of plant origin and other preparations in domestic enterprises, and increase their competitiveness.

According to the forecasts of the World Health Organization, over the past ten years, the share of herbal medicines in the total volume of medicines will be more than 60%. Therefore, at present, the share of herbal medicines in the world market is increasing. Worldwide, public interest in herbal medicines has increased significantly compared to synthetic medicines due to their milder action, less dependence and the absence of side effects.

The development of pharmaceutical production technologies in the Republic of Kazakhstan is a purposeful and strategically effective way to develop the production of herbal medicines, which is due to the unique stock of medicinal herbal raw materials in the country, a significant scientific and technical potential in the production of new medicinal products from herbal raw materials. Based on this, one of the main priority tasks on the path of development of the domestic pharmaceutical industry is the search for new sources of medicines, the development of original domestic pharmaceutical substances and the introduction into practice of medicines based on medicinal plant materials.

Lavatera thuringiaca L. is of practical interest as a promising medicinal plant rich in biologically active substances. According to the results of an analytical review of the literature, the medicinal plant *Lavatera thuringiaca* L. has not been systematically

studied in Kazakhstan. *Lavatera thuringiaca* L. is widespread in all regions of the Republic of Kazakhstan. *Lavatera thuringiaca* L. - belong to the genus *Lavatera* of the *Malvaceae* family. The only species of this family is *Lavatera thuringiaca* L., which grows in steppes, pastures, forests, roadsides, rivers, lakes of all regions of Kazakhstan [1].

This plant is a medicinal plant widely used in folk medicine for colds, coughs, diarrhea and some other diseases of the gastrointestinal tract, which has anti-inflammatory, antimicrobial and other pharmacological properties [2].

The medicinal properties of *Lavatera thuringiaca* L. are due to its chemical composition. The composition contains mucus (17.37%), polysaccharides (9%), fatty acids (15.8%), alkaloids, flavanoids, phospholipids, phenolipids, condensed and hydrolysable tannins, coumarins, these compounds indicate the feasibility of studying and implementing of this raw material in scientific medicine.

Russian scientists have identified phenolic compounds contained in the rhizomes, grass, stem, leaves, flowers of plant material *Lavatera thuringiaca* L. using high-performance liquid chromatography. In addition, the composition of flavonoids and phenolic acids in the flowers of *Lavatera thuringiaca* L., growing in Poland, has been studied. It has been established that the roots of *Lavatera thuringiaca* L. contain at least 9% of the amount of water-soluble polysaccharides and at least 3% of the total of monosaccharides, including reducing sugars. Phenolic compounds, flavonoids and polysaccharides in the composition of the plant raw material *Lavatera thuringiaca* L. Pavle Z. Mašković and others have been fully studied by Serbian scientists. They obtained extracts by Soxhlet, maceration, ultrasound, microwave and subcritical water extraction and studied their antimicrobial, cytotoxic, antioxidant effects. The chemical components of these extracts were determined by HPLC-DAD. When determining the component composition of extracts obtained by foreign scientists, the class of terpenes was not identified.

In this regard, for the first time in our study, a CO₂ extract was obtained under subcritical conditions from the aerial part of *Lavatera thuringiaca* L. and its component composition was studied, in which compounds of the terpene class were determined, and antimicrobial activity against pathogenic bacteria was determined. Flavonoids in the composition of the extract from *Lavatera thuringiaca* L. were not determined by HPLC. When studying the component composition of the pharmaceutical substance by GC/MS, saturated and unsaturated fatty acids, terpenes and their derivatives, coumarin derivatives, and other biologically active substances were identified.

Therefore, the study of the chemical composition of the medicinal plant material *Lavatera thuringiaca* L., the selection and standardization of effective technological aspects for obtaining extracts from plant materials and the optimal methods for searching for biologically active compounds in their composition is an urgent problem.

The purpose of the research work: the search for the main biologically active substances in the composition of the medicinal plant material *Lavatera thuringiaca* L.,

the development and standardization of the technology of the resulting pharmaceutical substance.

Objects of study: *Lavatera thuringiaca* L., growing on the territory of Kazakhstan and extracts obtained by maceration, percolation, CO₂ extraction under subcritical and supercritical conditions.

Subject of study: According to the scientific literature, analysis of the area of growth, chemical composition, use in official and traditional medicine and pharmacological properties of the object of study of the medicinal plant *Lavatera thuringiaca* L.; on this basis, the definition of the goals and objectives of scientific research, the determination of the pharmacognostic features of plant raw materials *Lavatera thuringiaca* L., the standardization of medicinal plant materials, obtaining the optimal technology of extracts and the study of their chemical composition and pharmacological properties, the development of regulatory documents, the collection of final materials that determine the theoretical and practical value dissertations.

Research tasks:

- Collection of plant raw materials *Lavatera thuringiaca* L. and study of the technology of harvesting and the area of its distribution;

- Determination of pharmacognostic features of medicinal plant materials *Lavatera thuringiaca* L.;

- Study of pharmaceutical and technological parameters of medicinal plant raw materials *Lavatera thuringiaca* L.;

- Optimal technology for obtaining an extract from plant raw materials *Lavatera thuringiaca* L. and its standardization;

- Study of the safety and pharmacological activity of the phytosubstance from *Lavatera thuringiaca* L.;

Scientific novelty of the research:

For the first time in Kazakhstan, a pharmacognostic analysis of medicinal plant raw materials *Lavatera thuringiaca* L. was carried out: macro- and microscopic analysis, commodity analysis, phytochemical analysis. For a comparative study of the chemical composition of the medicinal plant material *Lavatera thuringiaca* L., thick extracts were obtained by maceration, percolation, subcritical and supercritical carbon dioxide extraction, and their chemical composition was determined by gas chromatography using a mass spectrometric detector. As a result, the method of carbon dioxide extraction under subcritical conditions was chosen as the optimal technology for obtaining an extract from *Lavatera thuringiaca* L. plant raw materials; more than 40 chemical compounds were identified during the study of its phytochemical composition. Antimicrobial activity has been proven against test strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonasaeruginosa*, *Candida albicans*, *Streptococcus Pneumonia*, *Klebsiella pneumonia*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, as well as anti-inflammatory and antioxidant efficacy. The scientific novelty of the study is confirmed by a patent under registration number No. 35059 (Method for obtaining a carbon dioxide extract from the herb *Lavatera thuringiaca* L.

Key points of the dissertation research submitted for defense:

- The results of studying the distribution area, developing the technology for harvesting raw materials, determining the pharmacognostic features and pharmaceutical and technological parameters of *Lavatera thuringiaca* L. plant raw materials and its standardization.
- The results of research on the choice of the optimal technology for obtaining an extract from *Lavatera thuringiaca* L. raw materials, determining its component composition and standardization, assessing its safety and pharmacological activity.

Practical significance of the obtained results

Draft regulatory documents for plant raw materials *Lavatera thuringiaca* L. and extracts based on it have been developed, methods of analysis have been tested and implemented:

- The technology of collection and preparation of plant raw materials *Lavatera thuringiaca* L. is presented. The identification of medicinal plant materials is confirmed by the State Institution of the Republic of Kazakhstan "Institute of Botany and Phytointroduction". Registration reference number No. 01-08/273 (Appendix No. B).

- On the practical application of the technology for collecting and harvesting plant raw materials *Lavatera thuringiaca* L., an Implementation Act was presented in Zerde-Fito LLP (Appendix No. B).

- An Implementation Act was proposed for the "Method of obtaining a thick carbon dioxide extract from the aerial part of the plant material *Lavatera thuringiaca* L." in PoM Zhanafarm LLP (Appendix No. D).

- A draft technological instruction for the "Method of obtaining a thick carbon dioxide extract from the aerial part of the plant material *Lavatera thuringiaca* L." in PoM Zhanapharm LLP (Appendix No. D).

- An act of introducing the technological process for obtaining an extract by percolation from plant raw materials *Lavatera thuringiaca* L. was presented to the Department of Pharmaceutical Technology of the NJSC "Asfendiyarov Kazakh National Medical University" (Appendix No. E).

- An act of introducing the technological process for obtaining an extract by maceration from plant raw materials *Lavatera thuringiaca* L. was presented to the Department of Pharmaceutical Technology of the NJSC "Asfendiyarov Kazakh National Medical University" (Appendix No. G).

- Completed a scientific internship as part of a doctoral dissertation at the Bashkir State Medical University.

- Proposed Standard of the organization for the "Method of obtaining a thick carbonic extract from the aerial part of the plant material *Lavatera thuringiaca* L." in PoM Zhanapharm LLP (Appendix No. M).

A draft of regulatory documents has been developed for "CO₂ extract in subcritical conditions of the herb of *Lavatera thuringiaca* L." in PoM Zhanapharm LLP (Appendix No. N).

Personal contribution of a doctoral student

The doctoral student independently reviewed and analyzed information from domestic and foreign countries on the topic of the dissertation work, performed all the experimental work on the research tasks set. All the obtained research results are confirmed by the use of modern methods of analysis and equipment in research centers and laboratories.

The reliability and validity of the results of the research is confirmed by the fact that the work performed is intended to solve the current problem, carried out in the leading modern world-class research centers and the draft regulatory documents.

Approbation of the results of the dissertation:

The results of the research work performed on the topic of the dissertation are presented in the materials of the International Scientific Conference "Modern Aspects of Medicine and Pharmacy: Education, Science and Practice, dedicated to the 40th anniversary of the South Kazakhstan Medical Academy" (Shymkent, 2019); in the materials of the XXXVII International Scientific and Practical Conference (Penza, 2020) "Fundamental and applied scientific research: Topical issues, achievements and innovations"; in the proceedings of the III International Scientific and Practical Conference dedicated to the memory of Professor R. Dilbarkhanov "Formation and development prospects of the scientific School of Pharmacy: continuity of generations" (Almaty, 2020); in the materials of the IX International Conference "Publishing houses of pharmacy and dentistry: from theory to practice", dedicated to the memory of Professor Kiyashev D.K. in the framework of the 90th anniversary of the Asfendiyarov Kazakh National Medical University (Almaty, 2020); in the materials of the III International book edition of the countries of the Commonwealth of Independent States "The best young scientist - 2021" (Nur-sultan, 2021). Reported at the Department of Engineering Disciplines of the School of Pharmacy NJSC "Asfendiyarov Kazakh National Medical University" (Almaty, 2021); at the meeting of the Scientific Commission "Pharmacy and Technologies of Pharmaceutical Production" NJSC "Asfendiyarov Kazakh National Medical University" (Almaty, 2021).

Publications

The results of the dissertation research were published in 14 scientific papers, including: 1 publication in an international journal included in the Scopus database, 4 publications in journals recommended by the CQASES MES RK (Committee for Quality Assurance in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan), 6 publications in the materials of International scientific and practical conferences, 1 publication in the RSCI database, 1 publication in the publishing house "The Best Young Scientist - 2021", 1 patent for an invention.

Scope and structure of the dissertation: The dissertation is presented on 170 pages of typewritten text in a computer set, contains 61 tables, 64 figures, a list of references, including 128 sources, and 12 appendices. The work consists of an

introduction, a literature review, a section on research materials and methods, three sections of own research, findings and a conclusion.

Conclusion

1. Wild plant material *Lavatera thuringiaca* L. was collected during the summer period in the flowering phase in accordance with the requirements of GACP. The grass was dried in a special room of the Institute of Botany and Phytointroduction at room temperature $25\pm 2^{\circ}\text{C}$. It was checked that the collected raw materials do not contain solid particles of soil, dirt, dust, insects. The raw materials were placed in kraft paper bags of 10 kg each, indicating the name of the raw material, the place of procurement, the time of collection and the net weight. The study of the anatomical and morphological features of *Lavatera thuringiaca* L. raw materials revealed the following diagnostic features: when viewed from both sides of the leaf blade, the epidermal cells have straight or slightly curved walls. Anomocytic type stomal apparatuses are visible. The epidermis contains many stellate hairs. The upper epidermis consists of simple unicellular hairs and mucous idioblasts. The main vein in cross section has a round triangular shape. In the main vein, both sides of the epidermal cell are elongated with vertical walls. Star-shaped hairs along the vein. The phloem and around the parenchyma contain large amounts of calcium oxalate.

2. Identification of samples of plant raw materials *Lavatera thuringiaca* L. was carried out according to the following parameters: macro- and microscopic features of raw materials, qualitative and quantitative composition of biologically active substances. Qualitative and quantitative research revealed the presence of flavonoids, amino acids, alkaloids, polysaccharides, hydrolyzed and condensed tannins, essential oils (terpenes), phenolic acids, iridoids, coumarins and saponins.

According to the order of the Minister of Health of the Republic of Kazakhstan No. KR DSM-20 dated February 16, 2021, the quality indicators of *Lavatera thuringiaca* L. plant raw materials were determined and a quality specification was developed.

According to the order dated October 28, 2020, the results obtained over a long period of research on the raw materials of *Lavatera thuringiaca* L. allow us to establish a temperature of $25\pm 2^{\circ}\text{C}$, a relative humidity of $60\pm 5\%$, and a shelf life of 2 years.

3. Technological parameters of plant raw materials *Lavatera thuringiaca* L. were determined: specific, volume and bulk weight, porosity, free volume of the raw material layer, absorption coefficient of the extractant, extractive substances.

Based on the plant material *Lavatera thuringiaca* L., extracts were obtained by traditional and modern extraction methods. Used classical methods: maceration, percolation; modern methods: carbon dioxide CO_2 extraction under supercritical and subcritical conditions. The carbon dioxide extract obtained under subcritical conditions was chosen as the optimal extract, and the parameters of its extraction were determined. Optimal conditions for obtaining a subcritical carbon dioxide extract:

extraction time 11 hours, pressure 45-51 atm, extractant flow rate through the raw material 5-10 cm³/h, raw material fineness 1-3 mm and temperature 18-210 °C.

Analysis of the chemical composition of extracts obtained by maceration and percolation methods, sub- and supercritical carbon dioxide extraction, was carried out by gas chromatography (Agilent MSD ChemStation) using a mass spectrometric detector. According to the results of the study, the main compounds are presented in the following table:

Hold time, min.	Compound	Maceration		Percolation		Subcritical CO ₂ - extract, %	Supercritical CO ₂ -extract, %
		50%	70%	50%	70%		
12,8	N,N-Dimethylglycine	15,9	17,1	9,7	8,6	0,37	-
10,9	Pulegone	-	-	-	-	5,08	-
20,4	Phytol	9,12	6,39	5,6	12,2	2,49	-
25,7	Linoleic acid	7,56	8,42	13,2	7,1	6,95	-
26,7	Linolenic acid	26,9	21,3	20,1	23,6	9,38	-
17,6	Glycerin	4,32	4,36	5,7	3,0	-	-
11,2	cis-β-Farnesene	-	-	-	-	7,63	-
18,3	Coumaran	2,13	1,53	1,02	1,0	-	-
14,0	Berberone	-	-	-	-	1,93	-
14,7	Caryophyllene oxide	-	-	-	-	1,35	-
40,6	Stigmasterol	2,6	-	-	-	-	-
24,0	γ-Sitosterol	15,2	-	-	-	-	-
25,0	Oleic Acid	2,31	2,15	1,23	1,6	10,7	-
16,1	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-	-	-	-	-	6,97	-
16,2	α-Bisabolol oxide B	-	-	-	-	9,65	-
16,9	α-Bisabolol	-	-	-	-	1,36	-
17,3	Palmitic acid, ethyl ester	-	-	6,0	8,0	2,72	-
18,7	Bisabolol oxide A	-	-	-	-	8,26	-
23,1	Herniarin	-	-	-	-	5,61	-
25,9	1-Eicosanol	3,52	-	-	-	-	-
24,3	Squalene	-	-	-	-	-	1,28
22,9	Nonacosane	-	-	-	-	-	77,40
29,5	Octacosanol	-	-	-	-	-	5,07
19,5	Heneicosane	-	-	-	-	-	3,83

According to the results of the study, carbon dioxide extraction was chosen as the optimal method for extracting biologically active substances.

4. An optimal technology for obtaining a carbon dioxide extract has been developed: a technological scheme of production has been proposed, a description of the technological process has been given.

5. The quality indicators of the carbon dioxide extract based on the plant raw material *Lavatera thuringiaca* L. were determined according to the order of the Minister of Health of the Republic of Kazakhstan No. KR DSM-20 dated February 16, 2021: description, identification, dry residue, weight loss upon drying, heavy metals, microbiological purity, quantitative determination, packaging, labeling, transportation, storage, shelf life, main pharmacological action, retention time of bisabolol - 18.7; quantification - 8.26%.

The data obtained for a long-term test of a carbon dioxide extract based on plant raw materials *Lavatera thuringiaca* L. according to the order of the Minister of Health of the Republic of Kazakhstan No. KR DSM -165/2020 of October 28, 2020 showed that at a temperature of 25 ± 2 °C, relative humidity $60\pm 5\%$, there were no significant changes in the results of determining quality indicators. Studies to determine the stability of the extract are ongoing.

6. It has been established that the carbon dioxide extract based on the plant material *Lavatera thuringiaca* L. is safe and does not show allergic reactions. As a result of the study, no allergic reaction was observed on the skin area where the oily solution of the carbon dioxide extract of *Lavatera thuringiaca* L. was applied.

According to the classification of Hodge, Sterner and K.K. Sidorov extract belongs to the group of practically non-toxic class 5 compounds, $LD_{50} > 5000$ mg/kg.

Carbon dioxide extract based on plant raw materials *Lavatera thuringiaca* L. practically belongs to the group of non-toxic drugs, therefore, the possibility of being recommended for clinical trials with the aim of presenting it as a substance for pharmaceutical production has been proven.

Extracts based on plant raw materials *Lavatera thuringiaca* L. obtained by maceration, percolation and carbon dioxide extraction have a pronounced antimicrobial effect against clinically significant microorganisms: *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 8739, *Streptococcus pneumoniae* ATCC 660, *Klebsiella pneumoniae* 700603, *Staphylococcus aureus* ATCC 6538-P, *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus*.

Determination of the antioxidant activity of extracts obtained by maceration, percolation and carbon dioxide extraction from plant raw materials *Lavatera thuringiaca* L. was carried out by changing the intensity of chemiluminescence on a chemiluminometer KhL-003 on model systems (in vitro) in the reactions of formation of the most frequently occurring reactive oxygen species and reactions of free radical lipid peroxidation.

The anti-inflammatory effect of the carbon dioxide extract based on the plant material *Lavatera thuringiaca* L. was tested on a model with acute exudative

inflammation induced by carrageenan injection to assess the effect of the extracts on the cyclooxygenase system in male rats. Acute carrageenan tumors arose as a result of subplant injection of 0.1 ml of 1% carrageenan solution into the aponeurosis of the hind limbs of rats.

The results of the analysis of the anti-inflammatory activity of the carbon dioxide extract showed 7.5% at a dose of 25 mg/kg, 13% at a dose of 50 mg/kg, 16% at a dose of 100 mg/kg of the carbon dioxide extract of *Lavatera thuringiaca* L., while in the comparative preparation - 15%. The maximum activity at the received doses was 100 mg/kg, i.e. 16%.

Evaluation of the completeness of the tasks

While maintaining the internal unity of the dissertation work, studies were carried out in full on the tasks set to determine the area of distribution of plant raw materials *Lavatera thuringiaca* L., the development of an appropriate technology for harvesting raw materials, pharmacognostic features and the results of determining pharmaceutical and technological parameters and standardization; on the choice of the optimal technology for obtaining an extract from *Lavatera thuringiaca* L., the determination of its component composition and standardization, the assessment of safety and biological activity.

Recommendations and input for specific use of the results

Determination of the area of distribution of plant raw materials *Lavatera thuringiaca* L., development of an appropriate technology for the procurement of raw materials, pharmacognostic features and results of the determination of pharmaceutical and technological parameters and standardization; the choice of an effective technology for obtaining various types of extract from *Lavatera thuringiaca* L., the determination of its component composition and standardization, the determination of safety and antioxidant, antimicrobial and anti-inflammatory properties allow it to be recommended to domestic pharmaceutical industries as a pharmaceutical substance.

A draft of regulatory documents for plant raw materials *Lavatera thuringiaca* L. and a carbon dioxide extract based on it has been developed.

Evaluation of the scientific level of the work performed in comparison with the best achievements in this field

The results obtained for the completed dissertation work are confirmed by 1 patent for an invention, 4 articles in journals recommended by the CQASES MES RK (Committee for Quality Assurance in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan); 1 publication in an international journal included in the Scopus database "Determination of the chemical composition and antimicrobial activity of *Lavatera thuringiaca* L. medicinal herb material extracted under subcritical conditions by liquid carbon dioxide method". In addition, the main results of the research were reported at the International Scientific and Practical Conferences (Kazakhstan, Russia).

The scientific and methodological level of the dissertation work as a whole corresponds to the modern requirements for this category of work.