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 Study

Issues related to 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 priorities set forth in 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, particular importance is attached to conducting scientific and practical research aimed at addressing current problems arising in the healthcare sector, as well as at studying medicinal products needed by the market for the prevention and treatment of diseases. To achieve these strategic goals and objectives, it is necessary to carry out large-scale research on the effective use of local natural raw materials.

These priorities are also consistent with the priorities of the National Development Plan through 2029, as defined by the Decree of the President of the Republic of Kazakhstan No. 611 dated July 30, 2024. This Decree sets objectives for the development of domestic pharmaceutical manufacturing, the expansion of local production of medicinal products, the strengthening of cooperation with global pharmaceutical enterprises, the attraction of investment, and the transfer of new technologies and scientific developments to enhance the competitiveness of the pharmaceutical industry. In addition, particular attention is paid to the localization of medicinal product manufacturing and increasing the share of domestic products 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 in the development of the pharmaceutical industry is the creation of medicinal products that are structurally close to natural compounds, relatively safe, beneficial to human health, and affordable.

Thus, the effective use of domestic plant raw materials is currently of particular relevance in order to systematically reduce the Republic of Kazakhstan’s import dependence on foreign medicinal products.

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

In recent years, the global scientific community has been conducting studies to evaluate 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 an array of biologically active substances with antimicrobial and anti-inflammatory effects. In particular, the main biologically active components of this medicinal plant include menthone and piperitone, present in high concentrations, as well as limonene, eucalyptol, phenolic acids, and flavonoids.

In this regard, the development of the composition and technology of medicinal products derived from Asian mint (*Mentha asiatica* Boriss.) is of current relevance.

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

Research objectives:

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

Study design: The research was conducted in accordance with the methodology of an experimental study.

Research methods: Pharmacopoeial methods (physical and physicochemical, pharmaceutical-technological, and biological) as well as statistical methods were applied.

Object of the study: The plant 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 a dental film developed on the basis of Asian mint (*Mentha asiatica* Boriss.) extract.

Main provisions of the dissertation 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 containing the liquid extract of Asian mint (*Mentha asiatica* Boriss.);

- results of the evaluation of safety and biological activity of the liquid extract of Asian mint (*Mentha asiatica* Boriss.) and the dental film developed on its basis.

Scientific novelty:

For the first time:

As a result of the conducted studies, fundamental and applied scientific results were obtained and scientifically substantiated for the first time, enabling the solution of important scientific and practical problems in the fields of pharmacy and phytochemistry:

The identification of the plant raw material of Asian mint (Mentha asiatica Boriss.), collected in the Sairam-Ugam region of the Turkestan region, was carried out at the State Institution of the Republic of Kazakhstan "Institute of Botany and Phytointroduction" and confirmed by certificate No. 01-05/341. The quality indicators of the plant raw material were evaluated 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 several extraction methods, including circulation extraction, ultrasonic extraction, and vortex extraction, followed by a comparative study of their phytochemical composition. Based on the results obtained, the circulation extraction method was determined to be the optimal extraction technique, and the corresponding extraction technology was developed. The results were confirmed by the Patent of the Republic of Kazakhstan for a utility model No. 9819 entitled "Method for obtaining a liquid extract of Mentha asiatica Boriss. from Asian mint herb" (application No. 2024/0844.2 dated July 12, 2024; published November 22, 2024) (Appendix A). The obtained extract was evaluated for quality, and its pharmacological activity was studied under in vitro and in vivo conditions;

The composition and technology of a dental coating containing 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 Pharmacopoeia of the Eurasian Economic Union.

Practical significance of the study

The practical significance of the research lies in the fact that the obtained results are relevant for specialists in the fields of medicine and pharmacy and may serve as a scientific and methodological basis for the development of extracts, composition, and manufacturing technology 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 medicinal product derived from Asian mint (*Mentha asiatica* Boriss.) have been implemented at the production facilities of “AntiGen” Research and Production Center LLP (Almaty, Republic of Kazakhstan) (Appendix A2), as well as at the Medical University of Gdańsk (Gdańsk, Poland), which is confirmed by an implementation act (Appendix B).

The analysis of the medicinal plant raw material, as well as the determination of quality indicators of the dental films and its biological activity *in vitro*, were conducted at the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius

University (Bratislava, Slovakia). The results are documented by an implementation act (Appendix C).

Personal contribution of the doctoral candidate

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

The reliability and validity of the results are substantiated by their focus on solving current problems in pharmaceutical science and practice, the development of a new domestic medicinal product, the conduct of research in modern scientific centers, and the preparation of draft regulatory documentation.

Conclusion

The presented dissertation research 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 conducted research, the following conclusions were formulated:

1. The plant raw material of Asian mint (*Mentha asiatica* Boriss.) was collected in June–August 2023 in the Turkestan Region, at the Kazygurt branch of the Sairam-Ugam State National Nature Park (41°57′N, 70°03′E), in accordance with WHO GACP requirements (2003) and EEC Decision No. 15 dated January 26, 2018, after which a processing technology was developed. Macro- and microscopic studies made it possible to establish the main diagnostic features of the plant raw material: the stem cross-section 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 visible. The length of the calyx was 4–6 mm, the width was 2–2.5 mm, and 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 the pharmaco-technological indicators, the limiting density was 0.14 g/cm³, the bulk density was 0.17 g/cm³, the tapped density was 0.08 g/cm³, porosity was 2.21, internal porosity was 1.012, and free volume was 1.6, which characterizes the loose structure of the raw material as favorable for extraction. The highest yield of extractive substances was obtained using 90% ethanol 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 were obtained from the aerial parts of the plant by four methods: hydrodistillation, ultrasound-assisted extraction, circulation extraction using a Soxhlet apparatus, and vortex extraction.

According to the GC-MS results, the ethanol circulation extract obtained using the Soxhlet apparatus was recognized as the most optimal, with the following technological parameters: 90% ethanol, extraction duration of 300 minutes, and a total of 5 cycles. The extraction technology was confirmed by the Patent of the Republic of Kazakhstan for utility model No. 9819, "Method for obtaining a liquid extract from the herb of Asian mint *Mentha asiatica* Boriss." (application No. 2024/0844.2; July 12, 2024; published on November 22, 2024) (Appendix A).

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

3. A two-layer dental film incorporated into a collagen-gelatin-glycerin matrix was developed on the basis of liquid extract of Asian mint (*Mentha asiatica* Boriss.). A total of 5 samples were produced during the study. As a result, sample No. 3 was recognized as the most effective composition. The composition of the optimal sample No. 3 included, in the first 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 second 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 pain relief, while Asian mint (*Mentha asiatica* Boriss.) extract ensures prolonged anti-inflammatory and antimicrobial effects.

According to the comparative analysis, sample No. 3 showed optimal results in terms of uniformity, adhesion, strength, and elasticity. Therefore, this sample was selected as the most suitable composition for the dental film. The film production technology was developed step by step and included the stages of preparing the solutions of the main and second layers, incorporation of 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 effectiveness of the dental film were substantiated.

4. The obtained two-layer dental film had a homogeneous, smooth structure. Due to the natural chlorophyll compounds present in the Asian mint (*Mentha asiatica* Boriss.) extract, the upper layer had a light green color, whereas the lower layer was characterized by a yellowish-transparent hue. The film had a mild menthol odor characteristic of Asian mint. The study established that the geometric dimensions of

the film were 50×40×0.1 mm, the pH was 6.5±0.2, and the 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 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 24-month stability study, the appearance, color, odor, dimensions, pH level, mass uniformity, loss on drying, and microbiological purity of the film remained unchanged; the mass uniformity ranged from 7.1% to 7.3%, and the loss on drying ranged from 10% to 10.5%. These results proved that the developed dental film is a dosage form with high structural stability, a preserved terpene composition, and stable quality characteristics 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 Kazakh National Medical University and the B. Atchabarov Research Institute of Fundamental and Applied Medicine on the basis of the bioethics committee protocol dated November 7, 2023, No. 8 (144). At a dose of 500 mg/kg, signs of mild edema and parenchymatous dystrophy were observed in the kidney and stomach tissues; at a dose of 2000 mg/kg, toxic changes in the liver, kidney, and stomach tissues became more pronounced; and at a dose of 5000 mg/kg, marked necrotic lesions were recorded. The average number of necrotic foci in the liver per 10 fields of view was 0.1 ± 0.3 in the control group, 2.3 ± 1.1 at a dose of 500 mg/kg ($p<0.05$), 5.7 ± 1.4 at a dose of 2000 mg/kg ($p<0.01$), and 12.4 ± 2.1 at a dose of 5000 mg/kg ($p<0.001$). This indicated a dose-dependent nature of the extract's toxicity.

In vitro, the anti-inflammatory activity of the circulation liquid extract was evaluated in 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 toward normal HUVEC cells was relatively low, confirming a certain selectivity.

The wound-healing activity of the dental film with Asian mint (*Mentha asiatica* Boriss.) extract was studied in rats, with the "Curatick" dental film (manufacturer: South Korea) selected as the reference preparation. This preparation was used as a control agent in the evaluation of the wound-healing effect. As a result, in animals receiving the investigated film, the weight of the load required to rupture the scar was

1.5–2 times higher compared with the control group, while the 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, at 52.1%.

6. As a result of the development of the feasibility study for the production of the dental film for a pilot-industrial batch of 10,000 units, the direct production costs amounted to 17,932,700 tenge (raw materials – 17,765,000 tenge; packaging – 967,700 tenge; other expenses – 200,000 tenge). Taking into account administrative (40%) and commercial (30%) costs, the total production cost reached 30,485,590 tenge. At a profitability level of 30%, the total selling price was set at 39,631,267 tenge, and the price per unit was 3,963 tenge. The net profit from one batch amounted to 21,698,567 tenge, which substantiated the high economic efficiency of production and the feasibility of industrial-scale implementation.

In summary, it should be noted that, as a result of the comprehensive scientific studies carried out, the plant raw material of Asian mint (*Mentha asiatica* Boriss.) was comprehensively investigated and scientifically standardized, while, based on the comparison of different extraction methods, ethanol circulation extraction performed using the Soxhlet apparatus was identified as the most optimal option. On the basis of the obtained standardized extract, a scientifically substantiated two-layer dental film in terms of composition and technology was developed, and its quality, stability, safety, and pharmacological effectiveness were established, demonstrating the high prospects for introducing a domestic phytosubstance and dental film based on Asian mint (*Mentha asiatica* Boriss.) into pharmaceutical practice.

Publications

The results of the dissertation research have been 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; and 3 implementation certificates.

Approbation of the Study

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

- Journal “Bulletin of the International Medical University of Central Asia”, Kyrgyz Republic, Jalal-Abad, 2023;
- International Scientific Conference of the Institute of the Chemistry of Plant Substances named after Academician S.Yu. Yunusov, 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 presentation on the results of the research project of the Ministry of Science and Higher Education of the Republic of Kazakhstan and the Committee of Science for 2023–2025 “IRN AP19680441 – Pharmaceutical Development and Pharmacological Studies of Dental Films 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 (Second Prize 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.

Relationship of the research objectives to scientific projects

The dissertation research was carried out within the framework 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 Films for Use in Dental Practice.”

Structure and scope of the dissertation:

The dissertation, which presents the results of the scientific research, comprises 189 pages, including 28 tables, 56 figures, a bibliography of 114 references, and appendices. The dissertation includes an introduction, a literature review, sections devoted to the materials and methods of the study, sections presenting the research results, and a conclusion.