

ANNOTATION

of the doctoral dissertation for the degree of Philosophy Doctor (PhD) on the specialty 6D074800 - Technology of pharmaceutical production **Zhunuosova Maira Abylovna "Pharmaceutical development of medicines from plant raw materials of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L."**

Topicality of dissertation work

An important direction of the world pharmaceutical industry is the creation of similar in structure to nature, relatively safe, sufficiently useful for human health and affordable medicines.

For the realization of the goals and objectives in this area should be carried out full-scale research on the rational use of local natural resources. The Republic of Kazakhstan implements such state programs as the "Kazakhstan - 2050" Strategy", «The concept of Kazakhstan's entry into the list of 30 most developed countries in the world», «Strategic Development plan of the Republic of Kazakhstan until 2020», «Message of the President of the Republic of Kazakhstan "Kazakhstan way - 2050: unified goal, unified interests, unified future", one of the goals of which is to increase the number of names of medicinal preparations from domestic plant raw materials, development of domestic pharmaceutical production, construction of new production sites, and contribution in meeting the state's needs for medicinal preparation, by reorganizing the existing productions.

The need to ensure the population of the Republic of Kazakhstan with their own medicines is well-founded within the framework of «The State Program for Industrial and Innovative Development of the Republic of Kazakhstan for 2015-2019 year».

In this regard, one of the urgent problems of modern technology of pharmaceutical production is the development of new medicines based on raw materials of the Republic of Kazakhstan, including raw material resources of plant origin.

The National Science Report (2015) substantiates priority fundamental and applied research, including rational use of natural resources, processing of raw materials and products.

Based on the foregoing, the topicality of the of dissertation work is to develop medicines based on plant raw materials as a source of BAS, which cause less toxicity, bioavailability and a wide range of biological activity in comparison with synthetic medicinal preparations.

Flora of Kazakhstan is rich in promising little studied plants that are used in folk medicine, but for their introduction into medical practice, additional in-depth studies are required, using modern scientific methods.

Given the above, conducting research of polymorphic plants from the family *Dipsacaceae*, genus *Scabiosa* with the purpose of their incorporation in medical practice and the development of new medicines based on them is an urgent task and meets the requirements of modern pharmaceutical science.

Purpose of the study

Pharmaceutical development of plant raw materials and carbon dioxide extracts based on the plant raw materials of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.

Research objectives:

- 1) To study the raw material stocks of plants of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. in the territory of the Karaganda region;
- 2) Develop a technology of harvesting of plant raw materials of pale yellow scabious and iset scabious;
- 3) Pharmacognosy study of the herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- 4) Study of the indicators of the norm and quality, the shelf life of plant raw materials of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- 5) Study on the selection of parameters for the production of carbon dioxide extract from herbs of pale yellow scabious and iset scabious;
- 6) Study of the component composition, research for the presence of heavy metals and study thermal decomposition of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- 7) Develop a technology for the production of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- 8) Study of the indicators of the norm and quality, the shelf life of the carbon dioxide extracts obtained from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- 9) Study the biological activity *in vitro* of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. and extracts from wastes of carbon dioxide extraction from these species of raw materials.

Scientific novelty of research results

The novelty of the results of the study lies in the fact that for the first time:

- 1) the raw material stocks of plants of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. in the territory of Central Kazakhstan were investigated.
- 2) carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. was obtained.
- 3) the component composition of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. has been studied.
- 4) technology for the production of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. were developed.

Based on the obtained results, there are 2 the protection documents:

- Patent RK 33431 IPC A61K 36/35 (2006.01), A61K 133/00 (2006.01), A61K 31/04 (2006.01), B01D 11/00 (2006.01). "Method of obtaining CO₂ - extract from *Scabiosa isetensis* (L.) having cytotoxic activity». Application Date: 2017/0666.1, 11.08.2017 yr.; Publication Date 01.02.2019 yr.;
- Patent RK 33430 IPC A61K 36/35 (2006.01), A61K 133/00 (2006.01), A61K 31/04 (2006.01), B01D 11/00 (2006.01). "Method of obtaining CO₂ - extract from *Scabiosa ochroleuca* (L.) having antimicrobial activity». Application Date: 2017/0665.1, 11.08.2017 yr.; Publication Date 01.02.2019 yr.

The main provisions of the dissertation research for the defense:

- the raw material stocks of plants of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. in the territory of Central Kazakhstan;
- pharmacognostic analysis of the herbs *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- obtaining carbon dioxide extract from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- physicochemical parameters and spectral data of the carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- technology for the production of carbon dioxide extract from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- indicators of the norm and quality of the carbon dioxide extracts and plant raw materials of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.;
- results of the study of the antimicrobial, antiradical, cytotoxic activity of the carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and of *Scabiosa isetensis* L. and extracts from waste of carbon dioxide extraction from these species of raw materials.

The practical significance of the study:

As a result of the studies the raw material stocks of plants *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. in the territory of Central Kazakhstan was determined.

Based on the results obtained, the medicinal plant raw material of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. is recommended as medicines.

A rational technology for carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. was developed for the further development of dosage forms.

Projects of the analytical normative documents (AND) were developed: "Pale yellow scabious Herba", "Iset scabious Herba", "Carbo dioxide extractum of *Scabiosa ochroleuca* (L.) A. Love et D. Love) "Carbo dioxide extractum of *Scabiosa isetensis* (L.) A. Love et D. Love".

Projects of the experimental-industrial regulations (EIR) were developed: a) for the production of CO₂ - extract of iset scabious (*Scabiosa isetensis* (L.) A. Love et D. Love), obtained under subcritical conditions; b) for the production of CO₂ - extract of pale yellow scabious (*Scabiosa ochroleuca* (L.) A. Love et D. Love), obtained under subcritical conditions.

The results of research work on the pharmacognostic study of the aerial organs of *Scabiosa isetensis* L. and *Scabiosa ochroleuca* L. were introduced into the educational process of the Department of Botany in the discipline "Pharmacognosy" for students of the specialty 5B070100 - "Biotechnology".

The results of research work: on the selection of optimal conditions (temperature, pressure) of extraction with the achievement of the maximum yield of the extract introduced in LLP "Fito-aromat».

Information about the publications

Based on the materials of the dissertation **21** works were published, including:

- **3** articles in journals recommended by the Committee for Control in the Field of Education and Science of the Ministry of the Republic of Kazakhstan (CCFES MES RK);

- **1** articles in International journals indexed in Web of Science Core Collection (Clarivate Analytics) and Scopus databases (Impact Factor 0,46);

- **6** articles in International scientific conferences, including: 4 articles in materials of foreign conferences;

- **11** - in other scientific publications, including 2 articles in journals recommended by CCFES MES RK, 3 - articles in the edition entering into base eLibrary.

Approbation of work

The results and the main provisions of the scientific work are presented on:

1) VIII International scientific and practical conference «Исследование различных направлений науки», Moscow, January 29, 2016 yr.

2) Scientific and practical conference with international participation «Актуальные проблемы геронтологии и гериатрии» г. Самарканд, Узбекистан, 3-4 ноября, 2016 г.

3) Proceedings of Fifth International scientific and practical internet-conference «Medicinal Herbs: from past experience to new technologies», Poltava, May 30-31, 2016 yr.

4) PhD medical science research group 1st annual meeting «PhD day – 2016», Karaganda, December 9, 2016 yr.

5) International scientific and practical conference «Наука и образование в современном мире», Karaganda, February 19, 2017 yr.

6) V International scientific and practical conference «Современные аспекты использования растительного сырья и сырья природного происхождения в медицине», Moscow, March 15, 2017 yr.

7) Republican student scientific conference «Вклад молодежной науки в реализацию «Стратегии «Казахстан - 2050», Karaganda, April 13-14, 2017 yr.

8) VII All-Russian student scientific and practical conference «Актуальные проблемы науки в студенческих исследованиях», Almet'yevsk, May 12, 2017 yr.

9) XIII International scientific conference «Modern science in Eastern Europe», Morrisville, USA, December 22, 2017 yr.

10) International scientific and practical conference «Наука и образование в современном мире», Karaganda, February 16, 2018 yr.

11) Republican scientific and practical conference of students, undergraduates, doctoral students and young scientists (with international participation) «Молодежь и глобальные проблемы современности», Karaganda, March 30, 2018 yr.

12) XXX International scientific and practical conference: «Actual problems of modern science», Saint-Petersburg - Astana - Kiev - Vienna, May 30, 2018 yr.

13) Scientific results of the dissertation were approbated on scientific-expert commission "Pharmacy" and "Technology of pharmaceutical production" held (Protocol № 2 dated June 7, 2019 yr.).

The structure and scope of the dissertation

The dissertation is presented on 139 pages of computer text and consists of an introduction; literature review; 7 sections describing materials and methods of research; the main part containing the results and discussion of their own research; conclusions and practical recommendations; references, including 175 literary sources, of which 3 in the state language, 103 in the Russian and 69 in the foreign languages. The dissertation is illustrated by 47 tables and 63 figures and appendices.

Conclusions

1. Raw stocks of plants *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. in the territory of Central Kazakhstan were investigated. Thickets of pale yellow scabious are noted on the territory of the Korneevsky forests and iset scabious - in the Ulytau and Buyratau mountains. The operating reserve of the pale yellow scabious pale yellow at 31.25 centners, and the volume of possible harvest was 18.75 centners. The operational reserve of iset scabious was 87.83 centners, and the volume of possible collection of raw materials was calculated at the level of 52.70 centners.

2. Developed technology harvesting of plant raw materials *Scabiosa ochroleuca* L., *Scabiosa isetensis* L. According to the technological scheme of the billet, raw materials were collected by cutting 7-10 cm from the soil surface, the raw material was dried outdoors in the shade at an ambient temperature of 23-25 °C, the raw material was laid out in layers of 5-8 cm, on racks with gauze, raw materials were turned over at least 2 times a day.

3. Identification of plant raw materials of scabious pale yellow (*Scabiosa ochroleuca* L.) and iset scabious (*Scabiosa isetensis* L.) on external and microscopic characteristics, qualitative and quantitative content of α -santonin was carried out.

As a result of the pharmacognostical study of raw materials, the macroscopic features of the plant *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. are determined:

a) *Scabiosa ochroleuca* L.: plant 80-100 cm tall; the stem is erect, the stem is round in cross section, the stem is abundantly branched from the middle, the surface is bare,

b) *Scabiosa isetensis* L.: plant 35-40 cm tall; the stalk is upright, cross-sectional, rounded, cross-sectional, not branched, small - coarse, incomprehensible and curly and hairy, in the upper part with a denser pubescence with an admixture of rare and long hair, only in the lowest part and under the head - curly and fluffy, the color of the stem is green.

Microscopic features of the plant *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. were identified. The following diagnostic features are determined:

a) *Scabiosa ochroleuca* L.: strongly-tortuous cells of the epidermis, stomata of the diat type, a few bicoid trichomes are marked on the underside of the leaf.

b) the cells of the upper epidermis are small, of the prochenchymal type, the lower one is isodiametric with sinuous walls, the stomata of the diacite type, there

are no trichomes, and the elongated receptacles of the schizogenic type are marked on the transverse section of the leaf.

In the study of the mineral composition of the studied plants, quantitative data were obtained on 59 elements.

4. Parameters and quality standards of plant raw materials *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. were established.

The data obtained for determining the quality parameters of plant raw materials are included in the drafts of the analytical regulatory document for medicinal plant raw materials "Pale yellow scabious Herba", "Iset scabious Herba", which are approved in LLP "VivaFarm".

The shelf life of the plant raw material *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. was determined - 18 months (observation time).

5. The parameters for the preparation of carbon dioxide extracts from the herbs of scabious pale yellow and iset scabious have been established: working pressure 69-72 atmospheres, temperature 18-21 °C, extraction time 18 hours. The yield for *Scabiosa isetensis* L. was - 0.57%, *Scabiosa ochroleuca* L. - 0.46% in terms of air-dry raw materials.

6. The component composition of carbon dioxide extracts from the herbs of scabious pale yellow and iset scabious was determined. The main components of α -santonine, 1,8-cineol, α -thujone and β -thujone, n-hexadecanoic acid, campesterol, stigmasterol, hexadecanoic acid ethyl ester, phytol, linoleic acid ethyl ester, also terpenes and their derivatives: aromatic monoterpene o-Cymene, monocyclic monoterpene 4-terpinyl acetate, α -terpenyl acetate, monoterpene cyclic alcohol, cis-sabinene hydrate; terpinen-4-ol; in addition, antioxidants α -tocopherol and squalene, saturated, polyunsaturated and essential fatty acids; component of chlorophylls of green and red algae, diterpene alcohol, phytol have been identified.

The results of the determination of heavy metals showed that the content of heavy metals in the plant raw material under study does not exceed the quality index.

In the chain, of plant raw material - carbon dioxide extract from herb of iset scabious, cadmium transition amounted to $\leq 2.2\%$; lead - 0.03%.

In the chain of plant raw material - carbon dioxide extract - extracts from waste of carbon dioxide extraction of herb of scabious pale yellow, the transition of cadmium to carbon dioxide extract was - 3%; in alcohol extract from waste of carbon dioxide extraction - 12%; in the aqueous extract from waste of carbon dioxide extraction - 100%; the transition of lead to carbon dioxide extract amounted to 0.42%, to alcohol extract from waste of carbon dioxide extraction - 0.28%; 2.2% - in the aqueous extract from waste of carbon dioxide extraction.

The results of the study on the stability of carbon dioxide extracts from herbs of iset scabious and scabious pale yellow, to the effect of elevated temperatures established the temperature range from the onset of destruction to the complete burnout of the samples corresponding to 52–369 °C, which determines the possibility of storing carbon dioxide extracts under normal conditions.

7. A technology has been developed for the production of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. The

technological and hardware production schemes are proposed. Based on the results obtained, projects of the experimental-industrial regulations (EIR) for the production of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. in LLP "Fitoaromat" have been developed.

8. The parameters and quality standards of carbon dioxide extracts based on IR, UV spectroscopy, chromatography-mass spectrometry, TLC, HPLC, mineral analysis of ash, qualitative reactions were established. The physico-chemical and spectral indices of α -santonin, determined in these extracts, are described which will be used to identify and quantify α -santonin in drugs of carbon dioxide extracts from herbs *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. The projects of the analytical normative documents (AND) for a medicinal product has been developed: "Carbo dioxide extractum of *Scabiosa ochroleuca* (L.) A. Love et D. Love) "Carbo dioxide extractum of *Scabiosa isetensis* (L.) A. Love et D. Love ". The shelf life of carbon dioxide extracts of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L.) was determined - 18 months (observation time).

9. Based on the conducted experiments on the *in vitro* biological activity, the carbon dioxide extract from herb iset scabious at concentrations of 10 and 5 mg / ml exhibits cytotoxicity, the mortality of larvae is 78-88%, and the concentration of 1 mg / ml is non-toxic. The carbon dioxide extract from herb of *Scabiosa ochroleuca* L. has a high antimicrobial activity against strains of *Staphylococcus aureus* (0586), and moderate antimicrobial activity against strains of *Escherichia coli* (0524), *B. subtilis* (6633), *Candida albicans* (0475), *Candida albicans* (SIC 1).

Extracts from waste of the production of carbon dioxide extraction of herbs *Scabiosa ochroleuca* L., *Scabiosa isetensis* L. with respect to strains of *Staphylococcus aureus* (0586), *Bacillus subtilis* (6633), *Candida albicans* (0475), *Candida albicans* (SIC 1), *Escherichia coli* (0524) showed a weakly expressed activity equivalent to that of ethyl alcohol of 96% P.

The results of calculations of the kinetic criterion reflecting the amount of oxygen and active oxygen radicals reacted with the total content of antioxidants in the alcohol and aqueous extracts from waste of carbon dioxide extraction of herb *Scabiosa ochroleuca* L. also proves the presence of antioxidant activity.

All the results of the study of the biological activity of carbon dioxide extracts suggest that the activity of extracts is not associated with any individual substance; it is due to a synergistic effect, where the total composition of the extract determines biological activity.

Evaluation of the completeness of the solution of the tasks. Tasks on the study the raw material stocks of plants *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. in the territory of the Karaganda region and on the pharmacognostical study of plant raw materials of scabious pale yellow and iset scabious; on the development of technology for the preparation of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L., *Scabiosa isetensis* L.; on the study of physical and physico-chemical indicators, regulating the identification and quality of medicinal plant materials, of carbon dioxide extracts, of the wastes from the production of carbon dioxide extract, on the study the biological activity of carbon

dioxide extracts from herbs *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. and extracts from the wastes from the production of carbon dioxide extract; on the development of technology for the production of carbon dioxide extracts from herbs *Scabiosa ochroleuca* L., *Scabiosa isetensis* L.; on the study of indicators and quality standards of carbon dioxide extracts and raw materials of *Scabiosa ochroleuca* L., *Scabiosa isetensis* L.; on the development of a project of the normative documents on the species of raw materials and medicines of scabious pale yellow and iset scabious fulfilled.

Recommendations and background data on the specific use of the results.

A technology has been developed for the production of carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. The resulting carbon dioxide extracts may be used as the substance, ready medicine; waste from of carbon dioxide extraction of herbs *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L. can be offered as fillers, additives in the production of functional foods and medicines.

The temperature interval from the beginning of the destruction to the complete burnout of the samples corresponds to 52-369 °C determines the possibility of storing carbon dioxide extracts from herbs of scabious pale yellow and iset scabious in normal conditions. These results can be recommended in determining the quality of storage of the studied carbon dioxide extracts during storage and the possibility of using high temperatures in the manufacture of dosage forms. The results of this dissertation can be used in pharmacy and pharmaceutical technology.

Evaluation of technical and economic efficiency of implementation. The results obtained have a high technical and economic efficiency, since a technology has been developed for producing carbon dioxide extracts from herbs of *Scabiosa ochroleuca* L. and *Scabiosa isetensis* L., which is characterized by the safety and quality of the products obtained.

The introduction of medicines from herb of scabious pale yellow into the production of medicines, which has an antimicrobial effect and of medicines from herb of iset scabious, which has a cytotoxic effect, will expand the range of drugs based on domestic raw materials of plant origin.

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

According to the materials of the dissertation, there are 2 patents of the Republic of Kazakhstan on the invention, 3 articles in journals, recommended by the Committee on the Control of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan published, 1 article in a foreign scientific publication included in the databases of Web of Science Core Collection (Clarivate Analytics) and Scopus, 6 articles in materials of international conferences, including 3 in materials of foreign conferences, 1 in materials abroad.

In general, the scientific and methodological level of the thesis presented corresponds to the modern analogues published in the open scientific press.