

## ANNOTATION

**Dissertation work on the topic** «Pharmacognostic studies of the herb *Stachys sylvatica* L. and development of the technology of extracts of pharmacopoeial quality» for the degree of Doctor of Philosophy (PhD) in the specialty 8D07201 – «Technology of pharmaceutical production»

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**General characteristics of the work.** The dissertation work is devoted to pharmacognostic study of plant raw materials of *Stachys sylvatica* L. and obtaining the extract in accordance with pharmacopoeial standards.

**Relevance of the research topic.**

According to the comprehensive plan for the development of the pharmaceutical and medical industry for 2020-2025, the study of medicinal plants and the extraction of pharmacologically active substances on their basis is one of the most important scientific areas of modern pharmacy. Rich plant resources of Kazakhstan, including *Stachys sylvatica* L., as a natural source of many biologically active substances of pharmacological importance. The study of the chemical composition, pharmacological action and therapeutic potential of medicinal plants lays the foundation for the development of new drugs.

*Stachys sylvatica* L. although it has long been used in traditional medicine for its antibacterial, anti-inflammatory and wound-healing properties, the full components of the plant's bioactive substances and its therapeutic potential under-researched. In addition, the extraction methods and processes designed to utilise these substances for medicinal purposes have also not been adequately improved.

In the study of *Stachys sylvatica* L. one of the important tasks is to determine the chemical properties of biologically active substances contained in the plant and their pharmacological action. Research in this direction allows not only to create new medicines, but also to scientifically substantiate the possibilities of innovative technologies and the accumulated experience of folk medicine.

In this regard, pharmacognostic studies of plant raw materials, determination of their chemical composition and pharmacological properties are among the most important issues in the field of pharmacy and contribute to the innovative development of the pharmaceutical industry of Kazakhstan.

**Purpose of research:** Pharmacognostic analysis of plant raw materials of *Stachys sylvatica* L. and development on its basis of technology for obtaining extracts of pharmacopoeial quality.

**Research objectives:**

- Pharmacognostic analysis of *Stachys sylvatica* L. and standardisation of plant raw materials;
- Preparation of extracts from plant raw materials of *Stachys sylvatica* L. and evaluation of quality parameters;
- Safety assessment of *Stachys sylvatica* L. extract and study of biological activity profile;

- Transfer of technology for obtaining *Stachys sylvatica* L. extract and development of feasibility study.

**Objects of research:** plant material of *Stachys sylvatica* L. and the extract obtained from standardised raw materials.

**Methods of research:** pharmacopoeial, pharmacognostic, pharmaceutical-technological, pharmacological, biological and statistical methods.

**Scientific novelty**

For the first time:

– morphological and anatomo-diagnostic identification features of the above-ground part of the plant raw material of *Stachys sylvatica* L. growing in Almaty region;

– extract from plant raw material of *Stachys sylvatica* L. by ultrasonic maceration method, which is confirmed by the patent for utility model №7763 from 06.10.2022 (Appendix A);

– acute and subacute toxicity of *Stachys sylvatica* L. extract was studied, and its antimicrobial, anti-inflammatory, antiviral, antitumour and antihelminthic properties were revealed, indicating high biological activity of the extract;

– the study of *Stachys sylvatica* L. extract by GC-MS method revealed that the main volatile substances are esters of diterpenoids and fatty acids. According to the results of RP-HPLC/PDA analysis, 10 compounds were detected in the extract, the main ones being chlorogenic acid and verbascoside;

– extract obtained by ultrasonic maceration from plant material of *Stachys sylvatica* L., 17 compounds were detected by HPLC-ESI-QTOF-MS/MS. Flavonoids and their glycosides (chlorogenic acid and verbascoside) constitute the main group of compounds of plant raw materials of *Stachys sylvatica* L., their content is not less than 2.0%. Quality indicators and criteria of their suitability were established for the raw material of *Stachys sylvatica* L., and the extract was standardised;

– The extract of *Stachys sylvatica* L. used in the study showed high antimicrobial activity against Gram-positive bacteria, especially *Bacillus cereus*, with a minimum inhibitory concentration (MIC) in the range of 0.5-2 mg/ml. The bactericidal effect of the extract was most pronounced against *B. cereus*. The extract of *S. sylvatica* L. showed low cytotoxicity on VERO cells ( $CC_{50}$  0.810±0.013 mg/ml) and medium cytotoxicity on MRC-5 cells ( $CC_{50}$  0.0891±0.014 mg/ml). On MRC-5 cells, the *S. sylvatica* L. extract reduced the viral load of HCoV-229E virus by 1.56 log, showing no cytopathic effect, whereas on VERO cells it showed dose-dependent efficacy against HHV-1 virus, significantly reducing the cytopathic effect and reducing the viral load by 1.11 log. The extract was tested for antitumour activity against FaDu, H1HeLa and RKO cell lines. It showed weak cytotoxicity to FaDu and RKO cells and moderate cytotoxicity to H1HeLa cells. When tested for antihelminthic activity, the extract showed activity similar to albendazole at the concentrations tested.

**The main provisions of the dissertation research submitted for defense:**

The results of determination of quality indicators of plant raw materials of *Stachys sylvatica* L. in accordance with pharmacopoeial requirements of the Republic of Kazakhstan and EAEU;

The results of the choice of technology for obtaining an extract from the plant raw material of *Stachys sylvatica* L. and determination of its chemical composition by gas chromatography-mass spectrometry and HPLC;

The results of studies on the evaluation of some preclinical parameters and biological activity profile of the extract obtained from the raw material of *Stachys sylvatica* L., based on the conducted studies.

**Practical significance of the research:**

- Identification of the species of the plant of *Stachys sylvatica* L. is confirmed by certificate №01-05/309 from 23.09.2021, issued by the Institute of Botany and phytointroduction of the Committee of Forestry and Wildlife of the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan, Almaty (Appendix B);

- The technology of collection, harvesting and storage of plant raw materials of *Stachys sylvatica* L. confirmed by the act of introduction of LLP «Fitoleum», Esik, Kazakhstan (Appendix C), and introduced a regulatory document «*Stachys sylvatica* L.» from 03.09.2022 (Appendix D);

- The technology of obtaining the extract from plant material of *Stachys sylvatica* L. is confirmed by the act of introduction of LLP «Fitoleum» (Appendix E);

- The production process of pilot series of extracts obtained from plant material of *Stachys sylvatica* L. is confirmed by the technological act at the pharmaceutical company LLP «Fitoleum» (Appendix F);

- A normative document on the quality of extract obtained from *Stachys sylvatica* L. (Appendix G);

- Studies of biological activity of dry extract of *Stachys sylvatica* L. introduced in the educational process at the departments of «Pharmaceutical Microbiology» Lublin Medical University and the departments of «Pharmacognosy with a course of botany» Pharmacy School of the Kazakh National Medical University named after S.D. Asfendiyarov (Appendices H, I);

- Certificate of inclusion of information in the state register of rights to copyrighted objects №47427 12 June 2024 on the topic «Pharmacognostic studies of the herb *Stachys sylvatica* L. and development of the technology of extracts of pharmacopoeial quality» was received (Appendix J).

**Personal contribution of the author.** In the process of carrying out the thesis work, the author effectively used domestic and foreign sources of information, conducting comprehensive research and analyses on the topic of the study. All experimental work was carried out in full in accordance with the set tasks. The reliability of the obtained results was confirmed by new sources obtained using modern methods of analysis and equipment in scientific centres and laboratories, as well as reflected in scientific articles.

Reliability and validity of the obtained results, as well as the focus of the research on solving topical problems are confirmed by draft regulatory documents

performed in the world's leading research centres. The analysis of the research results is based on a significant amount of experimental material and was carried out using modern certified equipment and validated methods, which emphasises the author's personal contribution to science in the field of pharmaceutical production technologies.

#### **Approbation of the work**

The main data of the dissertation work were presented and published in the materials of international conferences:

- At the international scientific-practical conference «Modern Pharmacy: New Approaches and Current Research» organised within the framework of «Days of the University» named after S.D. Asfendiyarov KazNMU (Almaty, October 2021);

- Awarded II degree diploma in the IV international publication «Best Young Scientist – 2021», presented by the countries of the Commonwealth of Independent States (Nur-Sultan, 2021);

- At the conference «Innovative Technologies in Pharmacy» (Czech Republic, Prague, April 2021);

- At the XI International Scientific and Practical Conference «Priorities of pharmacy and dentistry: from theory to practice»;

- 1st place in the section «Pharmacy» at the I International Forum Asfen.forum. «New Generation – 2023» (Almaty, June 2023).

#### **Information about publications:**

The results of the dissertation work have been published in 11 scientific papers. In the international scientific journal, included in the databases Scopus and Web of Science Core Collection - 1, in publications submitted by the Ministry of Science and Higher Education of the Republic of Kazakhstan, the Committee for Quality Assurance in the field of science and higher education - 3, in the materials of international scientific conferences - 5, patent for utility model - 1, register of rights to copyrighted objects - 1.

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

The thesis is presented on 142 pages of printed text, includes 39 tables, 50 figures, a list of literature from 181 sources, as well as 14 appendices. The work consists of an introduction, literature review, a section devoted to research materials and methods, three sections of own research, conclusions and findings.