

ANNOTATION

of the dissertation work on the topic: «**Pharmaceutical development of a finished dosage form based on biologically active substances of steppe sage**»
for the degree of Doctor of Philosophy (PhD)
in the specialty 6D074800 – «Technology of pharmaceutical production»
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Relevance of the research topic

A large number of plants including medicinal ones grow on the territory of the Republic of Kazakhstan. Great interest from the point of view practical use in medicine are species of the genus *Salvia* L. (*Salvia*). Currently, 8 species of plants of the genus Sage grow in the Republic of Kazakhstan. The State Pharmacopoeia of the Republic of Kazakhstan includes sage officinalis (*Salvia officinalis* L.) which is recommended for use as an anti-inflammatory and antiseptic agent is also used for diseases of the upper respiratory tract, a wound healing agent for burns or skin diseases.

However, other species of the genus *Salvia* L. are not yet sufficiently studied and are not in demand for medical use. Thus, steppe sage (*Salvia stepposa* Des.-shost.) grows almost throughout the entire territory of the Republic of Kazakhstan, the chemical composition of which has not yet been studied. Species of the sage genus are overproducers of phenolpropanoids and rosmarinic acid. In addition, a study on the search for phenolpropanoids and rosmarinic acid in *Salvia* L. in Kazakhstan has not been previously conducted.

Thus, the study of promising plant raw materials of steppe sage for its use in the production of domestic medicines indicates the relevance of this dissertation work and its practical significance for the pharmaceutical industry.

The purpose of the dissertation research: Development of the composition, technology and standardization of a new drug based on the steppe sage extract.

Objectives of study:

- to conduct a pharmacognostic study of the herb *Salvia stepposa* Des.-Schost and determine the indicators and quality standards, the shelf life of herbal raw materials *Salvia stepposa* Des.-Schost;

- to conduct a comparative study of the chemical composition, technological properties, biological activity of extracts from steppe sage obtained by traditional methods and methods of ultrasonic and microwave activation;

- to conduct research on the choice of the optimal method for obtaining a dry extract from the steppe sage herb, to develop a technology for obtaining a substance with a pronounced biological effect. Determine indicators and quality standards, shelf life and investigate the biological activity of the dry extract of steppe sage;

- to develop the optimal composition and technology for obtaining a finished dosage form containing an extract of steppe sage which has pharmacological activity. Determine indicators and quality standards, shelf life of the finished dosage form;

- develop draft regulatory and technical documentation for raw materials, extract and finished dosage form.

Research methods

The methods used to conduct scientific research comply with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan (SP RK), the Pharmacopoeia of the Eurasian Economic Union, European Pharmacopoeia, United States Pharmacopoeia, British Pharmacopoeia, FS and other regulatory documents in force on the territory of the Republic of Kazakhstan.

To develop a method, technology for obtaining a biologically active substance, draw up regulatory documents and laboratory regulations, the rules of GMP, QMS, GOSTs and other regulatory documents in force on the territory of the Republic of Kazakhstan were used.

- Microwave, ultrasonic and traditional extraction;

- Chemical: qualitative reactions to various classes of biologically active substances;

- Physical and chemical: thin layer chromatography (TLC), high performance liquid chromatography (HPLC/UV, HPLC/MS), gas chromatography-mass spectroscopy (GC/MS);

- Pharmacopoeial methods: determination of color, taste, smell according to the method set forth in the GF RK, Vol. 1, p. 548; determination of solubility in various solvents is carried out according to the method of the Global Fund of the Republic of Kazakhstan, T. 1, p. 175;

- Statistical processing of the results obtained will be carried out in accordance with GPM.1.1.0013.15 (General Pharmacopoeia Article) "Statistical processing of the results of a chemical experiment", section 3 "Metrological characteristics of the analysis method" and section 4 "Metrological characteristics of the average result", the following indicators will be calculated : sample mean, \bar{x} ; sample size, n ; standard deviation, S ; standard deviation of the mean result, S_x ; Student's test, t ($P=0.95$, f); confidence interval half-width, ΔX ; relative error, ε %;

- Evaluation of antimicrobial activity in accordance with the requirements of the RF SP XIII with test cultures: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231).

- Evaluation of anti-inflammatory activity on the model of acute exudative reaction (peritonitis).

- Evaluation of the microbiological purity of tablets according to the SP RK, t I, 5.1.4 "Microbiological purity".

- Evaluation of the antioxidant activity of the studied samples. method for determining the iron-reducing potential [FRAP-method (Ferric Reducing Antioxidant Power assay)].

- Evaluation of the antiradical action against the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•). An ethanolic solution of DPPH (100 µM) was used to evaluate the antiradical activity in the DPPH-radical test.

- Preclinical (non-clinical) studies of steppe sage extracts and test samples of tablets based on steppe sage tests were approved by the ethical commission of NJSC "Karaganda Medical University" of 10.10.2022, Protocol No. 6, assigned number 7/1.

- Cytotoxicity study on *Artemia salina* crustaceans using a kit containing *Artemia salina* cysts.

Objects of study: medicinal raw materials: aerial part of the *Salvia stepposa* Des.-Schost; aqueous-alcoholic extracts of sage steppe obtained using traditional extraction, ultrasound and microwave; tablets based on dry extract of steppe sage.

Subject of study: Pharmacognostic and commodity analysis of the aerial part of the *Salvia stepposa* Des.-Schost; standardization; the optimal method for extracting steppe sage; the amount of extractive substances; biological activity and preclinical tests of dry extract of sage leaves; the concentration of rosmarinic acid in raw materials, extract, tablets; composition and technology for obtaining tablets, evaluation of tablet quality indicators, development of regulatory documentation for raw materials and finished dosage form.

General provisions for defense:

- results of pharmacognostic study of the herb *Salvia stepposa* Des.-Schost;
- experimental data on the technology of obtaining a dry extract of the steppe sage leaves *Salvia stepposa* Des.-Schost;

- results of a study of the biological activity and safety of a dry extract derived from the leaves of *Salvia stepposa* Des.-Schost;

- results of pharmaceutical development of tablets based on dry extract of *Salvia stepposa* Des.-Schost leaves.

Scientific novelty

For the first time in the Republic of Kazakhstan:

- a pharmacognostic analysis was carried out and the quality indicators of the plant raw material *Salvia stepposa* Des.-Schost were assessed, stability was studied, a draft RD was developed;

- carried out ultrasonic and microwave extraction of *Salvia stepposa* Des.-Schost determined the optimal method of obtaining while maintaining the maximum activity of the sample;

- developed a finished dosage form in the form of tablets based on *Salvia stepposa* Des.-Schost;

- developed a technology for obtaining a dry extract from the leaves of *Salvia stepposa* Des.-Schost and assessed quality indicators, studied stability, developed laboratory regulations;

- on the basis of experimental, physico-chemical, technological studies, the optimal composition and production technology of a new drug has been developed, which has a pronounced pharmacological activity on *Salvia stepposa* Des.-Schost which grows on the territory of the Republic of Kazakhstan;

- a quality specification was developed and standardization of the finished dosage form in the form of tablets was carried out, stability was studied, a draft RD was developed.

Practical significance of the obtained results

The significance of the work on an international scale lies in the application of modern innovative methods for obtaining extracts based on steppe sage growing on the territory of the Republic of Kazakhstan namely microwave and ultrasonic activation methods which will certainly have an impact on the development of science and technology in Kazakhstan.

The innovative and competitive advantage from the implementation of the work is associated with the development and implementation of an import substitution system for the types of pharmaceutical industrial products that are vital for the Republic of Kazakhstan. The use of modern technology in the creation of new pharmacologically active substances will significantly increase productivity and reduce costs.

Pharmacognostic and merchandising data of *Salvia stepposa* Des.-Schost have been obtained which in the future will allow the use of this raw material in the production of domestic medicines. The project of the RD "Sage steppe leaves" was developed.

The use of the developed technology for obtaining a dry extract of steppe sage by using microwave extraction made it possible to reduce the duration and increase the productivity of the technological process and at the same time significantly reduce the cost of the target product the quality of the dry extract was assessed, laboratory regulations were developed for the production of a dry extract of steppe sage leaves (MWSS -40).

The dry extract was used as a substance for the development of a new medicinal product based on steppe sage, the development of the optimal composition of the finished dosage form in the form of tablets was carried out, the quality was assessed, and the project of RD "Tablets for resorption of steppe sage" was developed. The dissertation work was carried out in accordance with the research plan of NJSC "KMU" within the framework of the scientific project AP19176476 "Development of a new drug based on the extract of steppe sage".

Personal contribution of researcher

The researcher work independently selected and analyzed the scientific literature on the topic of the dissertation, experiments were carried out, experimental data were obtained and systematized, publications were prepared.

Conclusions

1) For the first time, a pharmacognostic study of plant raw materials of steppe sage (*Salvia stepposa*) was carried out and quality standards and shelf life were determined.

Macroscopic features: plant 30-50 cm tall, stem 3 to 5 mm in diameter, green in the upper part, purple in the lower part, upright 4-angled in cross section, the surface is covered with straight and curved numerous simple white hairs colors.

Microscopic features: cells of the upper epidermis are polygonal with straight walls, above the veins of the leaf - almost rectangular, with even and thickened walls, covered with a cuticle layer on top; lower - prosenchymal cells with thickened and straight walls, stomata of the diacytic type. Numerous small glandular trichomes along the leaf veins. Essential oil glands are not numerous, rising above the surface.

According to the results of the studies carried out to determine the quality parameters of plant raw materials, the data obtained are included in the projects. The results of the determination of heavy metals showed that the content of heavy metals in the studied plant material does not exceed the limits of permissible values. A project of ND was developed: "Sage steppe leaves". The shelf life is set to 24 months.

2) For the first time steppe sage extracts were obtained under microwave activation conditions. It was found that microwave extraction was able to reduce the extraction time by 15 times in comparison with traditional methods. The parameters for obtaining a dry extract from the leaves of the steppe sage by the method of microwave activation were established: the ratio of the raw material to the extractant was 1:10, the irradiation power was 300 W, the extraction time was 16 minutes. The yield of dry extract was 23.7% in terms of air dry raw material.

The component composition of dry extracts from the leaves of the steppe sage was determined. The major component is rosmarinic acid.

Tests were carried out on the biological activity of a dry extract of the leaves of steppe sage (MWSS-40). Based on the experiments, dry extract of sage leaves at concentrations of 1 mg/ml and 0.5 mg/ml exhibits cytotoxicity, and at a concentration of 0.25 mg/ml it is non-toxic. *Salvia stepposa* leaf dry extract has high antimicrobial activity against *Staphylococcus aureus* strains (ATCC 6538) and moderate antimicrobial activity against *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231) strains. MWSS-40 has the highest antioxidant activity at a concentration of 0.75 mg/ml, at a concentration of 12.5 mg/ml it has antiradical activity, and at a dose of 25 mg/kg it has anti-inflammatory activity.

3) For the first time, a technology has been developed for obtaining a dry extract from the leaves of *Salvia stepposa*. A technological scheme of production is proposed. A laboratory regulation has been developed for the production of the substance of the dry extract of the leaves of the steppe sage MWSS-40. The

indicators and quality standards of the dry extract of the leaves of the steppe sage have been established. The shelf life of the dry extract of the leaves of steppe sage was determined - 24 months.

4) A technology has been developed for obtaining tablets for resorption based on a dry extract of steppe sage leaves (MWSS-40). It has been established that tablets based on steppe sage contain 5.5 times more rosmarinic acid in comparison with tablets based on sage officinalis. The optimal composition of tablets weighing 750 mg was developed: dry extract of steppe sage MWSS-40 - 50 mg., MCC 105 - 75 mg., EMDEX - 587.5 mg., Citric acid - 28 mg., Calcium stearate - 7.5 mg., peppermint oil - 1.5 mg., eucalyptus oil - 0.5 mg. The shelf life of lozenges based on dry extract of sage leaves was determined to be 18 months (observation time). A draft ND was developed: "Sage steppe tablets" and laboratory regulations for the production of steppe sage tablets.

Approbation of the results of the dissertation

The results and main provisions of the scientific work are presented at:

- XXXXV International Student Scientific and Practical Conference: Interdisciplinary Research. current state and development prospects (Russian Federation, Yekaterinburg, 2019).

- 60th International Scientific Conference of the Eurasian Scientific Association (Russian Federation, Moscow, 2020).

- International scientific conference of students and young scientists "Farabi Əlemi" (Kazakhstan, Almaty, 2019).

- VI International Scientific and Practical Conference "SCIENCE AND EDUCATION IN THE MODERN WORLD: CHALLENGES OF THE XXI CENTURY" (Kazakhstan, Nur-Sultan, 2020).

- VIII International scientific and practical conference "Medicinal plant growing: from past experience to modern technologies" (Ukraine, Poltava, 2020).

Publications

Based on the materials of the dissertation, 11 works were published, including:

- articles in journals recommended by the Committee for Quality Assurance in the field of Science and Higher Education of the Republic of Kazakhstan- 3;

- articles in foreign scientific publications included in the Scopus database - 2;

- abstracts in the materials of international scientific and practical conferences - 5;

- in other scientific publications - 1.

Scope and structure of the dissertation

The dissertation is presented on 142 pages of computer text and consists of an introduction; literature review; 6 sections describing materials and research methods; the main part containing the results and discussion of their own

research, conclusions; list of used literature, including 119 literary sources; 11 applications. The dissertation is illustrated with 46 tables and 49 figures.