

ANNOTATION

of dissertation work on the topic «**Development of a Parapharmaceutical Technology with Antimicrobial Activity Containing Essential Oil of *Hyssopus ambiguus* (Trautv.) Iljin**», submitted for the degree of Doctor of Philosophy (PhD) in the specialty 8D07201 – «Pharmaceutical Manufacturing Technology».

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Relevance of the research topic. Currently, inflammatory diseases of the ENT organs remain among the most common pathologies, significantly reducing the quality of life and requiring prolonged therapy. The increasing antibiotic resistance of microorganisms and the limited efficacy of existing pharmaceuticals highlight the need for new, safer, and more effective natural-origin drugs. In this context, essential oils from medicinal plants with pronounced antimicrobial, anti-inflammatory, and antioxidant activity attract particular interest.

Plants of the genus *Hyssopus* L. (hyssop) are notable due to their high content of biologically active compounds — cis- and trans-pinocamphene, isopinocampone, β -pinene, 1,8-cineole, elemol, γ -amorphene, limonene, bicyclogermacrene, germacrene D, myrcene, pinocamphone, and sabinene — which exhibit a wide spectrum of pharmacological activity. However, the species *Hyssopus ambiguus* (Trautv.) Iljin, native to Kazakhstan, remains poorly studied and is not used in pharmaceutical practice. To date, the State Register of the Republic of Kazakhstan contains no preparations based on this species, highlighting the scientific and practical novelty of this research.

Considering the high essential oil content in *Hyssopus ambiguus*, a promising pharmaceutical form for realizing its pharmacological potential is a spray for treating upper respiratory tract inflammatory diseases. Studying the antimicrobial properties of *Hyssopus ambiguus* essential oil and its combinations with essential oils from other local medicinal plants (families *Lamiaceae*, *Cupressaceae*, and *Pinaceae*) is of crucial importance for the development of a domestic parapharmaceutical product with multifactorial therapeutic activity.

The purpose of scientific research. Development of the composition, manufacturing technology, and standardization of a parapharmaceutical antimicrobial agent for upper respiratory tract infections containing essential oil of *Hyssopus ambiguus* (Trautv.) Iljin.

Objectives of study:

1. To conduct a pharmacognostic study of *Hyssopus ambiguus* (Trautv.) Iljin, isolate its essential oil, and determine its component profile using GC–MS;
2. To develop the composition and manufacturing technology of an essential oil composition (EOC) containing *Hyssopus ambiguus* (Trautv.) Iljin and to study its component profile;
3. To develop and experimentally justify the technology for producing a parapharmaceutical antimicrobial throat spray based on the EOC;

4. To evaluate the acute toxicity of the developed EOC in animal models. To screen the antimicrobial activity of *Hyssopus ambiguus* (Trautv.) Iljin essential oil, the EOC, and the developed parapharmaceutical spray;

5. To standardize the essential oil, EOC, and parapharmaceutical spray, assess the stability of the final product, and develop regulatory documentation and quality specifications;

6. To conduct a techno-economic assessment of the industrial feasibility of producing a parapharmaceutical antimicrobial spray based on *Hyssopus ambiguus* (Trautv.) Iljin essential oil.

Research Methods. Morphological and anatomical study of *Hyssopus ambiguus* (Trautv.) Iljin herb was conducted in accordance with generally accepted methods of the State Pharmacopoeia of the Republic of Kazakhstan, 1st edition.

The study of macroscopic characteristics of *Hyssopus ambiguus* (Trautv.) Iljin plant material was performed according to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan using a Levenhuk stereoscopic microscope.

Morphometric and microscopic characteristics, as well as histochemical analysis of the plant material, were carried out in accordance with the methods of the State Pharmacopoeia of the Republic of Kazakhstan using a Biomed-4 microscope and an ultraviolet lamp.

Gas chromatography with mass spectrometric detection (GC–MS) was applied for the isolation and identification of biologically active components.

Non-clinical studies using in vivo and in vitro methods were performed to assess pharmacological activity and acute toxicity.

Statistical processing of experimental data was carried out using the STATISTICA 12.6 software package.

Objects of study: Aerial parts of *Hyssopus ambiguus* (Trautv.) Iljin; essential oil of *Hyssopus ambiguus* (Trautv.) Iljin; essential oil compositions including *Hyssopus ambiguus* essential oil; parapharmaceutical throat spray based on EOC.

Subject of the study: essential oil of *Hyssopus ambiguus* (Trautv.) Iljin, essential oil-based compositions, and a parapharmaceutical spray for the prevention of inflammatory diseases of the upper respiratory tract.

The main provisions for defense:

1. Pharmacognostic characteristics of the herb *Hyssopus ambiguus* (Trautv.) Iljin, including morphological, anatomical, microscopic, and diagnostic features, confirming its identification traits and suitability as a medicinal plant raw material.
2. The composition and technology for obtaining an essential oil-based formulation containing *Hyssopus ambiguus* (Trautv.) Iljin essential oil have been developed; its component profile was established using gas chromatography–mass spectrometry (GC–MS), ensuring reproducibility of quality.
3. The antimicrobial activity and safety of *Hyssopus ambiguus* (Trautv.) Iljin essential oil and its essential oil-based composition have been

experimentally confirmed against a range of clinically significant microbial strains.

4. The composition of a parapharmaceutical throat spray containing the essential oil-based formulation of *Hyssopus ambiguus* (Trautv.) Iljin has been scientifically substantiated and developed, with rational technological parameters and quality indicators of the final product determined.

Scientific novelty of the work.

For the first time:

- A pharmacognostic analysis of *Hyssopus ambiguus* (Trautv.) Iljin plant material, growing in the Karaganda region, was carried out, and its diagnostic macro- and microscopic features, as well as quality indicators, were determined.
- The component composition of *Hyssopus ambiguus* (Trautv.) Iljin essential oil was established using GC–MS, and biologically active components responsible for antimicrobial activity were identified.
- The antimicrobial activity of *Hyssopus ambiguus* (Trautv.) Iljin essential oil and compositions based on it was experimentally substantiated.
- Based on a set of physicochemical, technological, and biological studies, the optimal parameters for obtaining essential oil compositions and a spray dosage form were scientifically justified.
- The relationship between the component composition of the essential oil composition and its pharmacological activity was established.

Practical significance of the obtained results

The results obtained in this work can be applied in pharmaceutical and parapharmaceutical practice in the development and standardization of products based on essential oils.

A draft of regulatory documentation and a quality specification for *Hyssopus ambiguus* (Trautv.) Iljin plant material and a finished parapharmaceutical product in the form of a throat spray were developed.

A parapharmaceutical spray based on an essential oil composition containing *Hyssopus ambiguus* (Trautv.) Iljin essential oil was developed, including the optimal formulation, production technology, and laboratory protocol.

Standardization and stability studies of the finished product were conducted. Acute toxicity of the essential oil composition was determined, and its safety was confirmed (toxicity class V).

Two certificates of entry in the State Register of Rights for objects protected by copyright were obtained: No. 40479 “Essential Oil Composition (essential oils of *Thymus marschallianus*, *Hyssopus ambiguus*, *Juniperus sabina*, and peppermint)” and No. 40482 “Throat Spray Based on Essential Oil Composition,” dated 15.11.2023.

Marketing analysis of the range of sprays for the treatment of inflammatory throat diseases sold in the Republic of Kazakhstan showed a low share of domestic products (13.79%), confirming the relevance of developing and implementing domestic parapharmaceutical products.

Personal contribution of a doctoral student

The candidate independently analyzed domestic and international scientific literature, formulated research aims and objectives, conducted pharmacognostic, physico-chemical, microbiological, and technological experiments, processed and interpreted results, and prepared scientific publications and conference abstracts.

Conclusions:

1. Pharmacognostic Study: Collection, preparation, and storage of *Hyssopus ambiguus* (Trautv.) Iljin plant material were performed according to GACP requirements. Macro- and microscopic analysis revealed biologically active compounds: phenolic compounds, polysaccharides, essential oil, terpenoids, flavonoids, tannins, with localization determined. Quality indicators and raw material specification were developed. Optimal storage conditions were established (25 ± 2 °C, $60 \pm 5\%$ relative humidity, shelf life 24 months) and pharmaco-technological parameters for effective extraction of bioactive compounds were defined.
2. Development of Essential Oil Composition (EOC): EOCs containing *Hyssopus ambiguus* essential oil were prepared. GC–MS analysis identified over 40 compounds, mainly 1,8-cineole, p-cymene, o-cymene, γ -terpinene, and I-menthone. Manufacturing scheme and quality specification were developed. Long-term tests confirmed stability and suitability for further use.
3. Parapharmaceutical Spray Technology Development: A parapharmaceutical spray based on the EOC was developed. Optimal composition: active ingredient – 0.25 g EOC; excipients – 5.25 g sea buckthorn oil and 4.5 g olive oil. Manufacturing scheme and quality specification were established. Stability tests at 25 ± 2 °C and $60 \pm 5\%$ RH confirmed no significant changes in quality indicators.
4. Acute Toxicity Assessment: Tests on model animals confirmed the safety of EOC and spray. According to Hodge, Sterner, and K.K. Sidorov classification, they are practically non-toxic (class 5, $LD_{50} > 5000$ mg/kg), allowing further clinical research and practical application. Antimicrobial Activity Screening: In vitro screening showed pronounced activity against museum and clinically relevant strains: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231, *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* K3 54678 & K3 77729, and *Pseudomonas aeruginosa* K3 47303.
5. Standardization and Quality Control: Methods for standardization of EOC and spray were developed; quality and stability indicators were determined. Validation confirmed analytical and technological methods'

accuracy, reproducibility, and suitability for quality control at all development stages.

6. Techno-Economic Assessment: Industrial production of EOC and parapharmaceutical spray was evaluated. Feasibility for industrial implementation, production costs, and potential profitability were established, confirming the practical significance of the work.

Approbation of the results of the dissertation

The main results and findings of the dissertation were reported and discussed at the following conferences:

- XV International Scientific and Practical Conference “Actual Problems of Ecology” (Karaganda, January 20–21, 2023);
- Student Scientific and Practical Conference with international participation within the framework of the VI Central Asian International Conference on Medical Education “Education of the Future: Winds of Change” (Karaganda, April 13–14, 2023);
- Republican Scientific and Practical Conference of Students, Master’s, PhD Students, and Young Scientists (with international participation) (Karaganda, February 27–28, 2025);
- International Biomedicine Forum: Research and Innovation (Karaganda, April 17–18, 2025).

Publications.

Based on the results of the research, 10 scientific works have been published, including:

3 articles in international peer-reviewed scientific journals indexed in the Scopus database;

1 article in a journal recommended by the Committee for Quality Assurance in the Field of Science and Higher Education of the Ministry of Education and Science of the Republic of Kazakhstan;

4 abstracts in the proceedings of international scientific and practical conferences (Kazakhstan);

2 certificates of entry in the State Register of Rights for objects protected by copyright.

Scope and structure of the dissertation

The dissertation is presented on 204 pages of typewritten text, includes 53 figures and 57 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 192 literary sources.