

## ANNOTATION

of dissertation (PhD) work by Shaimerdenova Zhanar Rakhimovna on the topic “New medicinal substances based on terpenoids of *Artemisia glabella* Kar. Et Kir. and their production technology” in candidacy for a degree of Doctor of Philosophy (PhD) in specialty 6D074800 - Technology of pharmaceutical production

**The urgency of the problem.** The increase in demand for drugs of plant origin requires the expansion of production capacities. At the same time, the development of effective, targeted technologies for the integrated use of medicinal plant raw materials, achieving relatively high yields, target components, expanding the range of recoverable biologically active substances and resource conservation becoming of particular importance.

The endemic plant of Kazakhstan *Artemisia glabella* Kar. et Kir. is a source of biologically active terpenoids with practically valuable properties - of essential oils, sesquiterpene lactones of arglabin, argolide, dihydroargolide.

In JSC “International Research and Production Holding “Phytochemistry” on the basis of sesquiterpene lactone arglabin, isolated from *Artemisia glabella*, the domestic antitumor drug “Arglabin” is being produced. On the basis of essential oil obtained from raw material of *Artemisia glabella*, a new phytopreparation “Eferol” was developed with anti-inflammatory, antibacterial and antifungal action. At JSC “IRPH “Phytochemistry”, two technologies were introduced into production based on raw material of *Artemisia glabella*: for the production of natural arglabin substance and for the production of essential oil.

The technology for the production of a substance based on natural arglabin is based on supercritical extraction of plant raw materials with carbon dioxide, followed by the isolation of the target substance using centrifugal partitioning chromatography. During the implementation of the technological process, it was established that the carbon dioxide extract of *Artemisia glabella*, in addition to the main component of arglabin, contains related compounds, including fractions enriched with essential oil and sesquiterpene lactones argolide, dihydroargolide.

Therefore, the development of resource-saving technologies, due to the complex processing of the carbon dioxide extract of *Artemisia glabella*, providing the quantitative extraction of biologically active substances, for the creation of new drugs is an urgent and priority task.

**The goal of the dissertation research:** Development of a complex technology for obtaining new drugs based on biologically active terpenoids.

**Research tasks.** To achieve the set goal, it is necessary to solve the following tasks:

- to develop a new method and technology for the extraction of essential oil which is the substance of phytopreparation “Eferol” based on the complex processing of carbon dioxide extract of *Artemisia glabella*.

- to study the physicochemical, technological characteristics and biological activity of the essential oil obtained on the basis of the complex processing of the carbon dioxide extract of *Artemisia glabella*.

- to develop a new method and technology for the production of sesquiterpene lactone argolide based on the complex processing of the carbon dioxide extract of *Artemisia glabella*, to synthesize new modified derivatives on its basis, to establish the structure and to study the biological properties.

- to develop technology for obtaining the epoxyargolide substance.

- to develop regulatory documentation for the substances of argolide, epoxyargolide, in the form of AND drafts, laboratory regulations for obtaining and pilot-industrial regulations for production.

**Research objects:** medicinal raw materials of *Artemisia glabella* Kar. et Kir.; the sum of extractive substances; carbon dioxide extract of *Artemisia glabella*; fractions obtained after separation of carbon dioxide extract of *Artemisia glabella* using centrifugal partitioning chromatography; substances: essential oil of *Artemisia glabella*, argolide, epoxyargolide; standard samples: argolide and 1,8-cineole.

**Research subject:** a new method and technology for the extraction of essential oil based on the complex processing of carbon dioxide extract of *Artemisia glabella*, quality indicators and biological activity of the obtained essential oil; a new method and technology for preparation of argolide based on the complex processing of carbon dioxide extract of *Artemisia glabella*, synthesis, structure and biological activity of epoxyargolide; technology for production of epoxyargolide substance, regulatory documentation for the essential oil substances of *Artemisia glabella*, argolide and epoxyargolide.

**Research methods:** modern physicochemical techniques for the isolation, purification and analysis of substances were used to carry out the dissertation work: hydrodistillation on a Clevenger apparatus, fast centrifugal partitioning chromatography, high performance liquid chromatography, gas chromatography with flame ionization and mass selective detectors, infrared and ultraviolet spectrophotometry, nuclear magnetic resonance, mass spectrometry, X-ray structural analysis, analysis of elemental composition, optical rotation detection, refractive index and melting point. And also, primary screening of samples of natural compounds was carried out in relation to antimicrobial, anti-inflammatory activity and cytotoxicity.

#### **Statistical processing of results**

Statistical processing of the results was carried out using the “GraphPad Prism v. 6.0” program. To check the normality of the distribution of the results of observations, methods of descriptive statistics were used, graphically and using statistical criteria (Kolmogorova-Smirnova). The mean value (mean), standard error of the mean (SE), standard deviation (SD), median were also calculated.

One-way analysis of variance (ANOVA) was used to identify statistically significant differences in variables between samples in determining cytotoxic and antimicrobial activities.

To analyze intergroup differences in assessing anti-inflammatory activity, the nonparametric Mann-Whitney U-test was used.

Differences were considered significant at the achieved significance level  $p < 0.05$ .

**The main provisions for the defense:**

- a new method and technology for producing essential oil based on the complex processing of carbon dioxide extract of *Artemisia glabella*, quality indicators of the essential oil substance of *Artemisia glabella* and its biological activity;

- a new method and technology for isolation of sesquiterpene lactone argolide based on the complex processing of carbon dioxide extract of *Artemisia glabella*, quality indicators of the argolide substance;

- synthesis of 7 new derivatives of argolide, their physicochemical parameters, spectral data and biological activity;

- production technology of epoxyargolide substance;

- regulatory documents for the essential oil substances of *Artemisia glabella*, argolide, epoxyargolide, in the form of AND projects, laboratory regulation for preparation and pilot-industrial regulations for production;

- implementation of the developed technologies and organization of production of pilot batches of essential oil substances of *Artemisia glabella*, argolide and epoxyargolide.

**Practical significance of the work:**

- the developed technology of essential oil extraction based on the complex processing of carbon dioxide extract of *Artemisia glabella* allows the rational use of medicinal plant raw materials of *Artemisia glabella*;

- the quality of the essential oil substance of *Artemisia glabella* was assessed, its compliance with the normative document was confirmed; pilot-industrial regulations for the production of the essential oil substance of *Artemisia glabella* based on the complex processing of carbon dioxide extract were developed and approved (OPR-FD 65005037R-07-18);

- the advantage of the developed technology for the production of argolide substance based on the complex processing of carbon dioxide extract of *Artemisia glabella* is an increase in the productivity of the technological process by 4.6 times and a significant reduction in its duration, elimination of toxic solvents, compliance with GMP requirements;

- epoxyargolide was proposed as a substance for the development of new drugs with antitumor and anti-inflammatory actions, recommended for further preclinical studies;

- AND project for essential oil substance of *Artemisia glabella* was developed;

- laboratory regulations for the production of epoxyargolide substance, pilot-industrial regulations for the production of argolide substances and for the essential oil of *Artemisia glabella* were developed and approved;

•developed technologies were implemented on the basis of LLP “Karaganda Pharmaceutical Plant” and the production of pilot batches of *Artemisia glabella* essential oil, argolide and epoxyargolide substances was organized.

**Main research results:**

1) A new method and resource-saving technology were developed for production of essential oil - the substance of phytopreparation “Eferol”, based on the complex processing of carbon dioxide extract of *Artemisia glabella*, the use of which allows the rational use of medicinal raw materials of *Artemisia glabella*.

2) It was established, that in terms of its component composition, physicochemical, technological characteristics and biological action, the essential oil obtained on the basis of complex processing of carbon dioxide extract corresponds to the essential oil obtained from the medicinal raw materials of *Artemisia glabella*; a pilot industrial regulation for the production of *Artemisia glabella* essential oil substance based on the complex processing of carbon dioxide extract was developed and approved; a draft analytical regulatory document for the essential oil substance of *Artemisia glabella* was developed.

3) For the first time, a method and resource-saving technology for the isolation of sesquiterpene lactone argolide based on the complex processing of carbon dioxide extract of *Artemisia glabella* was developed. The advantage of the developed technology is an increase in the productivity of the technological process by 4.6 times and a significant reduction in its duration, elimination of toxic solvents, compliance with GMP requirements.

4) Based on the isolated sesquiterpene lactone argolide, 7 new previously undescribed compounds were synthesized, the structure of which was unambiguously established on the basis of IR-, UV-, NMR-spectroscopy, and elemental analysis data. For the first time, as a result of a study of biological activity, it was established that epoxyargolide exhibits cytotoxicity against cells of hepatocellular carcinoma HepG2 and has an anti-inflammatory effect comparable to the reference drug “Diclofenac sodium” in the model of acute exudative reaction; epoxyargolide was proposed as a substance for the development of a new drug with antitumor, anti-inflammatory action and is recommended for further preclinical studies.

5) For the first time, a technology for producing epoxyargolide was developed.

6) For the first time, quality indicators were developed and standardization of argolide and epoxyargolide substances was carried out, their stability was studied; analytical normative documents for argolide and epoxyargolide substances were developed.

7) For the first time laboratory regulations for the production of epoxyargolide substance (LR-FD65005037R-01-18) and pilot-industrial regulations for the production of argolide substance (OPR-FD65005037R-06-18), essential oil of *Artemisia glabella* (OPR-FD65005037R-07-18) were developed and approved. Technologies for obtaining essential oil substances of *Artemisia glabella*, argolide, epoxyargolide was officially accepted and implemented on the

basis of LLP “Karaganda Pharmaceutical Plant”, and the production of their pilot batches was organized.

**Scientific novelty of work:**

- a new method and resource-saving technology for preparation of essential oil - the substance of phytopreparation “Eferol”, based on the complex processing of the carbon dioxide extract of *Artemisia glabella* are developed;

- in terms of its component composition, physicochemical, technological characteristics and biological action, the essential oil obtained during complex processing of carbon dioxide extract corresponds to the essential oil produced from plant raw material of *Artemisia glabella*;

- a new method and resource-saving technology for the production of sesquiterpene lactone argolide based on the complex processing of carbon dioxide extract of *Artemisia glabella*, providing a quantitative yield of the target substance of appropriate quality are developed;

- on the basis of the isolated argolide, 7 new compounds previously undescribed in the literature were synthesized, the structure of which was unambiguously established on the basis of IR-, UV-, NMR-spectroscopy, and elemental analysis data;

- for the first time, as a result of a study of biological activity, it was established that epoxyargolide exhibits cytotoxicity against cells of hepatocellular carcinoma HepG2 and has an anti-inflammatory effect comparable to the reference drug “Diclofenac sodium” in the model of acute exudative reaction;

- for the first time, a technology for producing epoxyargolide substance was developed;

- quality specifications were developed and the standardization of the essential oil substances of *Artemisia glabella*, argolide, epoxyargolide was carried out, their stability was studied.

**Relation of work to the plan of state scientific programs.** The dissertation work was carried out at JSC “IRPH “Phytochemistry” within the framework of the scientific and technical program O.0676 “Development of new pharmacological compounds - substances of original drugs and their standard samples” for 2015-2017; STP O.0820 “Development of new phytopreparations and their pharmacological and clinical studies” for 2018-2020; grant project No. AP05130575 “Development of effective methods for the isolation and identification of new biologically active compounds from essential oils of plants” for 2018-2020.

**The personal contribution of the author** consists in research carried out personally by the applicant and included in the thesis: in the development of methods and technologies for obtaining essential oil and sesquiterpene lactone argolide based on the complex processing of carbon dioxide extract of *Artemisia glabella*; in the study of quality indicators and biological properties of *Artemisia glabella* essential oil; in the synthesis of 7 new derivatives based on argolide, the establishment of their structure and the study of biological properties; in the technology of obtaining the substance of epoxyargolide; in the development of

regulatory documents for essential oil substances, argolide and epoxyargolide in the form of AND projects, laboratory and pilot-industrial regulations.

**Approbation of work.** The main provisions of the dissertation were reported at: IX All-Russian Scientific Conference “Chemistry and technology of plant substances” (Moscow, September 28-30, 2015); VII All-Russian conference with international participation “New achievements in the chemistry and chemical technology of plant raw materials” (Barnaul, April 24-28, 2017); All-Russian scientific conference with international participation “Modern problems of organic chemistry” (Novosibirsk, June 5-9, 2017); Seventh scientific conference with international participation “Modern trends in the development of health-saving technologies” (Moscow, 2019); XIII International Symposium on the Chemistry of Natural Compounds (Shanghai, October 16-19, 2019).

**Publications.** Based on the materials of the dissertation, an application for a patent of the Republic of Kazakhstan No. 2020/0165.1 dated March 11, 2020 was filed. The main provisions of the dissertation are reflected in the following publications:

- 3 articles in journals recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan;

- 3 articles in foreign scientific journals included in the Web of Science and Scopus databases;

- abstracts of 6 reports, including abstracts of 2 reports in materials of international conferences.

**The structure of the dissertation:** The dissertation is presented on 117 pages of typewritten text, includes 12 figures and 20 tables; consists of an introduction, 8 chapters, a conclusion, a list of used sources and applications. The list of references includes 122 literary sources.