

## ANNOTATION

of the dissertation work on the topic: “**Pharmacognostic study of medicinal herbal plant materials of *Lepidium latifolium* L. and pharmaceutical development of phytopreparations based on it**” for the degree of Doctor of Philosophy (PhD) in the specialty 6D074800 - «Technology of pharmaceutical production»

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### **Relevance of the research topic**

One of the topical issues current pharmaceutical industries of the Republic of Kazakhstan is the development and introduction into the production of import-substituting medicines, including plant origin.

The national project "Quality and affordable healthcare for every citizen" Healthy Nation " Ministry Health Republic of Kazakhstan is aimed at increasing the share of domestic pharmaceutical products from 17% in 2020 to 50% in 2025y. The main objectives of the project are to increase the scientific and human potential for the pharmaceutical and medical industries, as well as the development of domestic production of medicines and medical devices. In addition, the State Health Development Program Republic of Kazakhstan for 2020-2025 is aimed at improving the health of population, providing quality and affordable healthcare, as well as creating and introducing competitive import-substituting medicines. In this regard, is relevant to create new pharmaceutical substances from domestic plant materials and medicines based on them.

The territory of the Republic of Kazakhstan has a rich stock of medicinal plants, the rational use and processing of which will increase the volume of herbal medicines. The flora of Kazakhstan has more than 6,000 plant species, however, the degree of their study is at a low level, as well as their pharmacological actions have not been fully investigated. [1]. A few plant species are used as medicines. In this regard, the search for plants as potential sources of biologically active compounds, the identification and study of their chemical composition, the development of optimal technology for pharmaceutical substances and medicines based on them, the study of pharmacological activity are the main tasks of pharmaceutical science in Kazakhstan.

*Lepidium latifolium* L. is have practical interest as a promising medicinal plant, which has a rich set of biologically active substances. On the territory of Kazakhstan, the plant is found everywhere. According to literature data, *Lepidium latifolium* L. is used as a vegetable product, garnish, drink, and also as an herbal medicinal plant with anti-inflammatory, antibacterial, diuretic and tonic effects [2]. The Western Himalayan ecotype of this plant is used as a herbal product for the treatment of the gastrointestinal tract. In folk medicine, a decoction and infusion of the roots are used to treat dermatological diseases, wounds, disorders of the nervous and digestive systems [3].

The plant contains saponins, flavonoids, alkaloids, glycosides and tannins [1], leaves contain steroids [4].

The subsequent comprehensive study of the pharmacological properties of *Lepidium latifolium* L. will expand the range of domestic herbal medicines for use in medical practice as an antimicrobial and anti-inflammatory agent.

**The purpose of scientific research:** carrying out pharmacognostic analysis of medicinal plant raw materials *Lepidium latifolium* L., obtaining and researching herbal medicines based on it.

**Objects of study:** above-ground part of the medicinal plant material *Lepidium latifolium* L., extracts obtained by percolation, ultrasonic and carbon dioxide extraction and gel based on carbon dioxide extract.

**Subject of study:** the area of growth, determination of pharmacognostic features of plant raw materials of *Lepidium latifolium* L.; optimal technology for obtaining the extract and its standardization; technology for obtaining gel based on carbon dioxide extract of *Lepidium latifolium* L. and its standardization; investigation of the safety and pharmacological properties of carbon dioxide extract and gel based on it.

**Objectives of study:**

- to collect and pharmacognostic analysis of medicinal plant materials *Lepidium latifolium* L.;
- to develop the optimal technology of extract from *Lepidium latifolium* L. and standardize it;
- to carry out pharmaceutical development of a gel based on a carbon dioxide extract from *Lepidium latifolium* L.;
- to determine the safety and pharmacological activity of the carbon dioxide extract and the gel based on it in experimental animals;
- conduct a feasibility study for the production of the gel.

**Scientific novelty of the research**

For the first time in Kazakhstan:

- carried out pharmacognostic analysis of the above-ground part *Lepidium latifolium* L.: macro- and microscopic, merchandising, phytochemical analyzes;
- for a comparative study of the chemical composition of the medicinal plant material *Lepidium latifolium* L., thick extracts were obtained by percolation, subcritical and ultrasonic extraction, and their chemical composition was studied by gas chromatography with mass spectrometric detection. The carbon dioxide extract was chosen as the optimal one, in which more than 40 chemical compounds were identified;
- carried out pharmaceutical development of a gel based on carbon dioxide extract *Lepidium latifolium* L.;
- proven antimicrobial activity of carbon dioxide extract *Lepidium latifolium* L. and a gel based on it against clinically significant test strains *Staphylococcus aureus*, *Klebsiella pneumonia*, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, also anti-inflammatory action.

The scientific novelty of the study is confirmed by a patent for a utility model under the registration number №5249 from 16.04.2021 y. «Method for obtaining carbon dioxide extract from the above-ground part *Lepidium latifolium* L.» (Appendix N);

### **The main provisions of the dissertation research submitted to the defense**

- 1) Results pharmacognostic study of medicinal plant materials *Lepidium latifolium* L.;
- 2) Experimental data on the technology of obtaining and studying extracts from medicinal plant materials *Lepidium latifolium* L.;
- 3) Results pharmaceutical development of a gel based on carbon dioxide extract *Lepidium latifolium* L. and studies of its safety and pharmacological activity.

### **Practical significance of the obtained results**

- The technology of collection and preparation of plant raw materials is presented *Lepidium latifolium* L. Identification confirmed by the State Institution of the Republic of Kazakhstan «Institute of Botany and Phytointroduction. Reference registration number № 01-08/10 (Appendix A);
- The technology for collecting and preparation plant raw materials *Lepidium latifolium* L. has been introduced at LLP Zerde-Fito (Appendix B);
- The method for obtaining a thick carbon dioxide extract from the above-ground part of the plant material *Lepidium latifolium* L. has been introduced in LLP “PoM Zhanafarm” (Appendix C);
- A draft technological instruction for the "Method of obtaining a thick carbon dioxide extract from the aboveground part of plant raw materials *Lepidium latifolium* L.” is presented in LLP “PoM Zhanafarm” (Appendix G);
- Proposed Organization Standard for "Method of obtaining a thick carbon dioxide extract from the aboveground part of plant raw materials *Lepidium latifolium* L.” в LLP “PoM Zhanafarm” (Appendix D);
- Obtaining an extract by percolation from *Lepidium latifolium* L. implemented at the Department of Pharmaceutical Technology NJSC "Kazakh National Medical University named after. S.D. Asfendiyarov" (Appendix E);
- A draft Regulatory Document for CO<sub>2</sub> extract of *Lepidium latifolium* L. obtained under subcritical conditions from grass has been created (Appendix G);
- Technology for obtaining a gel based on carbon dioxide extract from aboveground part *Lepidium latifolium* L. implemented in LLP «DOSFARM» (Appendix I);
- A draft technological instruction for the production of a gel containing carbon dioxide extract *Lepidium latifolium* L. has been developed and approved in LLP «DOSFARM» (Appendix K);
- A draft Regulatory Document has been developed for a gel obtained on the basis of a carbon dioxide extract from *Lepidium latifolium* L. (Appendix L);
- Development of the optimal composition and technology for obtaining a gel based on a carbon dioxide extract *Lepidium latifolium* L. implemented at the Department of Pharmaceutical Technology NJSC "Kazakh National Medical University named after. S.D. Asfendiyarov" (Appendix M);

### **Personal contribution of researcher**

The researcher independently conducted a review and analysis of domestic and foreign literature on the topic of the dissertation work, carried out experimental work on all the tasks set. This is confirmed by the results of studies obtained in laboratory and production conditions using modern equipment and literature.

The reliability and validity results of the study is confirmed by the focus of the work performed on solving the current problem, the implementation in a modern research center and the draft regulatory documents.

### **Approbation the results of the dissertation research**

The main results of the dissertation research were published and reported in the materials:

- VII scientific-practical conference with international participation "Priorities of pharmacy and dentistry: from theory to practice" (Almaty, 2018 y.);
- International conference «Modern achievements of pharmaceutical technology and biotechnology: collection of scientific works» (Kharkiv, 2018 y.);
- scientific-practical conference, dedicated to the 80th anniversary KSMA (Bishkek, 2019 y.);
- III international scientific-practical conference "Abu Ali ibn Sino and innovations in modern pharmaceuticals" (Tashkent, 2020 y.);
- III international scientific-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 y.);
- IX International conference "Pharmacy and Dentistry Publishers: From Theory to Practice", dedicated to the memory of Professor D.K. Kiyashev in the framework of the 90th anniversary of the Kazakh National Medical University. S.D. Asfendiyarov (Almaty, 2020 y.);
- XVI international scientific-practical conference «Global science and innovations 2022: Central Asia» (Nur-Sultan, 2022 y.).

### **Publications**

The results of the dissertation research were published in 14 scientific papers, including:

- an article in an international journal included in the database Scopus -1;
- articles in journals recommended by the Committee for Quality Assurance in Education and Science - 4;
- abstracts in the materials of international scientific and practical conferences - 8;
- utility model patent -1.

### **Scope and structure of the dissertation research**

The dissertation work includes 165 pages of machine text, 50 tables, 48 figures, 130 domestic and foreign literature, as well as applications. The work consists of an introduction, a literature review, materials and methods, 5 sections of the experimental part, conclusions by sections and final conclusion.

### **Conclusion**

1. The collection, preparation and storage of medicinal raw materials of the *Lepidium latifolium* L. were carried out in accordance with the GACP standard. Drying of the herb *Lepidium latifolium* L. was carried out in a shady room at an ambient temperature of  $(25\pm 2)^{\circ}\text{C}$  and relative humidity  $(60\pm 5)\%$  on the basis of the Institute of Botany and Phytointroduction of Plants of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan. 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, indicating the name of the raw material, the place of procurement, the time of collection and the net weight.

A pharmacognostic analysis of the herbal medicinal product has been carried out *Lepidium latifolium* L.:

- according to macroscopic features, the stem is branched. The leaves are leathery, oblong, entire gray-green color. Basal leaves narrowed into a long petiole, elliptical, saw-toothed, 1-2 cm wide and 3-5 cm long. The upper leaves sessile, small. The flowers are small white color;

- according to microscopic features, the stem is round in cross section, has thickened tangential walls. The central part of the stem is occupied by the core, the cells of which are multifaceted. The core is represented by storage tissues. On a transverse section, a leaf of a dorsoventral structure. Stomata are numerous on both sides of the leaf. Anisocytic stomata are found on both epidermis of the leaf;

- the following biologically active substances have been identified: alkaloids, tannins, anthraquinones, flavonoids, polysaccharides, steroids, saponins, phenolic acids. It has been established that steroids and polysaccharides predominate quantitatively.

Pharmaceutical and technological parameters of raw materials were determined *Lepidium latifolium* L. for the optimal extraction technology in order to extract the maximum extraction of biologically active substances: specific gravity ( $1,64 \pm 0,01 \text{ g/cm}^3$ ), bulk mass ( $0,35 \pm 0,01 \text{ g/cm}^3$ ), porousness ( $0,71 \pm 0,01 \text{ g/cm}^3$ ), porosity ( $0,24 \pm 0,00 \text{ g/cm}^3$ ), free volume of raw material layer ( $0,78 \pm 0,01 \text{ g/cm}^3$ ), extractant absorption coefficient of the extractant (3,45), extractive substances (54,71%).

Quality indicators have been determined and a quality specification for plant raw materials has been developed *Lepidium latifolium* L. (order of the Ministry of Health of the Republic of Kazakhstan №KR DSM-20 from 16 February 2021 year).

Results obtained during a long-term study of raw materials *Lepidium latifolium* L. allow you to set the temperature ( $25 \pm 2$ )°C, relative humidity indicator ( $60 \pm 5$ )%, and 2 year shelf life (order of the Ministry of Health of the Republic of Kazakhstan №KR DSM -165/2020 from 28 October based on a long-term study of the stability of medicinal plant materials *Lepidium latifolium* L.).

2. Thick extracts obtained by traditional and modern methods. Traditional - percolation method using *ethanol* (70%), modern - by the method of carbon dioxide extraction under subcritical conditions and ultrasonic extraction.

The chemical composition of the obtained extracts was analyzed by gas chromatography (Agilent MSD ChemStation) using a mass spectrometric detector.

A subcritical carbon dioxide extract was chosen as the optimal extract, in which more than 40 compounds were identified, among them the main components: phytosterols (campesterol -2,80%, stigmasterol -1,76%,  $\beta$  - sitosterol - 12,71%), diterpenes (phytol 7,30%), triterpene (squalene -1,54%), Vitamin E – 5,54% and etc., the parameters of its extraction were determined: working pressure 51 atm, temperature 210C, and extraction time 11 hours, the flow rate of the extractant

through the raw material was 5-10 cm<sup>3</sup>/h, the degree of grinding of the raw material was 3-5 mm, while the yield was 1,35%.

Technological scheme was created for obtaining carbon dioxide extract from above-ground part *Lepidium latifolium* L.

A quality specification has been developed for the carbon dioxide extract of *Lepidium latifolium* L.: description, identification, dry residue, weight loss on drying, heavy metals, microbiological purity, quantitative determination, packaging, labeling, transportation, storage, shelf life, main pharmacological action, retention time  $\beta$ -sitosterol - 18,7 min; quantitative determination - 12,71%.

Data were obtained on a long-term test of a carbon dioxide extract based on plant raw materials *Lepidium latifolium* L., significant changes were not observed based on the results of determining quality indicators (Order of the Ministry of Health of the Republic of Kazakhstan №KR DSM-165/2020 from 28 October 2020 y.).

3. Pharmaceutical development of a gel based on carbon dioxide extract *Lepidium latifolium* L. An optimal composition and technology for obtaining a gel has been developed, which includes: a pharmaceutical substance for the practical use of plant origin - a carbon dioxide extract (3 g), excipients: letsigel (1 g) - gelling agent, glycerin (10 g) - plasticizer, nipagin (0.04 g), nipazol (0.01 g) - preservatives, purified water. A technology for obtaining a gel based *Lepidium latifolium* L. on a carbon dioxide extract has been developed

A quality specification for a gel based on carbon dioxide extract *Lepidium latifolium* L has been developed, also developed a project RD (order of the Ministry of Health of the Republic of Kazakhstan №KP DSM-20 dated February 16, 2021).

The results stability test of gel based on carbon dioxide extract *Lepidium latifolium* L. under long-term conditions: at a temperature of (25±2)°C, relative humidity (60±5)% showed that there were no significant changes in the results of determining the quality indicators (order of the Ministry of Health of the Republic of Kazakhstan №KP DSM -165/2020 dated October 28, 2020). Research to determine gel stability is ongoing.

4. Tests were carried out on the effectiveness and safety of carbon dioxide extract *Lepidium latifolium* L. and gel based on it. They have been found to be safe, and in the study of the allergenic effect, no reaction was observed on the skin area where the oily solution of carbon dioxide extract was applied *Lepidium latifolium* L. and gel based on it.

According to classification Hodge, Sterner and K.K. Sidorova extract belongs to the group of practically non-toxic compounds of the class 5, LD<sub>50</sub>>5000 mg/kg.

The carbon dioxide extract from the raw materials of *Lepidium latifolium* L. and the gel based on it practically belongs to the group of non-toxic drugs, therefore, the possibility of being recommended for clinical trials to expand the range of pharmaceutical production.

Carbon dioxide extract of plant raw material *Lepidium latifolium* L. and a gel based on it have antimicrobial effect against clinically significant microorganisms: *Staphylococcus aureus*, *Klebsiella pneumonia*, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, and have anti-inflammatory activity.

5. A feasibility study was carried out for the production carbon dioxide extract of *Lepidium latifolium* L. and a gel based on it.