

Annotation

of PhD thesis work of Kishkentayeva Anarkul Serikovna on a theme: “Development of technologies of pharmacological active substances based on grosheimin and its standardization” for Doctor of Philosophy (PhD) Degree on speciality: 6D074800 - Technology of pharmaceutical production

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Urgency of the problem. One of the priorities of development of pharmaceutical science and industry of the Republic of Kazakhstan is a search of approaches for full use of its own resources of the wild-growing and cultivated vegetable raw materials and development on its basis of the original phytodrugs, which are available at prices and at the same time are not lower in quality of their competitive analogs.

The perspective object for works in this direction is *Chartolepis intermedia* Boiss. which is included in the State pharmacopeia of the Republic of Kazakhstan as medicinal vegetable raw materials to obtain the biologically active sesquiterpene lactone grosheimin having high antitumoral, anti-inflammatory and bactericidal activities.

Chartolepis intermedia Boiss. is a renewable vegetable raw material, which has the steady operational stock permitting to obtain biologically active sesquiterpene lactone grosheimin commercially.

The perspective direction is also the chemical modification of grosheimin molecule which permits to obtain derivatives with higher biological activity or the advanced physical and chemical properties, for example, solubility in water. In addition these researches help to understand an action mechanism of this or that biologically active substance within “structure-activity” correlation.

Thus, the advancement of extraction technology of biologically active grosheimin from vegetable raw materials of *Chartolepis intermedia* Boiss., the obtaining on its basis of new compounds with the expressed biological activity, with the subsequent development on their basis of substances for production of original drugs, is an important and priority objective.

Purpose of research. Development of the energy and resource saving, environmentally friendly technology for obtaining of grosheimin from medicinal plant raw materials of *Chartolepis intermedia* Boiss., development of technologies of the receiving of new pharmacologically active substances based on grosheimin and their standardization.

Objectives of research:

- to research raw stocks in the territory of the Central Kazakhstan and make the evaluation of quality of medicinal vegetable raw materials *Chartolepis intermedia* Boiss.;
- to perform the ultrasonic extraction of *Chartolepis intermedia* Boiss. and determine the optimal conditions of quantitative extraction of grosheimin from vegetable raw materials;
- to develop the energy and resource saving, environmentally friendly technology process of grosheimin receiving from *Chartolepis intermedia* Boiss.;
- to synthesize the new modified grosheimin derivatives, establish the structure and research their biological properties;
- to develop technologies of the obtaining of substances of chloroacetylgrosheimin and cytisililgrosheimin hydrochloride;
- to develop the standard documentation on substances of chloroacetylgrosheimin and cytisililgrosheimin hydrochloride as the projects of the analytic-normative documentation and pilot regulations on production.

Research objects: medicinal raw materials: *Chartolepis intermedia* Boiss., amount of extractive substances: water-alcohol extract of *Chartolepis intermedia* Boiss. received using of ultrasonics, substances: grosheimin, chloroacetyl grosheimin, cytisinil grosheimin hydrochloride; a standard sample: grosheimin.

Research subject: the optimal conditions of ultrasonic extraction of *Chartolepis intermedia* Boiss. for quantitative extraction of grosheimin, technology of the grosheimin receiving from alcoholic extract, indicators of grosheimin substance quality, synthesis, structure and the biological activity of chloroacetyl grosheimin and cytisinil grosheimin hydrochloride, technologies of the obtaining of chloroacetyl grosheimin and cytisinil grosheimin hydrochloride substances, standard documentation on chloroacetyl grosheimin and cytisinil grosheimin hydrochloride substances.

Research methods: the complex of modern physical and chemical methods of extraction and analysis was used in the furtherance of this goal and solution of objectives: ultrasonic extraction, analytical high performance liquid chromatography (HPLC), gas chromatography (GC), infrared (IR) and ultraviolet (UV) spectrophotometry, nucleic magnetic resonance (NMR) spectroscopy and element analysis, melting point.

Research novelty of work:

- the raw stocks in the territory of the Central Kazakhstan were researched and the evaluation of indicators of quality of medicinal vegetable raw materials of *Chartolepis intermedia* Boiss. on compliance to the normative document was made;
- the ultrasonic extraction of *Chartolepis intermedia* Boiss. was firstly performed and the optimal conditions of quantitative extraction of grosheimin from vegetable raw materials were determined;
- the lean process of the grosheimin substance receiving was developed from *Chartolepis intermedia* Boiss. by virtue of an exception of the use of expensive solvents at stages of extraction and isolation;
- chloroacetyl grosheimin and cytisinil grosheimin hydrochloride were firstly synthesized on the basis of grosheimin and their structures were established with IR-, UV-, mass-, ^1H , ^{13}C NMR- spectra, two-dimensional ^1H - ^1H , ^{13}C - ^1H (COSY, COLOC) NMR spectroscopy and data of the element analysis;
- the research results of the biological activity of new grosheimin derivatives established that chloroacetyl grosheimin has a high cytotoxicity for acute monocytic leukemia, at moderate toxicity, whereas cytisinil grosheimin hydrochloride *in vivo* shows the expressed anthelmintic action against helminths from *Nematodae* family;
- technologies of the obtaining of substances of chloroacetyl grosheimin and cytisinil grosheimin hydrochloride were developed;
- the quality specifications were developed and standardization of substances of chloroacetyl grosheimin and cytisinil grosheimin hydrochloride was performed and their stability was studied.

Practical significance of work:

- it was determined that an annual volume of preparation of medicinal vegetable raw materials of *Chartolepis intermedia* Boiss. growing in the territory of the Central Kazakhstan averages out from 11.9 to 21.8 centner/hectare, on external signs, microscopic characteristics, the quantitative grosheimin content and results of the trade analysis of vegetable raw materials correspond to the normative document;
- the use of developed technology of the receiving of grosheimin substance by an exception of application of the expensive solvents at stages of extraction and isolation, permitted to reduce duration and increase productivity of the technological process, and meanwhile, to lower significantly the prime cost of target product - by 9 times; the quality evaluation of grosheimin substance was performed, its compliance to the normative document was confirmed; pilot regulations were developed and approved on the production of substances of grosheimin (Pilot regulation FD65005037P-11-15)

- chloroacetyl grosheimin was recommended as substance to develop a new antitumoral drug and to make the expanded preclinical trials;
- cytisil grosheimin hydrochloride recommended as a substance to develop a new anthelmintic drug;
- the projects of analytic-normative documentation for substances of chloroacetyl grosheimin and cytisil grosheimin hydrochloride was developed;
- pilot regulations were developed and approved on the production of substances of chloroacetyl grosheimin (Pilot regulation FD65005037P-12-17) and cytisil grosheimin hydrochloride (pilot regulation FD65005037P-13-17);
- the pilot substance production of lots of chloroacetyl grosheimin and cytisil grosheimin hydrochloride was organized on the basis of LLP "Karaganda pharmaceutical factory".

Validity and authenticity. The experimental works were performed using the modern and calibrated equipment permitting to receive the authentic and safe results.

The basic terms submitted for thesis defense:

- the annual volume of production in the territory of the Central Kazakhstan and quality indicators of medicinal vegetable raw materials of *Chartolepis intermedia* Boiss.;
- the optimal conditions of the ultrasonic extraction of raw materials *Chartolepis intermedia* Boiss. providing the quantitative grosheimin extraction;
- technology of the receiving of grosheimin from alcoholic extract, indicators of quality of grosheimin substance;
- synthesis, the physical and chemical indicators, spectral data and biological activity of chloroacetyl grosheimin and cytisil grosheimin hydrochloride;
- technology of the receiving of substances of chloroacetyl grosheimin and cytisil grosheimin hydrochloride;
- the normative documents for substances of chloroacetyl grosheimin and cytisil grosheimin hydrochloride as projects of the analytic-normative documentation and pilot regulations;
- organization of output of pilot lots of substances of chloroacetyl grosheimin and cytisil grosheimin hydrochloride.

Work approbation. Materials of thesis work were reported on: X International symposium on the chemistry of natural compounds» (Tashkent, on November 21-23, 2013); The VI All-Russian Conference with the international participation "New advances in chemistry and chemical technology of vegetable raw materials" (Barnaul, on April 22-24, 2014); 22nd Conference on Isoprenoids (Prague, on September 7-10, 2014); 6-th Russian-Korean Conference «Current Issues of Biologically Active Compound Chemistry and Biotechnology (Novosibirsk, on July 5-10, 2015); The International Scientific and Practice Conference (Achievements and prospects for the Development of Phytochemistry) (Karaganda, on April 10-11, 2015); 11th International Symposium on the Chemistry of Natural Compounds (Antalya, on October 1-4, 2015); 24th Conference on Isoprenoids (Bialystok, on September 9-12, 2018).

Published papers. 1 patent RK was received under materials of this thesis. 1 positive decision on issue of the RK patent was received. The key terms of this thesis are demonstrated in the following published papers:

- 4 papers in the journals recommended by Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan;
- 7 papers in the foreign scientific editions such as Web of Science and Scopus;
- 7 abstracts of reports among them 3 abstracts at the international conferences.

CONCLUSIONS:

1. Raw stocks were identified and, in addition, it was determined that the annual volume of preparation of medicinal vegetable raw materials of *Chartolepis intermedia* Boiss. In communities growing in the territory of the Central Kazakhstan averages out from 11.9 to 21.8 c/ha. According to external signs, microscopic characteristics, the quantitative content of pharmacologically active compound of grosheimin and results of the trade analysis of vegetable

raw materials of *Chartolepis intermedia* Boiss. correspond to the normative document.

2. The ultrasonic extraction of *Chartolepis intermedia* Boiss. was firstly performed and the optimal conditions providing quantitative grosheimin extraction from vegetable raw materials were defined.

3. For the first time, the energy and resource saving, environmentally friendly technology of the obtaining of grosheimin substance from alcoholic extract of *Chartolepis intermedia* Boiss. was developed. Its application, by virtue of an exception of the use of expensive solvents at stages of extraction and isolation, permitted to reduce duration and increase productivity of the technological process, as a result to lower the prime cost of the target product by 9 times; quality evaluation of grosheimin substance was made, its compliance to the normative document was confirmed; The pilot regulations were developed and approved on the production of grosheimin (Pilot regulation FD65005037P-11-15).

4. Chloroacetylgrosheimin and cytisnilgrosheimin hydrochloride were firstly synthesized on the basis of grosheimin and their structures were established with IR-, UV-, mass-, ^1H , ^{13}C NMR- spectra, two-dimensional ^1H - ^{13}C , ^{13}C - ^1H (COSY, COLOC) NMR spectroscopy and data of the element analysis.

The research results of the study of biological activity of obtained compounds samples established that chloroacetylgrosheimin had a high cytotoxicity for acute monocytic leukemia, at moderate toxicity, and cytisnilgrosheimin hydrochloride *in vivo* showed the expressed anthelmintic action for helminth from *Nematodae* family. Chloroacetylgrosheimin was recommended as substance to develop a new antitumor agent and recommended for the expanded preclinical trials, and cytisnilgrosheimin hydrochloride recommended as substance to develop a new anthelmintic drug;

5. The technologies were developed to obtain the pharmacological active substances based on grosheimin permitting to make necessary quantity of chloroacetylgrosheimin and cytisnilgrosheimin hydrochloride of the corresponding quality.

6. Specifications of quality were developed and standardization of substances of chloroacetylgrosheimin and cytisnilgrosheimin hydrochloride was made, their stability was studied; projects of the analytic-normative documentation were developed for chloroacetylgrosheimin and cytisnilgrosheimin hydrochloride substances.

7. The pilot regulations were developed and approved on the production of chloroacetylgrosheimin (Pilot regulation FD65005037P-12-17) and cytisnilgrosheimin hydrochloride (pilot regulation FD65005037P-13-17) substances; and for pre-clinical trials the output of pilot lots of chloroacetylgrosheimin and cytisnilgrosheimin hydrochloride was organized on the basis of LLP "Karaganda pharmaceutical factory".