

ANNOTATION

of thesis on "Pharmacognostic analysis and technological aspects of introduction of *Crocus alatavicus*" for the degree of Doctor of Philosophy (PhD) on specialty 6D074800 - Technology of pharmaceutical production
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Relevance of the research topic. At the current stage of development of the pharmaceutical industry one of its urgent tasks is the creation and implementation of import-substituting drugs, including drugs of plant origin. Production of competitive medicines will contribute to the successful implementation of the "Comprehensive Plan for the development of the pharmaceutical and medical industry in 2020-2025" and allow to ensure national drug safety of Kazakhstan.

Improving the health of the population is a priority in the implementation of the national project "Quality and Affordable Healthcare for Every Citizen: A Healthy Nation". Despite the huge potential and relatively high growth rate of the domestic pharmaceutical industry, there is still a dependence on imports: the share of domestically produced medicines is no more than 20%, while the President's instruction is to bring the share of domestic medicinal products to 50% by 2025.

Organising the production of medicines using medicinal plants native to the Republic of Kazakhstan will help to improve the provision of medicines to the population and address current health problems in the prevention and treatment of various diseases.

To ensure a sustainable raw material base for phytochemical production and stable quality of medicinal plant raw materials, it is necessary to introduce into culture medicinal plants in compliance with the Good Agricultural and Collection Practice for starting materials of herbal origin (GACP).

In this respect, the plant *Crocus alatavicus* of the genus *Crocus* L, which grows in Kazakhstan, is a valuable source of BAS and is widely used in ethnopharmacy and folk medicine as an antibacterial, anti-inflammatory, cardiovascular, sedative and potency enhancer, is of particular interest. It is necessary to notice that the plant of a genus *Crocus* L, anciently was applied as a spice, and during almost four millennia was used for treatment on more than 90 medical indications. Nutritional and medicinal properties of the plant were mentioned by Homer ("Iliad", books IX and XII), Virgil (Georgian, IV, 182), Hippocrates, Pliny ("Natural History", XXI, VI), Ovidius ("Metamorphoses") and in the Old Testament "Song of Solomon". In ancient Egypt, the first document attesting to the use of *Crocus satvus* L. was the 15th century BC Ebers Papyrus.

Expanding the range of sources of herbal raw materials, developing phytosubstances and modern preparations from herbal raw materials is a promising area of pharmaceutical research.

Organising full-cycle production within the requirements of GACP and GMP good practices will ensure the quality and safety of herbal medicines, increase their competitiveness and enable them to enter foreign markets, including within the EAEU.

Thus, the pharmacognostic study and development of the technology for the introduction of *Crocus alatavicus* in accordance with GACP requirements to create herbal medicinal products in the future is of scientific and practical importance.

Purpose of the research: comprehensive pharmacognostic study and development of technology for the introduction of *Crocus alatavicus* for the production of a full cycle of herbal substances of pharmacopoeial quality.

Objectives of the research: The following objectives were to be achieved:

- to carry out pharmacognostic analysis and standardisation of the plant material *Crocus alatavicus*;
- to study the biological activity and safety profile of *Crocus alatavicus* raw material;
- to develop the technology of introduction and procurement of *Crocus alatavicus* raw materials in accordance with the requirements of GACP;
- conduct a comparative analysis of morphological and anatomical signs and phytochemical composition of wild and introduced *Crocus alatavicus* species;
- Conduct a feasibility study on the introduction of a phytosubstance *Crocus alatavicus*.

Object of research: herbal raw material *Crocus alatavicus*.

Methods of research: physical, physico-chemical, pharmacognostic, pharmaco-technological, biological, statistical pharmacopoeial and non-pharmacopoeial methods.

Scientific novelty

For the first time:

- diagnostic morphological and anatomical signs of herbal raw material *Crocus alatavicus*, allowing to carry out identification within the limits of pharmacopoeial requirements are defined;
- research of chemical composition of *Crocus alatavicus* raw material by modern physico-chemical methods (HPLC-MS, NMR-MS and GC-MS) on presence of the maintenance of biologically active substances, products of primary and secondary metabolites of studied plant was carried out. HPLC-ESI-QTOF-MS method revealed the presence of 22 compounds such as flavonoids (kaempferol and its derivatives, quercetin, rutoside, astragalol, nicotiflorin, acacetin), organic acids (gluconic, malic, citric and carboxyvanilic acids), anthraquinone (endococin), monoterpenoids (DH crocusatin F, crocusatin F). Kaempferol derivatives were found to account for 96.5% of all identified flavonoids. Three carotenoids (crocine, β -carotene, zeaxanthin) and 7 monoterpenoids (β -isophorone, 4-oxoisophorone, β -pinene, 1,8-cineol, 4-oxysafranal, picrocine, safranal), 13 fatty acids (myristic, pentadecane, palmitic, palmitoleic, stearic, oleic, linoleic, arachidonic, eicosanic, eicosenoic, eicosendienoic, eicosotrienoic, linolenic acids), 4 phenolic acids (3,5-dimethoxy-4-oxycinnamic, 3,4-dioxyctic, 3-methoxy-4-oxycoric, 4-oxycoric acids) and 20 amino acids (glutamic acid, asparagic acid, alanine, proline, leucine, serine, tyrosine, arginine, phenylalanine, glycine, threonine, lysine, valine, isoleucine, tryptophan, histidine, methionine, diaminovaleric acid, cysteine, oxalyl diamino-propionic acid). The dominant compounds kaempferol and β -carotene are

presented as chemical markers of the studied object and used in the development of standardization of the herb *Crocus alatavicus* herb;

- the safety and biological activity profile of *Crocus alatavicus*: antibacterial, antifungal, antiviral and antitumour;

- the technology for introducing *Crocus alatavicus* by seed propagation according to GACP principles has been developed. The novelty is confirmed by the patent for utility model No.6737 "Method of seed multiplication of *Crocus alatavicus* " registered in the State Register of Utility Models of the Republic of Kazakhstan on 09.08.2021;

- comparative analysis of morphological features and chemical composition of the main biologically active substances of wild and introduced *Crocus alatavicus* was carried out.

The main provisions of the thesis research put to the defense:

- the results of comprehensive pharmacognostic analysis: morphological and anatomical diagnostic features, chemical composition and standardization of *Crocus alatavicus* raw material;

- results of safety studies and biological activity profile of *Crocus alatavicus* raw material;

- the results of the technology of introducing *Crocus alatavicus* by seed multiplication, harvesting raw materials according to the principles of GACP and the feasibility study for the production of herbal substances.

Practical significance of the research:

- the results of research methods of antibacterial, antifungal, antiviral and antitumor activity of *Crocus alatavicus* were introduced in the scientific and educational process of the Department of Pharmaceutical Microbiology of Medical University of Lublin (Poland) (Implementation act No.1, Appendix A);

- the results of pharmacognostic study of *Crocus alatavicus* raw material have been implemented into the educational process of the Department of Pharmaceutical and Toxicological Chemistry, Pharmacognosy and Botany (Implementation act No.2, Appendix B);

- the results of technology introduction *Crocus alatavicus* by way of seed multiplication and harvesting raw materials in accordance with the principles of GACP standard "Good agricultural and collection practices" (Good agricultural and collection practice (GACP) for starting materials of herbal origin) are introduced in Fitoleum LLP (Implementation act No.3 and No.4, Appendix C, D);

- developed quality specification, draft normative document for medicinal herbal raw material "*Crocus alatavicus* herb" (Appendix D).

Author's personal contribution. All results of the dissertation research were obtained by the author independently and are the personal contribution of the candidate to the science of pharmacy.

Reliability of the results, the main provisions, defended, findings and conclusions formulated in the thesis work is substantiated by a significant amount of experimental material, fully supported by the results of own research conducted in laboratory and industrial conditions using modern certified equipment and accurate measurement methods, as well as comparison with the literature data.

Approbation of the work

The main provisions of the thesis were reported and published in the proceedings of international conferences: International scientific and practical conference "Modern methods of correction of acne and other skin problems in cosmetic practice" (Kharkov, Ukraine, 2018); International conference "Phytovalley 2019, Austrian Institute for Drug Screening GmbH. (Innsbruck, Austria, 2019); IV International Scientific and Practical Conference "Global Science and Innovation 2019: Central Asia" (Astana, Kazakhstan, 2018); XIV International Scientific and Practical Conference "Years of Rural Development, Tourism and Folk Crafts (2019-2021)" (Dushanbe, Tajikistan, 2019); VIII scientific-practical conference with international participation "Priorities of pharmacy and stomatology - from theory to practice" (Almaty, Kazakhstan, 2019); International conference "Modern science. Management and standards of scientific research" (Prague, Czech Republic, 2020); III International scientific-practical conference "Formation and prospects of development of scientific school of pharmacy: continuity of generations", dedicated to memory of Prof. R. Dilbarkhanov; IX International scientific-practical conference "Proprieties of pharmacy and stomatology: from theory to practice", dedicated to the memory of Prof. Kiyashev Dauletkeldy Karimovich, held within the "90th anniversary of the Kazakh National Medical University named after S.D. Asfendiyarov" (Almaty, Kazakhstan, 2020).

Information on publications

According to the results of research were published 16 scientific papers, including: an article in the international peer-reviewed scientific journal, included in the database Scopus and Web of Science Core Collection - 1 (Appendix E); articles in journals recommended by the Committee on Quality Assurance in Education and Science Ministry of Education and Science - 5; abstracts and articles at international scientific conferences (Russia, Ukraine, Czech Republic, Tajikistan, Kazakhstan) - 8; articles in international journals (Russia) - 1; patent for utility model - 1 (Appendix F).

Connection of research tasks with the plan of scientific programs

This thesis is executed in a direction of pharmaceutical industry development, realization of the Complex plan of pharmaceutical and medical industry development on 2020-2025 years, and also within the limits of initiative project of "KazNMU named after S.D. Asfendiyarov" "Ethnopharmaceutical study of Flora of Kazakhstan" № 0115RK0245 from 10.07.2015 and intramural scientific and technical project "Development of full cycle of production of antiseptics based on herbal raw materials" № 012RK40178 from 04.11.2021.

Scope and structure of the thesis

This thesis is presented on 152 typewritten pages; it contains 29 tables, 50 figures, a list of references including 159 sources, and 13 appendices. The work consists of an introduction, a literature review, a section devoted to the materials and methods of research, three sections of our own research and conclusions.