ABSTRACT

for the dissertation for the degree of Doctor of Philosophy (Ph.D) in specialty 8D07105 – "Chemical Technology of Organic Substances"

Development and Validation of a Technological Scheme for Obtaining a Plant-Based Gel

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Research Topic: The dissertation investigates the validation of technology for obtaining plant substances from the roots and aerial parts of *Limonium gmelinii* plants, selected through comparative analysis of various extraction methods: ultrasonic extraction (USE), supercritical fluid extraction (SFE), and classical maceration (CM); standardization of substances, study of their antibacterial and immunomodulatory activities; pharmaceutical development of gels with the extracted plant substances, validation of their production technology, and study of their anti-inflammatory activity.

Relevance of the Study: The COVID-19 pandemic, which emerged in 2020, revealed the need to strengthen Kazakhstan's pharmaceutical sector as a key element of national security. Within the framework of the "Kazakhstan-2050" program and the "Healthy Nation" project, there are plans to increase the share of domestic pharmaceutical products to 50% by 2025. To achieve this goal, nationwide tasks have been set to expand the scientific and human resource potential for the pharmaceutical and medical industries, and to develop domestic production of medicines and medical devices.

Kazakhstan's rich biodiversity and diverse flora provide favorable conditions for the development of domestic herbal medicines, which are globally recognized as a safe and effective alternative to synthetic drugs.

Plants of the genus *Limonium gmelinii* (*L. gmelinii*) are a promising species in terms of economic and environmental feasibility for their introduction into medicine due to their high biological activity, resource availability, and ease of harvesting. *L. gmelinii* is already recognized as a pharmacopeial raw material for producing plant substances with antioxidant, anti-inflammatory, wound-healing, and hepatoprotective properties. These substances have been used to develop medicinal forms such as syrups, ointments, and tinctures, which have shown favorable therapeutic effects in in vivo research and preclinical trials.

Given the above, the use of this plant material for the development of new medicinal forms with a modern scientifically grounded approach, and in compliance with Good Manufacturing Practices (GMP) principles and requirements, is a relevant task. This approach addresses one of the main challenges in the development of herbal medicines — the lack of proper quality control and standardization, which directly affects their safety and efficacy. The complex and variable chemical composition of plant materials complicates quality assurance. A key component of GMP methodology is validation, the goal of which is to prove the suitability of the selected technology, method, process, equipment, or system in the production of medicines. Validation of the production technology of substances and dosage forms based on them confirms the consistency of the drug manufacturing process, ensuring consistent quality for each unit of the dosage

form and packaging, regardless of the batch. Applying this concept to the production of herbal medicines, which are challenging to standardize, will undoubtedly improve their quality and guarantee it throughout the production process.

Based on the above, the relevance of this dissertation lies in the development and validation of technology for producing domestic medicines based on the promising plant material *L. gmelinii* in accordance with GMP requirements, and the study of their biological activity, with the goal of introducing them into medical practice.

Object of the Study: Medicinal plant raw materials — roots and aerial parts (AP) of *L. gmelinii* plants; substances extracted from the roots and AP of *L. gmelinii* plants; gels with plant substances from the roots and AP of *L. gmelinii* plants.

Subject of the Study: Optimization of the technology for obtaining substances from the roots and AP of *L. gmelinii* plants, their validation, biological properties, development of the optimal composition and technology for obtaining gel compositions, their standardization, stability study, validation of the technological process for obtaining gels, and the study of their anti-inflammatory activity.

Objective of the Dissertation Research: Pharmaceutical development and validation of the technology for obtaining soft dosage forms in the form of gels with plant substances extracted by various methods from the roots and aerial parts (AP) of *L. gmelinii* plants, analyzed both for their chemical composition and newly studied immunomodulatory activity to select the most rational and acceptable of them.

Research Methods: Pharmacognostic, chemical, and physico-chemical methods of analysis were used, such as macroscopic, microscopic, and phytochemical analyses; microbiological control; UV spectroscopy, FTIR spectroscopy, HPLC-DAD, HPLC-quadruple time-of-flight mass spectrometry, ^{1}H NMR spectroscopy, horizontal diffusion cell method, viscometry, in vitro antibacterial activity studies, immunoreferent analysis methods (ELISA) and flow cytometry, in vivo studies of anti-inflammatory activity on a model of formalin-induced paw edema in experimental mice, as well as statistical methods.

Scientific Novelty:

1.A comparative analysis of various methods for obtaining plant substances from the roots and AP of *L. gmelinii* plants by CM, USE, and SFE methods was conducted for the first time.

2.The immunomodulatory activity of plant substances from the roots and AP of *L. gmelinii* plants was established for the first time.

3.A comparative analysis of the component composition of plant substances obtained by different methods, in particular USE and CM, was carried out for the first time. In substances S1 and S2 obtained from roots, and in substances S3 and S4 obtained from aerial parts, the identity of their chemical compositions was established by ultrasonic electron microscopy (S1, S3) and CM (S2, S4) methods. As a result of the analysis of the chemical composition of plant substances using

the LC-QToF-MS method, 62 compounds were identified based on their fragment ions and in comparison with data from previously published studies. It was also shown that substances S1 and S2 contain a larger amount of sulfated flavonoid derivatives, in contrast to substances S3 and S4 with a predominance of flavonoid aglycones.

4.The positions of monosaccharide bonds in polysaccharide complexes isolated from plant substances obtained from the roots and APs of plants of the species L. gmelinii were determined for the first time by analyzing partially methylated alditol acetates (MAA). All plant substances contained rhamnose (Rha-terminal and Rha-3,4), arabinose (Ara-terminal), glucuronic acid (GlaA), mannose (Man), galactose (Gal), and glucose (Glc). Xylose (Xyl-terminal) and galacturonic acid (GalA) were also found in the polysaccharide complex of NPs.

5.The transfer and validation of the technology for obtaining plant substances from the roots and APs of plants of the species *L. gmelinii* by the USE method in pilot-industrial conditions of Antigen LLP were carried out for the first time.

6.Soft dosage forms in the form of gels with plant substances isolated from the roots and aerial parts of plants of the species *L. gmelinii* were developed for the first time, using carbomer as a gelling agent. For the first time, the composition of excipients and active substances was formulated using ICH Q8 methods. The technology was validated at the level of laboratory batches.

7.The organoleptic and rheological properties of the developed gels were studied for the first time, the kinetics of active pharmaceutical ingridients release from them was studied, and their anti-inflammatory activity was established in *in vivo* tests.

Main Points for Defense:

1 The optimal conditions for obtaining plant substances from the roots and aerial parts of *L. gmelinii*, based on yield and tannin content, are a single 45-minute ultrasonic extraction (UZE) with 50% ethanol at a raw material to solvent ratio of 1:5 for roots and 1:7 for aerial parts. Additionally, supercritical fluid extraction (SFE) for 240 minutes at a pressure of 35 MPa and temperature of 45°C, using ethanol as a co-solvent at 60%, proved effective.

2 The polysaccharide complex obtained from the aerial part of Limonium gmelinii differs from the complex extracted from the roots by the presence of xylose (terminal Xyl) and galacturonic acid (GalA), as identified by the derivatization of acetylated methyl glycosides.

3 Methanol fractions of the extracts exhibited antibacterial activity against *Staphylococcus epidermidis* ATCC 12228, whereas the water fractions, containing polysaccharides, showed no antibacterial activity, indicating the predominant role of phenolic compounds in the antibacterial effect of Limonium gmelinii extracts.

4 Based on the analysis of release kinetics and rheological properties, the optimal gel formulations with plant substances from *L. gmelinii* were gels containing 1% carbomer and 10% propylene glycol, which demonstrated anti-inflammatory effects in *in vivo* experiments on mice. The manufacturing process of

the gels is stable and reproducible, as confirmed by the validation of laboratory batches.

Practical Significance: The dissertation materials can be used for organizing technology transfer divisions in universities/research institutes, training and advanced training of pharmaceutical industry workers, as well as training master's and PhD students in pharmaceutical faculties.

Optimal conditions for obtaining plant substances from the roots and aerial parts (AP) of *L. gmelinii* using ultrasonic extraction (USE) and supercritical fluid extraction (SFE) methods have been developed for further pharmaceutical formulation. The conditions developed using the USE method are more advantageous from an economic and practical standpoint. For obtaining plant substances under production conditions at LLP "Antigen" using the USE method, pilot-industrial regulations have been developed and approved, validation has been conducted, and process stability has been studied. Comprehensive studies of the semi-solid dosage forms (SSDF) in the form of gels with plant substances from the roots and AP of *L. gmelinii* showed satisfactory consumer and technological qualities, stability during storage, and anti-inflammatory effects in a formalin-induced paw edema model in mice in vitro. The experimental data obtained will allow for continued work on the production of gels under pilot-industrial conditions with subsequent implementation in medicine.

Correspondence to the Directions of Science Development or State Programs

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The author's personal contribution includes direct execution of the experimental part of the work, participation in the analysis, synthesis, and interpretation of the obtained experimental data.

Publications: The main results of the dissertation research were published in co-authorship in 9 scientific works, including 2 articles in international peer-reviewed journals *Plants* (Q1, 90th percentile) and *Antioxidants (Basel)* (Q1, 90th percentile), and 1 article in the journal *International Journal of Biology and Chemistry*, which is included in the list of 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, as well as materials and abstracts of 6 presentations at international scientific conferences.

Approbation of the dissertation work.
The results and main provisions of the scientific work were presented at the following events: XI All-Russian Youth Scientific Conference (Syktyvkar, Russia, 2019), 47th IUPAC WORLD CHEMISTRY CONGRESS (Paris, France, 2019),

67th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (Innsbruck, Austria, 2019), Book Collection of the Commonwealth of Independent States "Best Young Scientist - 2020" (Astana, 2020), GA – 70th Annual Meeting (Thessaloniki, Greece, 2022), Scientific-practical conference "Problems of Synthesis of New Medicines in Pharmaceutical Chemistry" (Tashkent, Uzbekistan, 2024).

The personal contribution of the author to the preparation of each publication:

1. Article “Study of the hexane fraction isolated from the substance obtained from the roots of *Limonium gmelinii* by GC-MS” — obtaining and discussing experimental data, data analysis, and processing.
2. Article “Plant Extract of *Limonium gmelinii* Attenuates Oxidative Responses in Neurons, Astrocytes, and Cerebral Endothelial Cells *In Vitro* and Improves Motor Functions of Rats after Middle Cerebral Artery Occlusion” — experimental part on obtaining plant substances and studying their physicochemical properties, data analysis of their chemical composition.
3. Article “Phytochemical Profiles and *In Vitro* Immunomodulatory Activities of Extracts Obtained from *Limonium gmelinii* Using Different Extraction Methods” — development of methodology, review preparation, data analysis, obtaining, discussing, and processing results, communication with the journal.

Volume and structure of the dissertation. The dissertation is presented in 204 pages of typed text and includes an introduction, 3 sections, a conclusion, 32 tables, 54 figures, and a list of 228 sources.

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