ANNOTATION

of the dissertation work on the topic

"Pharmaceutical substantiation for obtaining medicinal products from the plant raw material of Asian Mint (*Mentha asiatica* Boriss.)"

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

8D07201 – "Pharmaceutical Production Technology"

Gazizova Aida Arkhatkyzy

Relevance of the Research Topic

The issues of protecting public health, which is a key indicator of the socio-economic well-being of the Republic of Kazakhstan, are currently among the most pressing. In this regard, one of the important directions identified by the Resolution of the Government of the Republic of Kazakhstan No. 454 dated June 12, 2024, "On Approval of the Concept for the Development of Healthcare Infrastructure for 2024-2030," is the comprehensive development of the domestic pharmaceutical industry. As a "first step" in this direction, special importance is attached to conducting scientific and practical research aimed at addressing urgent problems arising in the healthcare sector, as well as studying medicinal products required by the market for the prevention and treatment of diseases. In order to achieve these strategic goals and objectives, it is necessary to carry out large-scale studies on the efficient use of local natural raw materials.

These priorities are also aligned with the priorities of the National Development Plan through 2029, as defined by Decree of the President of the Republic of Kazakhstan No. 611 dated July 30, 2024. This Decree sets tasks for the development of domestic pharmaceutical manufacturing, expansion of local production of medicinal products, strengthening cooperation with global pharmaceutical enterprises, attracting investment, and implementing the transfer of new technologies and scientific developments in order to enhance the competitiveness of the pharmaceutical industry. At the same time, special attention is given to the localization of medicinal product manufacturing and to increasing the share of domestically produced goods in the internal market.

One of the most important mechanisms for implementing these strategic objectives is the development of scientific and practical research aimed at solving urgent problems arising in the healthcare sector.

One of the main directions of pharmaceutical industry development is the creation of medicinal products that are natural in origin, relatively safe, beneficial to human health, and affordable in cost.

Thus, at present, in order to systematically reduce the Republic of Kazakhstan's import dependence on foreign medicinal products, the effective use of domestic plant raw materials has become especially relevant.

In the process of creating new pharmacologically active compounds, natural resources of plant origin serve as an important source of medicinal products. At the same time, it should be noted that defining the criteria for selecting material for scientific research is of great importance.

In recent years, the global scientific community has conducted studies aimed at evaluating the broad use of plants in dentistry, which has made it possible to identify plant species with potential biological activity. One such plant is Asian mint (*Mentha asiatica* Boriss.), a representative of the *Lamiaceae* family. Its composition contains significant concentrations of biologically active substances exhibiting antimicrobial and anti-inflammatory pharmacological effects. In particular, menthone and piperitone, limonene, eucalyptol, phenolic acids, and flavonoids are among the main bioactive components of this medicinal plant.

However, although the botanical, phytochemical, and certain pharmacological properties of Asian mint (*Mentha asiatica* Boriss.) have been described in the literature, the pharmaceutical substantiation for its use in the development of a dosage form intended for dental application has not yet been fully established. Specifically, the indicators for raw material standardization have not been systematized, the optimal extraction method ensuring the preservation of biologically active compounds has not been sufficiently substantiated, the technological parameters for incorporating the extract into a dental film have not been determined, and data on the quality, stability, and preclinical safety of the finished product remain limited. In this regard, the development of the composition and technology of a dosage form containing Asian mint (*Mentha asiatica* Boriss.) extract intended for dental use is considered relevant.

The aim of the dissertation research is to develop the composition and technology of a dental film with Asian mint (*Mentha asiatica* Boriss.) extract, evaluate its quality, and study its pharmacological activity.

Research objectives:

1. To conduct a pharmacognostic analysis of the plant raw material of Asian mint (*Mentha asiatica* Boriss.) and its standardization;
2. To develop a technology for obtaining extracts from the raw material of Asian mint (*Mentha asiatica* Boriss.) and carry out their quality control;
3. To develop the composition and technology of a dental film with Asian mint (*Mentha asiatica* Boriss.) extract;
4. To compile a quality specification for the dental film and study its stability;
5. To conduct preclinical studies of the extract of Asian mint (*Mentha asiatica* Boriss.) and the medicinal product;
6. To develop a feasibility study for the production of the dental film.

Research methods: pharmacopoeial (physical and physicochemical, pharmaco-technological, biological) and statistical methods.

Object of the research: raw material of Asian mint (*Mentha asiatica* Boriss.), its extracts, and the dental film.

Subject of the research: the composition, technology, quality indicators, stability, and pharmacological activity of the dental film obtained on the basis of Asian mint (*Mentha asiatica* Boriss.) extract.

Main provisions of the dissertation research submitted for defense:

- results of the pharmacognostic study of the medicinal plant raw material of Asian mint (*Mentha asiatica* Boriss.);

- results of studies on obtaining extracts from the medicinal plant raw material of Asian mint (*Mentha asiatica* Boriss.);
- results of the pharmaceutical development of a dental film with liquid extract of Asian mint (*Mentha asiatica*Boriss.);
- results of determining the safety and biological activity of the liquid extract of Asian mint (*Mentha asiatica* Boriss.) and the dental film prepared on its basis.

Description of the Main Research Results

In the course of the dissertation research, a comprehensive pharmacognostic study of the plant raw material of Asian mint (*Mentha asiatica* Boriss.) was carried out, as a result of which its morphological, anatomical, and pharmaco-technological indicators were determined. The quality of the raw material was assessed in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan, and its properties favorable for extraction were scientifically substantiated. The phytochemical study established that Asian mint contains pharmacologically significant terpene compounds, especially piperitone oxide.

During the research, several methods for obtaining extract from Asian mint raw material were comparatively studied, among which the method of ethanol circulation extraction using a Soxhlet apparatus was recognized as the most effective. The liquid extract obtained by this method was standardized, and its antimicrobial, anti-inflammatory, and antioxidant activity was experimentally confirmed. Based on the obtained results, a technology for obtaining the extract was developed and protected by a utility model patent.

Based on the standardized liquid extract, the optimal composition and technology of a two-layer dental film in a collagen-gelatin-glycerin matrix were developed. The quality indicators of the finished dosage form were studied, including appearance, homogeneity, pH, swelling and dissolution time, as well as loss on drying; their compliance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan and the EAEU Pharmacopoeia was established. Using GC/MS analysis, the main terpene compounds included in the film were identified.

Preclinical studies showed the safety and pharmacological activity of Asian mint extract and the dental film developed on its basis. The anti-inflammatory activity of the extract, as well as the wound-healing and anti-inflammatory effect of the dental film, were confirmed in *in vitro* and *in vivo* experiments. In addition, the feasibility study of a pilot-production batch of 10,000 units demonstrated the expediency of introducing the developed dental film into industrial production.

Justification of Scientific Novelty:

For the first time:

As a result of the conducted studies, results of both a fundamental and applied nature were obtained and scientifically substantiated for the first time, making it possible to solve important scientific and practical tasks in the field of pharmacy and phytochemistry:

The identification of the plant raw material of Asian mint (*Mentha asiatica* Boriss.), collected in the Sairam-Ugam region of Turkestan Region, was

carried out at the state institution of the Republic of Kazakhstan “Institute of Botany and Phytointroduction” and confirmed by certificate registration number No. 01-05/341. The quality of the plant raw material was assessed in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan (SP RK);

Liquid extracts from the plant raw material of Asian mint (*Mentha asiatica* Boriss.) were obtained using various extraction methods: circulation extraction, ultrasonic extraction, and vortex extraction, followed by a comparative study of their phytochemical composition. Based on the study results, the circulation extraction method was identified as the optimal extraction method, and the corresponding extract production technology was developed. The results were confirmed by utility model patent of the Republic of Kazakhstan No. 9819 “Method for obtaining liquid extract of *Mentha asiatica* Boriss. from Asian mint herb” (application No. 2024/0844.2 dated 12.07.2024; published on 22.11.2024) (Appendix A). The obtained extract was assessed for quality, and its pharmacological activity was also studied under *in vitro* and *in vivo* conditions;

The composition and technology of a dental coating using Asian mint (*Mentha asiatica* Boriss.) extract were developed, and quality indicators were determined in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan and the EAEU Pharmacopoeia.

Practical Significance of the Obtained Results

The practical significance of the scientific work lies in the fact that the obtained results are relevant for specialists in the field of medicine and pharmacy and may serve as a scientific and methodological basis for the development of extracts, compositions, and technologies of medicinal products based on Asian mint (*Mentha asiatica* Boriss.), as well as for analytical and design activities.

The developed composition and technology of the dental film from Asian mint (*Mentha asiatica* Boriss.) were implemented at the production base of SPC “AntiGen” LLP (Almaty, Republic of Kazakhstan) (Appendix Ә), as well as at Gdańsk Medical University (Gdańsk, Poland), which is confirmed by an implementation act (Appendix Б).

The analysis of medicinal plant raw material, as well as the determination of the quality indicators of the medicinal product and its biological activity *in vitro*, were carried out at the Department of Pharmacognosy and Botany of the Faculty of Pharmacy, Comenius University (Bratislava, Slovakia). The results were formalized by an implementation act (Appendix B).

Personal Contribution of the Doctoral Candidate

The doctoral candidate conducted an extensive review and analysis of domestic and foreign scientific literature and also independently performed experimental studies in accordance with the set objectives. This is confirmed by the obtained results achieved under laboratory and production conditions using modern equipment and scientific sources.

The reliability and validity of the obtained results are confirmed by their focus on solving urgent problems of pharmaceutical science and practice, the development of a

new domestic medicinal product, the conduct of research in modern scientific centers, as well as the development of draft regulatory documentation.

Conclusions

The presented dissertation work is aimed at conducting a pharmacognostic study of the plant raw material of Asian mint (*Mentha asiatica* Boriss.), optimizing the extraction technology, determining the chemical composition and safety of the obtained extract, as well as developing a dental film based on the optimal extract and providing a feasibility study for its production.

Based on the results of the studies carried out, the following conclusions were formulated:

1. The plant raw material of Asian mint (*Mentha asiatica* Boriss.) was harvested in June–August 2023 in Turkestan Region, in the Sairam-Ugam State National Nature Park, Kazygurt branch (41°57′N, 70°03′E), in accordance with the requirements of WHO GACP (2003) and EEC No. 15 dated 26.01.2018, after which a technology for its processing was developed. Macro- and microscopic studies made it possible to establish the main diagnostic characteristics of the plant raw material: the transverse section of the stem is quadrangular, the epidermis is single-layered, the leaf is hypostomatic, the stomata are of the anomocytic type, and essential oil glands and multicellular trichomes are clearly expressed. The calyx length was 4–6 mm, the width was 2–2.5 mm, while the corolla was 2–2.3 times longer than the calyx. Histochemical analysis showed the predominant localization of essential oils, flavonoids, and phenolic compounds, whereas sesquiterpene lactones, starch, and alkaloids were not detected.

According to pharmaco-technological indicators, the limiting mass was 0.14 g/cm³, the bulk density was 0.17 g/cm³, the loose bulk density was 0.08 g/cm³, the porosity was 2.21, the internal porosity was 1.012, and the free volume was 1.6, which characterizes the loose structure of the raw material favorable for extraction. The highest yield of extractive substances was obtained using 90% ethyl alcohol and ranged from 3.01±0.01% to 3.5±0.02%, averaging about 3.2%. GC/MS analysis showed the predominance of terpenes in the leaves, including piperitone oxide (9.51%), which confirmed that the raw material of Asian mint (*Mentha asiatica* Boriss.) is rich in pharmacologically valuable components.

2. Extracts from the aerial parts of the plant were obtained by four methods: hydrodistillation, ultrasonic extraction, circulation extraction in a Soxhlet apparatus, and vortex extraction.

According to GC/MS results, the ethanol circulation extract obtained using the Soxhlet apparatus was recognized as the most optimal, with the following technological parameters: 90% ethyl alcohol, extraction duration 300 minutes, total of 5 cycles. The technology for obtaining the extract is confirmed by utility model patent of the Republic of Kazakhstan No. 9819 “Method for obtaining liquid extract from the herb of Asian mint *Mentha asiatica* Boriss.” (application No. 2024/0844.2; 12.07.2024; published on 22.11.2024) (Appendix A).

The content of piperitenone oxide in its composition was 72.44%, which determined pronounced antimicrobial, anti-inflammatory, and antioxidant activity. In addition, such sesquiterpenes as trans-caryophyllene, γ-muurolene, and α-guaiene provided a synergistic effect. The obtained extract was standardized, and it was established that its stability period is 24 months.

3. Based on the liquid extract of Asian mint (*Mentha asiatica* Boriss.), a two-layer dental film incorporated into a collagen-gelatin-glycerin matrix was developed. During the study, a total of 5 samples were prepared. As a result, sample No. 3 was recognized as the most effective composition. The composition of the optimal sample No. 3 included: in the 1st layer – Asian mint (*Mentha asiatica* Boriss.) extract 5 mL, collagen 10 mL, gelatin 10 g, glycerin 2 mL, potassium sorbate 0.5 g, and purified water 24.5 mL; in the 2nd layer – lidocaine hydrochloride 1 mL, collagen 10 mL, gelatin 10 g, glycerin 2 mL, potassium sorbate 0.5 g, and purified water 24.5 mL. The main advantage of the proposed system is characterized by the combination of rapid and prolonged action: lidocaine hydrochloride provides rapid analgesia, while Asian mint (*Mentha asiatica* Boriss.) extract maintains anti-inflammatory and antimicrobial effects for a long time.

According to the results of the comparative analysis, sample No. 3 showed optimal results in terms of homogeneity, adhesion, strength, and elasticity. In this regard, this sample was selected as the most suitable composition of the dental film. The technology for producing the film was developed step by step and included the stages of preparing the solutions of the main and second layers, incorporating auxiliary and active substances, layer formation, drying, cutting, packaging, and labeling. The finished product was dried at a temperature of 25–30°C and relative humidity of 30–40%, after which it was cut into fragments measuring 5×4 mm. The developed technology ensured uniform distribution of the extract in the polymer base and preservation of its biological activity. As a result, the high structural stability and pharmacological efficacy of the dental film were substantiated.

4.The obtained two-layer dental film had a homogeneous, smooth structure. Due to the natural chlorophyll compounds in the extract of Asian mint (*Mentha asiatica* Boriss.), the upper layer had a light green color, whereas the lower layer had a yellowish-transparent shade. The film had a mild menthol odor characteristic of Asian mint. During the study, it was established that the geometric dimensions of the film were 50×40×0.1 mm, the pH value was 6.5±0.2, and mass uniformity was 7.1%. The swelling time was 3 minutes, the complete dissolution time was 11 minutes, and the loss on drying was 10%. During qualitative identification, the formation of a yellow-orange precipitate in the reaction with 2,4-dinitrophenylhydrazine confirmed the presence of piperitone oxide in the film.

According to GC/MS analysis, 40 compounds were identified in the composition of the film. The main components were determined to be 2-hexenol (19.61%), dl-limonene (18.86%), menthofuran (6.94%), cyclohexene (5.64%), piperitone oxide (4.642%), eucalyptol (3.783%), dihydrocarvone (3.217%), and terpinen-4-ol (2.893%). The total content of terpene compounds was 57.07%, and their amount in 100 g of the

finished dental film was calculated as 2.85 g. During the stability study over 24 months, the appearance, color, odor, dimensions, pH level, mass uniformity, loss on drying, and microbiological purity of the film remained unchanged; mass uniformity varied within 7.1–7.3%, and loss on drying ranged within 10–10.5%. These results proved that the developed dental film is a dosage form with high structural stability, preserved terpene composition, and stable quality indicators during long-term storage.

The obtained results showed that the quality indicators of the two-layer dental film comply with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan and the EAEU Pharmacopoeia.

5. The acute and chronic toxicity of Asian mint (*Mentha asiatica* Boriss.) extract was studied in the vivarium of Asfendiyarov KazNMU NJSC and the B. Atchabarov Research Institute of Fundamental and Applied Medicine on the basis of bioethics committee protocol No. 8 (144) dated 07.11.2023. At a dose of 500 mg/kg, signs of mild edema and parenchymal dystrophy were detected in the kidney and stomach tissues; at a dose of 2000 mg/kg, toxic changes in the liver, kidney, and stomach tissues intensified; at a dose of 5000 mg/kg, pronounced necrotic lesions were recorded. The average number of necrotic foci in the liver per 10 fields of view was: in the control – 0.1 ± 0.3, at a dose of 500 mg/kg – 2.3 ± 1.1 ($p<0.05$), at a dose of 2000 mg/kg – 5.7 ± 1.4 ($p<0.01$), and at a dose of 5000 mg/kg – 12.4 ± 2.1 ($p<0.001$). This indicated the dose-dependent nature of the extract toxicity.

In vitro, the anti-inflammatory activity of the circulation liquid extract was evaluated on RAW264.7 macrophages: at a non-cytotoxic dose of 31.25 μg/mL, it inhibited LPS-induced NO production by 31.8%. According to the MTT test results, the circulation extract demonstrated dose-dependent cytotoxicity against A549, Caco-2, LNCaP, and MDA-MB231 cells, whereas its toxicity against normal HUVEC cells was relatively low, which confirmed a certain selectivity.

The wound-healing effect of the dental film with Asian mint (*Mentha asiatica* Boriss.) extract was studied in rats, with the dental film “Curatick” (manufacturer: South Korea) chosen as the reference product. This product was used as a control agent in evaluating the wound-healing effect. As a result, in animals treated with the studied film, the weight load required to rupture the scar was 1.5–2 times higher compared to the control group, while wound depth decreased by 1/3–1/2, and the process of complete epithelialization improved.

When studying the anti-inflammatory effect of the film in the formalin-induced edema model, the dental film showed 47.1% inhibition of edema after 4 hours, which was close to the effect of the standard anti-inflammatory drug indomethacin – 52.1%.

6. As a result of a preliminary technical and economic assessment conducted for an experimental-industrial batch of 10,000 units of dental coating, the direct production costs amounted to 18,932,700 tenge (main and auxiliary raw materials – 17,765,000 tenge; packaging and wrapping materials – 967,700 tenge; other production costs – 200,000 tenge). Under a conservative scenario, with administrative expenses calculated at 40% and commercial expenses at 30%, the total production cost reached 32,185,590 tenge. With a planned profit margin of 30%, the projected release price of

the batch amounted to 41,841,267 tenge, while the projected release price per unit was 4,184.13 tenge. Thus, the obtained results demonstrated the preliminary economic feasibility of the developed technology at the experimental-industrial level; however, additional market and financial analysis is required to confirm its full commercial efficiency.

Summing up, it should be noted that as a result of the conducted comprehensive scientific research, the plant raw material of Asian mint (*Mentha asiatica* Boriss.) was comprehensively studied and scientifically standardized, and, based on the comparison of various extraction methods, ethanol circulation extraction performed in a Soxhlet apparatus was identified as the most optimal option. On the basis of the obtained standardized extract, a two-layer dental film scientifically substantiated in terms of composition and technology was developed, and its quality, stability, safety, and pharmacological efficacy were determined, which demonstrated the high prospects for introducing a domestic phytosubstance and dental film based on Asian mint (*Mentha asiatica* Boriss.) into pharmaceutical practice.

Approbation of the Dissertation Results

The main results of the dissertation work were presented and discussed at the following scientific events:

- Journal "Bulletin of the International Medical University of Central Asia," Jalal-Abad, Kyrgyz Republic, 2023;
- International Scientific Conference of the S.Yu. Yunusov Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan, 2023;
- V (2023) and VI (2024) International Scientific and Practical Conferences "Formation and Prospects for the Development of the Scientific School of Pharmacy: Continuity of Generations," dedicated to the memory of Professor R. Dilbarkhanov, with a report on the results of the implementation of the scientific project of the Ministry of Science and Higher Education of the Republic of Kazakhstan and the Science Committee for 2023–2025 "IRN AP19680441 – Pharmaceutical Development and Pharmacological Studies of Dental Coatings for Use in Dental Practice," Almaty, Republic of Kazakhstan;
- XIII International Scientific and Practical Conference "Priorities of Pharmacy and Dentistry: From Theory to Practice," dedicated to the 135th anniversary of S.Zh. Asfendiyarov, 2024, Almaty, Republic of Kazakhstan;
- Participation in the "Young Scientists" competition held within the framework of the International Forum "ANaMed Forum – New Generation 2025," as well as in the VII International Scientific and Practical Conference "Formation and Prospects for the Development of the Scientific School of Pharmacy: Continuity of Generations," dedicated to the 80th anniversary of Professor R. Dilbarkhanov, June 4–5, 2025 (2nd degree diploma), Almaty, Republic of Kazakhstan;
- International Scientific Conference "Contemporary Pharmacy: Issues, Challenges and Expectations," organized by the Faculty of Pharmacy of the Lithuanian University of Health Sciences, 2025, Kaunas, Lithuania.

Publications

The results of the dissertation research were published in 14 scientific works, including: 2 articles in international peer-reviewed journals indexed in the Scopus database; 3 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan; 5 articles and abstracts in the proceedings of international scientific and practical conferences; 1 utility model patent; 3 implementation acts.

Volume and Structure of the Dissertation

The dissertation, which presents the results of the scientific research, consists of 188 pages, including 28 tables, 56 figures, a list of references containing 114 sources, and appendices. The dissertation includes an introduction, a literature review, sections devoted to research materials and methods, sections presenting the research results, as well as a conclusion.