

ANNOTATION

dissertation work on the topic:

"Development of technology of antimicrobial and anti-caries agent with essential oil of oregano (*Origanum vulgare* L.)"

for the degree of Doctor of Philosophy (PhD) in the specialty 6D074800 -
Pharmaceutical production technology

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Relevance of the research topic. Dental caries is the most common chronic infectious disease of the oral cavity. Worldwide, it is estimated that 2 billion people suffer from permanent tooth decay and 520 million children suffer from primary tooth decay. The main ethological component of dental caries are bacterial species (mainly streptococci, lactobacilli and bifidobacteria). 1 ml of human saliva contains approximately 20 to 400 million microorganisms belonging to more than 700 species, including *Streptococcus mutans*, discovered by Klerke in 1924.

Cariogenic bacteria can easily form biofilms on the surface of teeth, rapidly producing lactic acid and therefore causing cavities. Currently, the incidence of caries continues to grow. During the last fifty years there has been no innovation in the field of dental caries prevention. The involvement of the microorganism in both oral and non-oral diseases has generated interest in knowing its susceptibility to antimicrobial agents. However, antibiotics are not suitable for routine use as antiplaque agents and their use in pharmaceuticals should be limited. Current treatments are not sensitive; they are not species specific and kill pathogenic species. Therefore, there is a need to recover and develop new therapeutic strategies that prevent or abolish biofilm formation in more precise ways, selectively targeting cariogenic bacterial biofilms and specifically targeting the prevention and treatment of dental caries in clinical practice.

Phytotherapy is a promising alternative approach. It is known that essential oils and extracts of medicinal plants or phytochemicals inhibit the formation of dental biofilms by reducing the adhesion of microbial pathogens to the surface of the teeth, which is a major event in the initiation and development of caries.

Purpose of study: development of technology of antimicrobial and anti-caries agent with essential oil of oregano (*Origanum vulgare* L.) and its standardization.

Objectives of study:

1 Conduct an analysis of literature data on the state of development in the field of creating antimicrobial, anti-caries and anti-inflammatory drugs and screening studies of samples of essential oils for anti-caries activity and to justify the choice of the object of study;

2 to carry out the collection and pharmacognostic analysis of medicinal plant raw materials of oregano (*Origanum vulgare* L.).

3 to develop a technology for obtaining antimicrobial and anti-caries substances obtained from oregano (*Origanum vulgare* L.) and standardize them;

4 to carry out pharmaceutical development of a gel based on essential oil and ethanolic extract of oregano and study its stability; to study the specific activity and safety of the developed composition of the dosage form

5 to conduct a feasibility study for the production of the gel.

Research methods

Materials and methods used for scientific research comply with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, the Pharmacopoeia of the Eurasian Economic Union, European Pharmacopoeia, The United States Pharmacopoeia, British Pharmacopoeia, regulatory documents regulating the quality of medicines in the Republic of Kazakhstan.

In the process of working on the research topic, both general scientific (analysis, induction, comparison, generalization) and empirical research methods (observation, description, experiment, modeling, measurement) were used.

The studies were carried out on the basis of the School of Pharmacy under the guidance of Doctor of Chemical Sciences, Professor Atazhanova G.A. All the necessary materials, equipment and reagents were available to carry out the experimental part. The procedures described in the literature were used in the experiments.

As part of the implementation of the work, the following research methods were used:

1. Essential oils were isolated by hydrodistillation using the Clevenger apparatus from the air-dry mass of plant materials.
2. Methods for obtaining biologically active compounds under conditions of ultrasonic extraction.
3. Screening of antimicrobial activity by disk diffusion method.
4. Testing the effectiveness of essential oils on the formation of *Streptococcus mutans* biofilms in vitro using colorimetric analysis.
5. Physical and chemical methods for the analysis of organic compounds: chromato-mass spectrometry, gas-liquid chromatography, high-performance liquid chromatography, thin-layer chromatography, UV spectroscopy.
6. Statistical processing of the obtained results was carried out according to the variance-statistical analysis using Student's criterion, in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, European Pharmacopoeia, The United States Pharmacopoeia, British Pharmacopoeia. The electronic programs Microcal Origin and Excel were used for calculations.

Objects of study

Medicinal raw materials of oregano (*Origanum vulgare* L.); essential oil; extracts.

Research subjects

Microwave and ultrasonic extraction; biological activity and preclinical tests of dry extract and essential oil of oregano; gel, soft dosage form; composition and technology of gel production; standardization.

The main provisions for defense:

- isolation of essential oils from plants of Kazakhstan. Search for a promising essential oil with anti-caries and antimicrobial effects;
- experimental studies of obtaining a substance (essential oil and ethanol extract) from oregano (*Origanum vulgare* L.);

- development of the composition of a combined drug of antimicrobial, anti-inflammatory and anti-caries action based on oregano (*Origanum vulgare* L.) in the form of a gel;

- regulatory documents for dental gel in the form of a draft RD and laboratory regulations for receipt.

Description of the main results of the study

- results of a study of the antimicrobial activity of essential oils; results of testing the inhibitory activity of essential oils against the formation of *S. mutans* biofilm;

- results of anatomical and morphological study of oregano;

- results of screening for antimicrobial activity of oregano extracts;

- optimal composition and technology for obtaining antimicrobial and anti-caries gel based on essential oil and ethanol extract of oregano; laboratory regulations for the preparation of the gel, quality specifications and draft normative documents, pilot regulations for soft dosage forms based on the substance from the herb oregano; methods for the qualitative and quantitative determination of the active substance carvacrol in a soft dosage form of a gel based on an extract from the herb oregano ordinary by gas-liquid chromatography;

- rheological, biopharmaceutical, physical and chemical pharmacological parameters of the gel, results of a study on acute toxicity of dental gel.

Rationale for scientific novelty:

- for the first time, a study was made of the anti-caries effect of experimental samples of plant essential oils, where it was found that the essential oil of oregano (*Origanum vulgare* L.) inhibits the formation of a biofilm of *Streptococcus mutans*, i.e. has a pronounced anti-caries effect and is a promising pharmaceutical substance for the development of a new dental drug;

- for the first time, the composition of a new combined drug of antimicrobial and anti-caries action based on oregano (*Origanum vulgare* L.) in the form of a gel was developed;

- for the first time, a technology for obtaining antimicrobial and anti-caries action based on substances obtained from oregano (*Origanum vulgare* L.) was developed;

- for the first time, methods for quality control of the developed medicinal product based on oregano (*Origanum vulgare* L.) were developed; the shelf life and storage conditions of the combined gel were determined.

The scientific novelty of the dissertation research is confirmed by the patent of the Republic of Kazakhstan for the invention No. 35343 dated 11/05/2021 "Antimicrobial dental gel".

The practical significance of the results obtained:

On the basis of pharmaceutical substances (essential oil and dry extract of *Origanum vulgare* L.), a soft dosage form has been developed in the form of a gel with antimicrobial, anti-inflammatory and anti-caries effects.

According to the results of preclinical (non-clinical) tests, the gel does not have toxic properties and is recommended as an antimicrobial, anti-inflammatory and anti-caries agent.

A laboratory regulation has been developed for obtaining dental gel.

The dissertation work was carried out in accordance with the research plan of NAO MUK within the framework of scientific projects AP09562096 "Development of obtaining a new dental gel of antimicrobial and anti-caries action based on oregano" (contract No. 256/12-2 of June 15, 2021) and AP14971364 "Development a new dental product based on *Origanum vulgare* L" (contract No. 303-ZHG-2-22-24 dated October 20, 2022).

The personal contribution of the doctoral candidate to the dissertation work consists in the analysis of literary and patent sources, carrying out experimental and computational work, analysis, interpretation, generalization and discussion of the obtained experimental results.

Conclusions:

1. Biofilm accumulation by *Streptococcus mutans* bacteria on hard dental tissues leads to dental caries, which remains one of the most common oral diseases. Therefore, the development of new antibacterial agents is critical. We have analyzed the component composition of essential oils of 14 species of common Kazakh plants (*Achillea nobilis* L., *Achillea millefolium* L., *Artemisia austriaca* L., *Hyssopus ambiguus* (Trautv.) Iljin., *Matricaria chamomilla* L., *Mentha piperita* L., *Melissa officinalis* L., *Nepeta cataria* L., *Origanum vulgare* L., *Pinus sylvestris* L., *Thymus marshallianus* Willd., *Thymus crebrifolius* Klok., *Thuja orientalis* Endl., *Ziziphora clinopodioides* Lam) and studied their effect on the formation of *Streptococcus mutans* biofilms.

As a result of screening samples of essential oils isolated from available plant sources in Central Kazakhstan for the formation of *Streptococcus mutans* biofilms, for the first time it was revealed that the essential oil of *Origanum vulgare* L. reduces the formation of *S. mutans* biofilm by 98% compared to untreated bacteria.

2. The collection and preparation of herbal raw materials of oregano were carried out in accordance with the Good Practice for the Collection of Medicinal Plants in the env. Shchuchinsk, Burabay district, Akmola region (52°56' N 70°12' E) and in the East Kazakhstan region.

Due to the fact that the operational reserves of oregano are in the range from 4.3 - 22.9 tons, it is recommended to annually collect raw materials in the Kazakh part of Altai, namely in the vicinity of the Ivanovsky (12.9 + 0.9), Naryn (16.5 + 1.9), Listvyaga mountain ranges (22.99+1.7) tons. The total area of oregano thickets in the East Kazakhstan region is 120.8 ha with a stock density of dry raw materials of 25.6 c/ha.

Preparation of raw materials *Origanum vulgare* L. It is recommended to dry the raw materials in the open air without exposure to direct sunlight, placing it on drying frames in layers of 10 - 15 cm at a temperature not exceeding 18 ° C.

The diagnostic features of raw oregano are the shape and arrangement of the leaves on the stem, the branching of the shoots and their color. Tetrahedral, straight, branched in the upper part. The shape and size of the leaf plates, the shape of the edge, apex and base, the color of the lower and upper sides, the degree of pubescence, the location of the glands. The leaves are oblong, opposite, petiolate, entire, pointed at the apex, both sides are painted green.

Pharmaceutical and technological parameters of oregano raw materials for the optimal extraction technology were studied: specific gravity ($1.73 \pm 0.02 \text{ g/cm}^3$), bulk density ($0.35 \pm 0.01 \text{ g/cm}^3$), porosity ($0.37 \pm 0.01 \text{ g/cm}^3$), free volume of the raw material layer ($0.81 \pm 0.01 \text{ g/cm}^3$), absorption coefficient of the extractant (3.45).

Ethyl alcohol (70%) with an optimal yield of 55.69% was chosen for the major yield of the sum of extractives.

3. Extracts were obtained from raw oregano by various methods: infusion, ultrasonic cavitation and microwave extraction.

A new method for obtaining a dry extract of oregano (*Origanum vulgare* L.) has been developed, which, due to the use of ultrasonic extraction, is characterized by high productivity of the technological process, low consumption of the extractant, and the exclusion of labor-intensive and time-consuming procedures, which makes it affordable, rational and economical.

A technological scheme has been developed for obtaining essential oil from the aerial part of oregano. A quality specification for essential oil has been developed: description, identification, heavy metals, microbiological purity, quantitative determination, packaging, labeling, transportation, storage, shelf life, main pharmacological action, carvacrol retention time - 20.3 minutes; quantification - 65%. Data on long-term testing of essential oil based on herbal raw materials of oregano (*Origanum vulgare* L.) were obtained, no significant changes were observed based on the results of determining quality indicators.

4. Pharmaceutical development of a gel based on essential oil and dry extract of oregano (*Origanum vulgare* L.) was carried out. An optimal composition and technology for obtaining a gel has been developed, which includes: a pharmaceutical substance for practical use of plant origin - a dry extract obtained as a result of ultrasonic cavitation - 0.6; essential oil of common oregano - 1.2; Na-CMC - 2.2; glycerin - 2.2; natural sweetener - 0.3; purified water - up to 100. A technology has been developed for obtaining a gel based on pharmaceutical substances from an ordinary soul. A quality specification for a gel-based product has been developed, and a draft ND has been developed. The results of testing the stability of the gel based on essential oil and dry extract under long-term conditions: at a temperature of (25 ± 2)°C, relative humidity (60 ± 5)% showed that no significant changes were observed in the results of determining the quality indicators. Stability studies gel continues.

Methods have been developed for the qualitative and quantitative determination of the active substance carvacrol in a soft dosage form of a gel based on the essential oil of an extract from the oregano herb by ordinary gas-liquid chromatography. At all stages of standardization of the object in the series of raw materials - substance - drug, carvacrol, a standard sample isolated by us from the essential oil of oregano, was used as an external standard to assess its authenticity and quantify the active substance.

Reproducible results of physicochemical, biopharmaceutical, pharmacological parameters of the gel based on essential oil and ethanol extract from oregano herb were obtained.

It was studied that a sample of dental gel at a dose of 25 mg/kg had anti-inflammatory activity, which was expressed in a significant decrease by 41.4%, respectively, in the amount of inflammatory exudate in the abdominal cavity in rats compared to the control. The anti-inflammatory activity of the dental gel with essential oil and ethanolic extract of oregano is comparable to that of the reference drug diclofenac sodium.

For the first time, a study of the anti-caries and antibacterial effects of prototypes of dental gel was carried out. A selection of dental gel samples was made, showing a relatively high anti-caries and antibacterial effect on test strains at minimal concentrations of essential oil and oregano extract.

When determining acute toxicity, the investigated anticaries gel showed low toxicity. For mice and rats, the LD50 was 2000 mg/kg intragastrically. According to the generally accepted classification of substance toxicity, the new gel developed by us can be attributed to the class of low-toxic substances (IV class of toxicity, LD50 > 5000 mg / kg, intragastric administration), that is, to practically non-toxic compounds.

5. A feasibility study was carried out for the production of an antimicrobial and anti-caries agent with the essential oil of oregano (*Origanum vulgare* L.), which shows the feasibility of manufacturing products on an industrial scale.

Approbation of the results of the dissertation. The main provisions of the dissertation work were reported and published in the materials of international and republican conferences: "Scientific community of the XXI century" (Anapa, 2020); "Science and education in the modern world: challenges of the XXI century" (Nur-Sultan, 2020); "Modern trends in the development of health-saving technologies" (Moscow, 2020); "Priorities of pharmacy and dentistry: from theory to practice" (Almaty, 2022); "International environmental problems" (Karaganda, 2023 G.).

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

- 4 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Republic of Kazakhstan;
- 2 articles in foreign scientific publications included in the Scopus database;
- abstracts of 6 reports at international conferences.

The volume and structure of the dissertation.

The dissertation is presented on pages of 164 pages of typewritten text, includes 38 figures and 43 tables; consists of introduction, 8 chapters, conclusion, list of references and appendices. The list of references includes 238 literary sources.