

ANNOTATION

of dissertation work on the topic: "**Development of a technology for producing a substance based on the alkaloid lupinine from *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens raw material**" for the degree of Doctor of Philosophy (PhD) in the educational program 8D07201 – "Technology pharmaceutical production"

Bekisheva Pernesh Zhaidarbekovna

Relevance of the research topic. The strategic direction of the Republic of Kazakhstan's policy is the development of knowledge-intensive technologies based on its own productive forces, raw materials, and scientific and technical potential, as well as pharmaceutical production by systematically reducing its dependence on imported medicines.

In accordance with Section 4 of the "Concept for the Development of the Healthcare Sector of the Republic of Kazakhstan until 2026", approved by Decree of the Government of the Republic of Kazakhstan No. 945 dated November 24, 2022, it is intended to ensure the satisfaction of the country's domestic needs, reduce dependence on the global pharmaceutical market and improve the biosecurity of the population through the development of domestic production of innovative medicines and strengthening national sanitary and epidemiological control.

The head of state set a goal to increase the share of domestic production in the pharmaceutical market to 50% by 2026. To this end, as part of the implementation of the Comprehensive Plan for the Development of the Pharmaceutical Industry for 2020-2026, the establishment of the production of broad-spectrum pharmaceuticals with various chemical compositions based on chemical substances in the Republic of Kazakhstan is of particular national importance.

At this stage, the effective implementation of the national pharmaceutical policy in the Republic of Kazakhstan is aimed at providing the population with high-quality, safe, and affordable medicines, reducing dependence on foreign pharmaceuticals, and developing domestic pharmaceutical production. Achieving these objectives requires the search for new medicinal compounds, the development of domestically produced chemically derived drugs, and their introduction into medical practice.

High dependence on foreign pharmaceuticals requires the development of domestic pharmaceutical production based on science. In this area, the study of medicinal plants rich in biologically active substances is relevant. Rich in alkaloids, *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens, which grows in Kazakhstan, is considered a promising plant with a high chemical composition and pharmacological potential, is widely distributed, and is suitable for industrial use.

One of the key research objectives is to study the biologically active substances contained in the *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens plant, including the isolation and chemical transformation of the alkaloid lupinine and the determination of its pharmacological action. The presence of an active hydroxyl group in the lupinine molecule allows for the synthesis of new derivatives. Therefore, the search for functional antiviral and antibacterial conjugates among modified alkaloid derivatives that stimulate the respiratory system is an urgent and important priority.

Therefore, a pressing and practically important issue in pharmacy is the study of methods for producing herbal remedies that are widely available and expanding the range of antiviral and antibacterial drugs based on local, environmentally friendly raw materials containing alkaloids.

The purpose of research. Obtaining, standardization and development technology of production of new compounds based on lupinine alkaloids as substances for biologically active preparations.

Objects of study: *Anabasis salsa* (C. A. Mey.) Benth. ex Volkens) plant material, thick extract, lupinine alkaloid, and lupinine derivatives.

Subject of study: A new method and technology for isolating the lupinine alkaloid from *Anabasis salsa* (C. A. Mey.) Benth. ex Volkens) extract, quality indicators and biological activity of the obtained lupinine; synthesis, structure, and biological activity of the lupinine derivative; technology for producing the lupinine derivative substance; regulatory documentation for the raw materials, extract, and lupinine derivative substances Lup-43 and Lup-41.

Research methods: physicochemical, chemical, pharmacognostic, pharmaceutical-technological, pharmacological, microbiological, biological and statistical methods

Objectives of study:

1. To establish the main morphological and anatomical characteristics of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens raw materials and determine their numerical parameters;

2. To develop an optimal technology for extracting the aerial parts of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens, study the chemical composition of the extracts, and evaluate their quality;

3. To isolate and purify the alkaloid lupinine from *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens extract and synthesize 1,2,3-triazole compounds based on lupinine;

4. To determine the qualitative parameters of substances, establish their stability and shelf life, develop regulatory documentation, and conduct pharmacological studies.

Scientific novelty of the research

A pharmacognostic analysis and evaluation of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens plant material, grown in Central Kazakhstan, was conducted for the first time, and draft regulatory documentation was developed. Phytochemical studies of the aboveground and underground parts (stems, inflorescences, and roots) of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens using ESI-QTOF-MS/MS resulted in the first identification of 26 compounds.

A method for obtaining thick extracts from *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens material using percolation and maceration was developed, and the quantitative content of the alkaloid lupinine in the resulting extracts was determined for the first time.

A method for isolating the alkaloid lupinine from *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens extract using centrifugal partition chromatography has been developed for the first time.

Methods for synthesizing its 1,2,3-triazole derivatives based on the alkaloid lupinine have been optimized and standardized, and a technology for its production has been developed. As a result of studying the biological activity of the synthesized compounds (1*S*,9*aR*)-1-({4-[4-(benzyloxy)-3-methoxyphenyl]-1*H*-1,2,3-triazol-1-yl}methyl)octahydro-2*H*-quinolizine (Lup-43) and {1-[(1*S*,9*aR*)-octahydro-2*H*-quinolizin-1-yl)methyl]-1*H*-1,2,3-triazol-4-yl}methyl 3-tert-butyl-2-hydroxy-5-ethylbenzoate (Lup-41), it was found that substance Lup-43 has an inhibitory effect on AChE, and substance Lup-41 has antimicrobial and antiviral activity against the H3N2 influenza virus strain. The stability and acute toxicity of the substances were studied, and draft regulatory documents were developed.

The scientific novelty of the dissertation research lies in the patents for utility models of the Republic of Kazakhstan No. 10151 dated 14.06.2024 "Method for obtaining an extract of the *Anabasis salsa* plant with antimicrobial activity", No. 10483 dated 27.02.2025 "Use of an ethanol extract of the *Anabasis salsa* plant as an antimicrobial agent" and No. 10740, registered on 03.04.2025 "{1-[(1*S*,9*aR*)-octahydro-2*H*-quinolizin-1-yl)methyl]-1*H*-1,2,3-triazol-4-yl}methyl 3-tert-butyl-2-hydroxy-5-ethylbenzoate with antiviral activity".

The main provisions for defense:

- A pharmacognostic study of raw materials and a phytochemical examination of various organs (stems, flowers, roots) of *Anabasis salsa* (C. A. Mey.) Benth. ex Volkens) allowed for the identification of the raw material, its quality, and the identification of the producing species of the *Anabasis* L. plant.

- A technology for obtaining a thick extract from the aerial parts of *Anabasis salsa* (C. A. Mey.) Benth. ex Volkens) and the isolation of the alkaloid lupinine for further chemical modifications.

- A technology for obtaining lupinine substance from an extract of the aerial parts of *Anabasis salsa* (C. A. Mey.) Benth. ex Volkens) using centrifugal distribution chromatography.
- A technology for the synthesis of new lupinine derivatives for the production of pharmaceutical substances was proposed.
- Identification of the structure of the synthesized substances using modern physicochemical methods.
- the dependence of the biological activity of compounds on their structure;
- research results on the technology for producing biologically active substances, determining quality indicators, shelf life, and safety;

Practical significance of the obtained results:

As a result of the conducted research, reserves of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens raw materials were identified in Kazakhstan, pharmacognostic studies were conducted and implemented in the educational process at the Faculty of Biology and Geography of the Karaganda National Research University named after Academician E.A. Buketov. A technology and quality specification for obtaining *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens plant materials were developed, draft regulatory documents were prepared. Based on *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens, a thick extract was obtained by percolation, the chemical composition was determined, and raw materials were standardized. A technology for isolating the alkaloid lupinine from this plant extract was proposed, its structure was proven using modern physicochemical methods, and quality indicators were studied.

A technological process for obtaining *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens extract using the percolation method was implemented at the School of Pharmacy of Karaganda Medical University and incorporated into the educational process. Utility model patents No. 10151 "Method for Obtaining an *Anabasis salsa* Plant Extract with Antimicrobial Activity" and No. 10483 "Use of an Ethanol Extract of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens as an Antimicrobial Agent" were obtained for the aerial parts of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens.

The synthesis of new derivatives based on the alkaloid lupinine was optimized, and the results of studies of the chemical structure, biological activity, and safety of the resulting compounds were presented. The synthesized compounds were tested in the microbiology laboratories of Karaganda Medical University and the virology laboratory of the Scientific Center for Anti-Infectious Drugs. Acute toxicity studies revealed that the 1,2,3-triazole derivative of lupinine exhibits no toxic properties.

Based on the dissertation work on the chemical synthesis of biologically active substances based on new 1,2,3-triazole derivatives of lupinine, regulatory documents have been developed at the School of Pharmacy of Karaganda Medical University. Laboratory procedures for obtaining the substances have been developed.

Substantiation of methods for producing high-quality pharmaceutical substances based on 1,2,3-triazole derivatives of lupinine (substances Lup-43, Lup-41) and an extract of the *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens plant can serve as a basis for the creation of new domestic drugs in the future and contribute to the development of the pharmaceutical industry in our country.

Author's Personal Contribution

All results of this dissertation were obtained independently by the author, demonstrating the candidate's personal contribution to pharmaceutical science. The reliability of the results, the main points presented for defense, and the conclusions formulated in this dissertation are substantiated by a significant amount of experimental data and are fully supported by the results of the author's own research conducted in laboratory and industrial settings, using modern certified equipment and precise measurement methods, as well as by comparison with literature data. Linking the research objectives to the research program plan

Relationship of research objectives with the research program plan

This work was carried out within the framework of grant projects No. AR23487712 "Design and Synthesis of Leader Compounds and Their Supramolecular Clathrates in the Creation of Next-Generation Bioactive Substrates by Transforming the Quinolizidine Backbone of Lupinine" and No. 7966-F-24 "Spatial Structure and Stereochemistry of Quinolysine Alkaloid Derivatives and Guaiane Sesquiterpenoids" for 2024-2026.

Dissertation Results Approbation

The main provisions of the dissertation have been presented and published in the following proceedings: International Conference "Pharmacy – Moving Forward!" (Karaganda, 2023); International Conference "V International Symposium "INNOVATIONS IN LIFE SCIENCES" (Belgorod, 2023); "VII International Book Publication of the Commonwealth of Independent States "Best Young Scientist – 2023" (Astana, 2023); "Pharmacy of Kazakhstan" (Almaty, 2023); "XI International Scientific Practical Conference of Young Scientists "Modern Trends in the Development of Health-Saving Technologies" (Moscow, 2023); "International Scientific Symposium "From Plant to Medicine" (Moscow, 2025).

Publications.

Based on the research results, 14 scientific papers were published, including:

- 1 article in a journal recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan;
- 4 articles in international scientific journals included in the Web of Science and Scopus databases;
- 6 paper abstracts, including 5 paper abstracts in international conference proceedings.
- 3 utility model patents of the Republic of Kazakhstan.

Scope and structure of the dissertation

The dissertation is presented on 154 typewritten pages, computer-typeset, and contains 37 tables, 47 figures, a bibliography of 148 sources, and 20 appendices. The work consists of an introduction, a literature review, a section on research materials and methods, three sections on the author's original research, conclusions, and a summary.

Conclusions

1. For the first time, morphological and anatomical diagnostic properties of *Anabasis salsa* (C.A.Mey.) Benth. ex Volkens) raw materials were established based on research results. Histochemical tests on shoot cross-sections, inflorescence surface preparations, and root cross-sections revealed flavonoids and alkaloids, and their localization was established.

Based on the experimental results, the pharmacognostic and technological properties of *Anabasis salsa* (C.A.Mey.) Benth. ex Volkens) plant materials, grown in the Karaganda region of the Republic of Kazakhstan, were determined, and an extractant was selected for the extraction. To select optimal conditions for the extraction process, the technological parameters of *Anabasis salsa* (C.A.Mey.) Benth. ex Volkens) raw materials were studied, and 70% ethanol was chosen as the extractant, as it provides a high yield of extractable substances.

2. The chemical composition of *Anabasis salsa* (C.A.Mey.) Benth. ex Volkens) extracts was studied for the first time using HPLC-UV and HPLC-MS/MS; a total of 26 compounds (alkaloids, flavonoids and their glycosides, phenolic compounds, amino acids) were identified. All identified compounds had been previously detected in other species of the genus *Anabasis* L.; however, most of the presented components were described for the first time for the plant material of *Anabasis salsa* (C.A.Mey.) Benth. ex Volkens). The quantitative content of the alkaloid lupinine in the aboveground and underground parts of *Anabasis salsa* (C.A.Mey.) Benth. ex Volkens) raw materials was studied. Moreover, the content of lupinine in AS-70P and AS-90P extracts obtained by the percolation method is predominant and fluctuates between 0.1769 and 0.0098%. In the underground part, the content of the alkaloid lupinine in the ASK-90 and AS-KH extracts obtained by the maceration method ranges from 0.006-0.0018%. The lupinine content in the AC-90tP extract obtained from the alkalized aboveground part is 0.0079%.

3. A new method for isolating lupinine from *Anabasis salsa* (C. A. Mey.) Benth. ex Volkens extract using centrifugal partition chromatography has been developed, ensuring stable production of the required quantity of product of appropriate quality.

Optimal conditions for modifying the lupinine alkaloid structure at position C-10 have been developed, yielding a potentially bioactive 1,2,3-triazole derivative. The developed conditions made it possible to synthesize the corresponding (1*S*,9*aR*)-1-({4-[4-(benzyloxy)-3-methoxyphenyl]-1*H*-1,2,3-triazol-1-yl}methyl)octahydro-2*H*-quinolizine (Lup-43) and {1-[[((1*S*,9*aR*)-octahydro-2*H*-quinolizin-1-yl)methyl]-1*H*-1,2,3-triazol-4-yl}methyl-3-tert-butyl-2-hydroxy-5-ethylbenzoate (Lup-41) in good yield.

4. It was established for the first time that extracts of *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens), namely 70% ethanol extract of the aerial parts, exhibit a higher effect against the reference strains of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. The lowest inhibitory and bactericidal concentrations were 0.625-1.25 mg/ml and 2.5 mg/ml for these bacteria, respectively. Other gram-positive microorganisms were also highly sensitive to this extract, namely: *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus cereus* (MIC = 2.5-5 mg/ml and MBC = from 10 mg/ml to >20 mg/ml). The lupinine sample also demonstrated activity against all Gram-positive bacteria (MIC = 5-10 mg/ml and MBC = 5 mg/ml to 20 mg/ml). Among the studied extracts, *Anabasis salsa* (C.A. Mey.) Benth. ex Volkens root extract proved to be the most active antifungal agent, exhibiting higher activity against all reference strains of the genus *Candida*.

For the first time, a biological activity study established that the compound Lup-43 has AChE activity, while the compound Lup-41 exhibits antimicrobial and pronounced antiviral activity against the H3N2 influenza virus strain.

A draft regulatory document was developed, and the raw materials were standardized. The stability of the substances "Herringberry thick extract", "Lupinine substance", "Lup-43 substance", and "Lup-41 substance" was studied. Laboratory regulations for obtaining the substances "Lup-43 substance" and "Lup-41 substance" have been developed and approved.