

ANNOTATION

of dissertation work on the topic: "**Chemical composition and biological properties of ultrasonic extract of creeping thyme of the flora of Central Kazakhstan, prospects for its use in medicine**" for the degree of Doctor of Philosophy (PhD) in the specialty 6D110400 - "Pharmacy"

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Relevance of the research topic. The medicinal properties of creeping thyme (*Thymus serpyllum* L.) have been known since ancient times and have been used in folk medicine for many centuries. Creeping thyme grass is included in the State Pharmacopoeias of Kazakhstan, the Russian Federation, Ukraine, Britain and other countries, in official medicine it is used as a medicinal plant material with antibacterial, astringent, anti-inflammatory, sedative, anticonvulsant, expectorant, antispasmodic, choleric, analgesic, diuretic, wound healing and anthelmintic action, is used in the form of decoctions and infusions.

And at present, creeping thyme attracts the close attention of scientists around the world, due to its pharmacological properties. In recent years, interest in ethnobotanical, phytochemical, and pharmacological studies of thyme has increased. In world practice, it has been proven that creeping thyme in nature, depending on the geographical region, climatic conditions and habitat, is represented by several chemotypes, i.e. the qualitative composition and quantitative content of the essential oil and the main groups of biologically active substances change, therefore, the pharmacological properties also change.

Despite the fact that the creeping thyme herb is included in the State Pharmacopoeia of the Republic of Kazakhstan, the chemical composition of this domestic medicinal plant is practically not studied. Therefore, the study of the chemical composition and biological properties of creeping thyme, depending on the territory and growing conditions, is of great importance for the use of this medicinal plant in the pharmaceutical industry and applications in medicine.

In addition, the method of obtaining biologically active substances from plant materials is of great importance. To date, in the pharmaceutical industry, a liquid extract of creeping thyme is produced by the classical repercolation method, the technology used is multi-stage, laborious and time-consuming.

As a result, the pharmacognostic study of creeping thyme growing in the territory of Central Kazakhstan, the development of a new rational method and technology for obtaining the amount of extractive substances from the domestic medicinal plant creeping thyme, the study of its chemical composition and biological properties, is an important and priority task.

The purpose of scientific research. Study of the chemical composition and biological properties of ultrasonic extracts of two chemotypes of creeping thyme (*Thymus serpyllum* L.) of the flora of Central Kazakhstan, substantiation of the prospects for their use in medicine.

Objectives of study:

1. Conduct a pharmacognostic study of creeping thyme (*Thymus serpyllum* L.) growing in the territory of Central Kazakhstan.
2. To develop a new method and technology for obtaining the amount of extractive substances from creeping thyme using ultrasonic extraction.
3. To investigate the chemical composition of polyphenolic compounds of ultrasonic extracts of two chemotypes of creeping thyme using modern instrumental methods.
4. To study the biological properties and acute toxicity of ultrasonic extracts of two thyme reprints chemotypes, to scientifically substantiate the possibility of their use in medicine.
5. Develop regulatory documents for the substance of creeping thyme ultrasonic extract in the form of a draft ND and laboratory regulations for obtaining.

Research methods: to achieve the set goal and solve problems, modern physicochemical methods of extraction and analysis were used: ultrasonic extraction, high performance liquid chromatography (HPLC/UV, HPLC/MS), gas chromatography (GC/FID), chromatography-mass spectroscopy (GC/MS) ultraviolet (UV) spectrophotometry.

Objects of study: medicinal plant materials: two samples of creeping thyme grass (*Thymus serpyllum* L.), collected in the populations of the Karaganda region of the Republic of Kazakhstan; sums of extractive substances: dry ultrasonic extracts of two samples of creeping thyme (*Thymus serpyllum* L.), collected in the populations of the Karaganda region of the Republic of Kazakhstan.

Subject of study: biomorphological features, diagnostic features, commodity indicators and chemical composition of two samples of creeping thyme (*Thymus serpyllum* L.); method and technology for obtaining dry ultrasonic extract of creeping thyme; chemical composition and biological properties of dry ultrasonic extracts of two samples of creeping thyme; normative documentation for the substance of the ultrasonic extract of creeping thyme.

The main provisions for defense:

- biomorphological features, diagnostic features, results of phytochemical and commodity analysis of air-dry raw materials of two samples of creeping thyme (*Thymus serpyllum* L.) collected in the populations of the Karaganda region of the Republic of Kazakhstan;
- a new method and technology for obtaining thyme ultrasonic extract creeping;

- chemical composition of polyphenolic compounds of ultrasonic extracts of two chemotypes of creeping thyme herb;
- results of biological properties and acute toxicity of ultrasonic extracts of creeping thyme;
- regulatory documents for ultrasonic extract of creeping thyme, in the form of a draft ND and laboratory regulations for obtaining.

Scientific novelty of the research

- for the first time, a comparative pharmacognostic study of medicinal plant raw materials of creeping thyme (*Thymus serpyllum* L.), collected in the populations of the Karaganda region of the Republic of Kazakhstan, according to external signs, microscopic characteristics, results of commodity analysis, both samples correspond to the SP RK, while significant differences between the two chemotypes of grass creeping thyme in terms of the quantity and component composition of the essential oil, as well as in terms of the quantitative content of the main groups of biologically active substances;
- developed a new method for obtaining the amount of extractive substances from creeping thyme using ultrasound;
- for the first time the chemical composition of polyphenolic compounds of ultrasonic extracts of two chemotypes of creeping thyme was studied by HPLC-UV and HPLCMS/MS, in total 15 phenolic compounds were identified and quantified, five of which are phenolic acids, ten are flavonoids.
- an effective, economical and environmentally safe technology for obtaining the substance of the ultrasonic extract of creeping thyme has been developed;
- as a result of the bioscreening, it was established for the first time that the ultrasonic extract of creeping thyme, collected in the mountain forest massif of Karkaralinsk, has a pronounced bactericidal effect against *Helicobacter pylori*, also exhibits a pronounced antimicrobial activity against 5 strains of gram-positive bacteria (two lines of strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus subtilis*), 2 strains of gram-negative bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis*). In addition, it has an expectorant effect comparable to the comparison drug "Bronchicum C".
- for the first time, according to bioscreening data, it was revealed that the ultrasonic extract of creeping thyme, collected in the Korneevsky forests, has a pronounced bactericidal effect against *Helicobacter pylori*, but exhibits a pronounced antimicrobial activity only against 3 strains of gram-positive bacteria (two lines of strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, at the same time, it causes a growth retardation of cultures of *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*), 2 strains of gram-negative bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis*), also causes a

growth retardation of the *Candida albicans* fungus culture. In terms of expectorant action, it is inferior to the reference drug "Bronchicum C".

- according to the results of the study of acute toxicity in the experiment in vivo, it was found that the ultrasonic extract of two chemotypes of creeping thyme belong to the group "Practically non-toxic" (V class of toxicity) and do not have mutagenic activity;

- the quality specification was developed and standardization of the substance of the ultrasonic extract of creeping thyme was carried out.

Practical significance of the obtained results

- indicators of pharmacognostic research and results of commodity analysis of creeping thyme (*Thymus serpyllum* L.) are included in the draft RD for medicinal plant raw materials "creeping thyme herb";

- for the first time, ultrasonic extracts of two chemotypes of creeping thyme growing in the Karaganda region are recommended as a promising substance for the creation of domestic drugs for the treatment and prevention of *Helicobacter pylori* - associated diseases;

- developed, tested and implemented a technology for obtaining an ultrasonic extract of creeping thyme, which is characterized by a significant reduction in the duration and increase in the productivity of the technological process, an increase in the yield of the finished product and the content of active substances, the absence of toxic solvents;

- a draft ND was developed for the substance ultrasonic thyme extract creeping;

- developed and approved laboratory regulations for obtaining the substance of creeping thyme ultrasonic extract (LR-005491-MK-04-21);

- on the basis of the Scientific Research Center of NCJSC "KMU" the production of experimental batches of the substance of the ultrasonic extract of creeping thyme for pharmacological research was organized.

Personal contribution of a doctoral student

The dissertator independently reviewed and analyzed domestic and foreign literature on the topic of the dissertation work, performed experimental work on all the tasks set. The reliability and validity of the results of the study is confirmed by the focus of the work performed on solving the current problem, the implementation in a modern research center and the draft regulatory documents.

Conclusions

1. For the first time, according to the results of a comparative pharmacognostic study of medicinal plant raw materials of creeping thyme (*Thymus serpyllum* L.), collected in the populations of the Karaganda region of the Republic of Kazakhstan, it was found that creeping thyme is represented by two chemotypes. Two samples of creeping thyme herb have an external similarity and identical anatomical

diagnostic features, while significant differences have been established in the yield and component composition of the essential oil. Also, the differences between the two chemotypes of creeping thyme herb in terms of the quantitative content of the sum of flavonoids, phenolcarboxylic acids, tannins, triterpene compounds, water-soluble polysaccharides, pectin substances, amino acids and organic acids were determined. According to the results of commodity analysis, both chemotypes of creeping thyme herb meet the requirements of the SPh RK. The data obtained are included in the draft ND for medicinal plant raw materials Creeping Thyme.

2. A new method and technology for obtaining a dry ultrasonic extract from creeping thyme has been developed. The advantage of the developed technology is an increase in the productivity of the technological process by 2.5 times and a significant reduction in its duration, an increase in the yield of the finished product.

3. For the first time, the chemical composition of polyphenolic compounds of ultrasonic extracts of two chemotypes of creeping thyme was studied by HPLC-UV and HPLC MS / MS, in total 15 phenolic compounds were identified and quantified, five of which are phenolic acids, ten are flavonoids. The obtained ultrasonic extracts revealed similarities in the qualitative composition of phenolic compounds, but significant differences were found in the quantitative content of phenolic acids and flavonoids, except for rosmarinic acid. The dominant polyphenolic compounds in the studied extracts are cynaroside, rosmarinic acid, naringenin and epicatechin.

4. For the first time it has been established that ultrasonic extracts of two chemotypes of creeping thyme exhibit bactericidal or bacteriostatic activity against 9 strains of gram-positive bacteria, 6 strains of gram-negative bacteria and 5 cultures of fungi at a concentration of 0.0625 to 20 mg/ml, but differ in the strength of the effect on test strains microorganisms, show the maximum bactericidal activity against *Helicobacter pylori*. Ultrasonic extracts of two chemotypes of creeping thyme are proposed as a substance for the creation of domestic drugs for the treatment and prevention of *Helicobacter pylori* - associated diseases.

5. Ultrasonic extract of creeping thyme, collected in the mountain forest massif of Karkaralinsk, has an expectorant property comparable to the comparison drug "Bronchicum C", ultrasonic extract of creeping thyme, collected in the Korneev forests, is inferior in expectorant action to the comparison drug "Bronchicum C".

6. According to the results of the study of acute toxicity in the experiment in vivo, it was found that ultrasonic extracts of creeping thyme belong to the "Practically non-toxic" group (V class of toxicity). According to the results of the Ames test, it was determined that ultrasonic extracts of creeping thyme do not have mutagenic activity.

7. A draft ND was developed and standardization of the substance of the ultrasonic extract of creeping thyme was carried out, its stability was studied. The

laboratory regulations for obtaining the substance of creeping thyme ultrasonic extract (LR-005491-MK-04-21) have been developed and approved. On the basis of the Scientific Research Center of the NCJSC "MUK", the production of experimental batches of the substance of the ultrasonic extract of creeping thyme for pharmacological research was organized.

Approbation of the results of the dissertation

The main results of the dissertation were presented at: V scientific and practical conference "Modern aspects of the use of plant materials and raw materials of natural origin in medicine" (Moscow, March 15, 2017); international conference of young scientists "The world of science and youth: trends and new horizons" (Karaganda, April 12, 2017); International Scientific and Practical Conference "Modern Biology. Theoretical, applied aspects and interdisciplinary connections" (Karaganda, October 12-13, 2017); V international scientific conference of young scientists and students "Prospects for the development of biology, medicine and pharmacy" (Shymkent, December 8-9, 2017); 18th International Congress on Infectious Diseases (Buenos Aires, March 1-4, 2018); IV (XII) International Botanical Conference of Young Scientists (St. Petersburg, April 22–28, 2018); XXV International scientific and practical conference of young scientists and students "Topical issues of new drugs development" (Kharkiv, April 18-20, 2018); international conference of young scientists and students "The world of science and youth: an era of rapid change" (Karaganda, April 28, 2018); IX All-Russian scientific conference of students and graduate students with international participation "Young pharmacy - the potential of the future" (St. Petersburg, April 22-23, 2019).

Publications. Based on the materials of the dissertation, 1 Eurasian patent, 1 patent of the Republic of Kazakhstan were obtained. The main provisions of the dissertation are reflected in the following publications:

- 3 articles in journals recommended by the Committee for Quality Assurance in the Field of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan;
- 1 article in an international scientific publication included in the Scopus Q3 database;
- abstracts of 9 reports, including abstracts of 7 reports in the materials of international conferences.

Scope and structure of the dissertation

The dissertation is presented on 138 pages of typewritten text, includes 14 figures and 31 tables. The work consists of an introduction, materials and methods, 5 chapters, a conclusion, a list of references and applications. The list of references includes 167 literary sources.