

ANNOTATION

Dissertation work on the topic "**Phytochemical analysis of some *Thymus L.* plant species and preparation of a new phytosubstantiation**"

for the degree of Doctor of Philosophy (PhD) in the specialty

6D110400 - "Pharmacy"

Zhumakanova Bagda Sagynaykyzy

General characteristics of the work. The dissertation work is devoted to the comparative study of the phytochemical composition of medicinal plant materials *Thymus marschallianus* Willd. and *Thymus seravschanicus* Klovov L. and the development of herbal pharmaceutical substances of pharmacopoeial quality.

Relevance of the research topic. Currently, one of the main goals for the domestic pharmaceutical industry is import substitution. As part of the Comprehensive Plan for the Development of Pharmaceutical Production for 2020-2025, the main focus is on the creation of production of medicines based on plants growing in the Republic of Kazakhstan. According to the World Health Organization (WHO), approximately 80% of the world's population uses herbal medicines. This trend is explained by a decrease in undesirable effects on the human body, the absence of physiological dependence in the process of long-term therapy with such drugs and economic accessibility.

To achieve the goals and objectives of the directions of development of the pharmaceutical industry, it is necessary to conduct comprehensive research on the rational use of domestic natural raw materials as a source of pharmacologically active substances. In this regard, it is of interest to study the chemical composition of some plant species of the genus *Thymus L.* of the *Lamiaceae* family, which grow on the territory of our country as promising herbal pharmaceutical substances.

There are 27 species of *Thymus L.* in the flora of Kazakhstan and two of them: *Thymus vulgaris L.* and *Thymus serpyllum L.* They are included in the State Pharmacopoeia of the Republic of Kazakhstan as a medicinal herbal remedy of expectorant, antimicrobial, analgesic action. More than ten medicines in their composition contain extracts of *Thymus L.* In addition, *Thymus* extracts are also present in the nomenclature of biologically active additives, which are used as a tonic, anti-inflammatory, soothing agent.

The study of various species of *Thymus L.* as potential sources of biologically active substances is an important area of research, especially given their potential applications in medicine and pharmacy. Lack of information about *Thymus marschallianus* Willd and *Thymus seravschanicus* Klovov L. as sources of biologically active compounds, it means that there are many opportunities for further research and expansion of the range of medicinal plants. To unlock the potential of these species, the identification and study of their chemical composition, including the analysis of their essential oils and other biologically active compounds, as well as the study of the profile of pharmacological activity is relevant.

Purpose of research: comparative phytochemical study of the herb *Thymus marschallianus* Willd and *Thymus seravschanicus* Klokov L. and the development of herbal pharmaceutical substances of pharmacopoeial quality based on them.

Research objectives: to achieve this goal, the following tasks should be solved:

1. To study the identification parameters and phytochemical composition of medicinal plant materials in the comparative aspect of two species of the genus *Thymus* L.;
2. To establish pharmaceutical and technological parameters and standardize medicinal plant materials *Th. marschallianus* Willd. and *Th. seravschanicus* Klokov L.;
3. To develop a technology for obtaining and validating the technological process for obtaining extracts of *Th. marschallianus* and *Th. seravschanicus*;
4. To study the safety and pharmacological activity profile of extracts of *Th. marschallianus* and *Th. seravschanicus*;
5. To carry out a feasibility study for the production of an herbal pharmaceutical substance.

Objects of research: *Th. seravschanicus* Klokov L. and *Th. marschallianus* Willd. plants, extracts of *Th. seravschanicus* and *Th. marschallianus*.

Research methods: pharmacopoeial and non-pharmacopoeial methods: physical, physico-chemical, pharmacognostic, pharmacological-technological, biological, statistical.

Scientific novelty.

For the first time:

- diagnostic morphological and anatomical signs of medicinal plant materials *Th. marschallianus* Willd. and *Th. seravschanicus* Klokov L. have been determined. in a comparative aspect, allowing the identification of the studied species;

- the chemical composition of hydroethanol extracts of the studied two types of *Thymus* L. was studied using modern physico-chemical methods RP-HPLC/PDA, HPLC/ESI-QTOF-MS/MS and GC-MS). Comparative phytochemical studies of hydroethanol extracts of *Th. marschallianus* and *Th. seravschanicus* were carried out. The RP-HPLC/PDA and HPLC/ESI-QTOF-MS methods showed some differences in the composition of the two extracts. HPLC/ESI-QTOF-MS methods were identified as part of *Th. marschallianus* - 21, *Th. seravschanicus* - 15 compounds. As part of the hydroethanol extract *Th. marschallianus* identified 11 flavanoids, of which 9 flavones and 2 flavanones. Among the flavones, luteolin and their glycosides, luteolin-7-*O*-rutinosides, luteolin-7-*O*-glucosides, luteolin-7-*O*-glucuronides, luteolin-7-*O*-dipentosides and luteolin-7-*O*-(6"-3-hydroxy-3-methylglutaryl)-glucosides were identified, apigenin and their glycosides, apigenin-7-*O*-glucosides, apigenin-7-*O*-glucuronides and apigenin-7-*O*-rhamnoglucuronides, as well as diosmetin glucuronides. The presence of eriodictyol and naringenin among the flavonones has been confirmed. Hydroethanol extract extract *Th. seravschanicus* contains eight flavonoid compounds, six of which are flavones: luteolin-7-*O*-rutinose, luteolin-7-*O*-glucoside, luteolin-7-*O*-glucuronide, luteolin-7-*O*-(6"-3-hydroxy-3-methylglutaryl)-glucoside, apigenin-7-*O*-glucuronide and diosmetin glucuronide. Compounds of eriodictyol and naringenin from the flavonone group have been

identified. The quantitative determination of the composition of extracts of *Th. marschallianus* and *Th. seravschanicus* polyphenolic compounds was carried out by the RP-HPLC/PDA method. Among the phenolic acids in *Th. marschallianus* revealed the predominance of protocatechic acid (2.08 ± 0.01 mg/g of dry extract), in *Th. seravschanicus* rosemary (3.33 ± 0.01 mg/g of dry extract) and protocatechic acid (3.06 ± 0.01 mg/g of dry extract) dominated. The composition of *Th. seravschanicus* has a significantly higher content of flavonoids than in *Th. marschallianus*. In general, the content of most compounds in *Th. seravschanicus* was three times higher than in *Th. marschallianus*. The total content of polyphenols (TRS) was shown in terms of gallic acid (GAE), this indicator was slightly higher in *Th. seravschanicus* (228.83 ± 39.44 mg GAE/g) compared to *Th. marschallianus* (186.01 ± 16.11 mg GAE/g).

- volatile compounds of hexane fractions of extracts of both *Thymus* L. species were analyzed by GC-MS method and *Th. seravschanicus* – 14 and *Th. seravschanicus* - 15 compounds were identified. Both types were characterized by the presence of two monoterpene alcohols: thymol and carvacrol. The volatile fraction of *Th. marschallianus* was distinguished by the presence of several sesquiterpenoids (β -bisabolene, spatulenol, viridiflorol) and eugenol (phenylpropanoids). The volatile fractions of *Th. seravschanicus* consisted mainly of monoterpenoids. In addition to the indicated thymol and carvacrol, the presence of *p*-cymol, limonene, carvone and thymoquinone was confirmed. In two extracts, hexane fractions were characterized by the presence of fatty acids and their esters. *Th. marschallianus* hexadecanoic, linoleic, linolenic acids and their ethyl esters were found in the volatile fraction. In the volatile fraction of *Th. seravschanicus*, all methyl esters of fatty acids were identified.

- the profile of pharmacological activity was studied, it was found that both studied species have safety and have a certain level of biological activity. Both extracts showed similar antioxidant properties in terms of DPPH removal activity: EC₅₀ shows for *Th. marschallianus* - 24.23 ± 0.29 g/ml and for *Th. seravschanicus* - 21.47 ± 1.63 g/ml. Antioxidant activity of the two extracts, determined by AII (index of antioxidant activity): for *Th. marschallianus* - 2.45 ± 0.03 and *Th. seravschanicus* - 2.78 ± 0.21 . Sensitivity of the hydroethanol extract *Th. seravschanicus* (MIC=0.625-10 mg/ml) to standard strains of Gram-positive and Gram-negative bacteria was higher than *Th. marschallianus* (MIC = 2.5-10 mg/ml). It should be noted that the aqueous alcohol extract of *Th. seravschanicus* showed high activity against *Helicobacter pylori* ATCC 43504 (MIC = 0.625 mg/ml), for *Th. marschallianus*, the MIC index against this type of microorganism was 2.5 mg/ml. It was found that both extracts exhibit the same activity against standard strains of yeast fungi of the genus *Candida* (MIC = 5 mg/ml), while the fungicidal activity of MFC/MIC extracts varied in the range 2-4;

- a new method for obtaining extracts from raw materials of *Th. marschallianus* and *Th. seravschanicus* has been developed, which allows to obtain extracts with maximum release of biologically active substances on the scale of pilot production at Fitoleum LLP, Esik, Republic of Kazakhstan;

- herbal pharmaceutical substances of pharmacopoeia quality were obtained and a feasibility study was conducted.

The main provisions of the dissertation research submitted for defense:

The results of a comprehensive pharmacognostic study of medicinal plant materials *Th. marschallianus* and *Th. seravschanicus* in a comparative aspect;

The results of the experimental substantiation of the development of an optimal technology for the production of herbal pharmaceutical substances based on medicinal plant materials *Th. marschallianus* and *Th. seravschanicus* with maximum BAS yield;

The results of studies of the chemical composition of extracts of *Th. marschallianus* and *Th. seravschanicus* in a comparative aspect;

Results of safety studies and pharmacological activity profile of hydroethanol extracts of *Th. marschallianus* and *Th. seravschanicus*;

Technology transfer and validation assessment of pilot production of extracts of *Th. marschallianus* and *Th. seravschanicus*;

Feasibility study of the production of phyto-substation *Th. seravschanicus*.

Practical significance of the study.

Expansion of pharmacopoeial *Thymus L.* species for introduction into the medical practice of healthcare in the Republic of Kazakhstan.

Technological instructions for the collection and harvesting of raw materials have been developed and implemented, pilot series have been produced in the pharmaceutical company Fitoleum LLP, Esik, Republic of Kazakhstan (Regulatory document " *Thymus marschallianus* herb" dated 05/21/2018 and Regulatory document " *Thymus seravschanicus* herb" dated 05/21/2018);

A method for obtaining an herbal pharmaceutical substance from *Th. marschallianus* and *Th. seravschanicus* has been developed, which allows to isolate biologically active substances with maximum yield at the enterprise of Fitoleum LLP on a pilot scale (Act of Implementation dated 08.10.2018);

Technological regulations for the production and production of herbal pharmaceutical substances from *Th. marschallianus* and *Th. seravschanicus* have been developed and approved at Fitoleum LLP, Esik, Republic of Kazakhstan, validation of the technological production process has been carried out (Appendices M and H);

An extraction method with activity against gram-negative bacteria *Helicobacter pylori* has been developed, the priority of the research is protected by utility model patent No. 6830 "Method for obtaining an extract from thyme", registered in the State Register of Utility Models of the Republic of Kazakhstan on 02/04/2022 (Appendix II) and copyright certificate No. 41427 dated 12/21/2023 (Appendix P);

The results of the study of the antibacterial, antifungal and antioxidant activity of hydroethanol extracts of *Thymus marschallianus* and *Thymus seravschanicus* were introduced into the scientific and educational process of the Department of Pharmaceutical Microbiology of the Medical University of Lublin (Poland), the results of the pharmacognostic study of these types of medicinal plant materials were introduced into the educational process of the Department of Pharmaceutical and Toxicological Chemistry, Pharmacognosy and Botany of the School of Pharmacy of Asfendiyarov KazNMU (Acts of implementation, Appendices C and T).

Personal contribution of the author. All the results of the dissertation research were obtained by the author independently, and are a personal contribution of the doctoral student to pharmaceutical science.

The reliability of the results, the main provisions submitted for defense, conclusions and conclusions formulated in the dissertation work is justified by a significant amount of experimental material, fully confirmed by the results of their own research conducted in laboratory and production conditions, using modern certified equipment and accurate measurement methods, as well as comparison with literary data.

Approbation of the work

The main provisions of the dissertation work were reported and published in the materials of international conferences: International scientific and practical Conference "Modern methods of correction of acne and other skin problems in cosmetology practice" (Kharkiv, Ukraine, 2018); VII scientific and practical conference with International participation "Priorities of pharmacy and dentistry – from theory to practice" (Almaty, Kazakhstan, 2019); International Conference "Modern Science. Management and Standards of Scientific Research" (Prague, Czech Republic, 2020);

Information about publications

According to the research results, 9 scientific papers have been published, including: an article in an international peer-reviewed scientific journal included in the Scopus database and the Web of Science Core Collection - 1; articles in journals recommended by the Committee for Quality Assurance in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan - 4; abstracts and articles on international scientific and practical conferences (Ukraine, Kyrgyzstan, Kazakhstan) - 4; patent for a utility model - 1, author's certificate for the establishment of a pharmacological profile – 1.

Connection of research tasks with the plan of scientific programs

The dissertation work was carried out in the direction of the development of the pharmaceutical industry, the implementation of a comprehensive plan for the development of the pharmaceutical and medical industry for 2020-2025, as well as within the framework of the initiative project of the Asfendiyarov Kazakh National Medical University "Study of the ethnopharmaceutical flora of Kazakhstan" No. 0115RK0245 dated 07/10/2015.

Scope and structure of the dissertation

The dissertation work is presented on 150 pages of typewritten text in a computer set, contains 44 tables, 48 figures, a list of references, including 163 sources, as well as 18 appendices. The work consists of an introduction, a literature review, a section devoted to materials and methods of research, three sections of own research, findings and conclusions.