ABSTRACT

of the dissertation submitted for the degree of Doctor of Philosophy (PhD)
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Study of the pharmacological properties of a new polyphenolic complex based on *Rheum cordatum* Losinsk.

General characteristics of the study. The dissertation is devoted to the investigation of the pharmacological properties of a new polyphenolic complex based on *Rheum cordatum* Losinsk.

Relevance of the research topic

Metabolism-associated fatty liver disease is currently considered one of the most widespread chronic liver diseases, closely linked to the global pandemic of alimentary obesity and metabolic syndrome. According to the data of the Global Burden of Disease (GBD 2021) project, the number of patients with metabolically associated fatty liver disease (MAFLD) increased from 564.4 million to 1.3 billion people between 1990 and 2021, while its prevalence among the global population rose from 10.6% to 16.1%.

The rapid growth in disease prevalence is driven by the increasing incidence of alimentary obesity, diabetes mellitus, and metabolic syndrome, making non-alcoholic fatty liver disease (NAFLD) one of the key medical and social challenges of modern healthcare. Despite significant advances in global research, pharmacotherapy for NAFLD remains limited. In 2024–2025, the first drugs, resmetirom (a THR-β agonist) and semaglutide (a GLP-1 agonist), were approved, demonstrating efficacy in patients with liver fibrosis and hepatic steatosis. However, these agents are characterized by high cost, the need for long-term safety monitoring, and narrowly targeted mechanisms of action. Previously used treatments, including vitamin E and pioglitazone, are recommended only for limited patient populations, while ursodeoxycholic acid has not demonstrated efficacy in NAFLD. Thus, there remains a clear need to identify new pharmacological agents with multimodal mechanisms of action and low pharmacotoxicity.

Polyphenolic complexes derived from plants of the genus Rheum L. are of particular interest due to their pronounced antioxidant, anti-inflammatory, and antimicrobial properties. These pharmacological effects are especially relevant for NAFLD, where oxidative stress, lipotoxicity, inflammation, and alterations of the gut microbiota play a key pathogenic role. The genus Rheum (Polygonaceae) comprises more than 60 species and has been widely used in the traditional medicine of China, India, and Central Asia. The most extensively studied species include *R. palmatum*, *R. officinale*, and *R. tanguticum*, which are rich in anthracene derivatives, flavonoids, and phenolic acids. In contrast, *Rheum cordatum* Losinsk., which grows in Kazakhstan, remains poorly studied despite its high potential content of valuable biologically active compounds.

Despite several individual studies devoted to *Rheum cordatum*, until now there have been no investigations that included: a comparative phytochemical analysis of extracts obtained using modern extraction methods; evaluation of in vitro antioxidant and antimicrobial activities; and assessment of hepatoprotective properties in vivo using a hepatic steatosis model.

The environmental aspect of pharmaceutical production is also of significant importance. One of the key principles of green chemistry is the prevention or minimization of negative environmental impacts, including the rational use and disposal of organic solvents. Conventional extraction methods (e.g., using hexane, acetone, or chloroform) are associated

with increased environmental and technological risks related to waste contamination and high energy consumption. Supercritical carbon dioxide ($ScCO_2$) extraction enables the production of high-purity extracts, minimizes the use of organic solvents, and reduces environmental burden, fully meeting the requirements of sustainable pharmaceutical manufacturing.

The implementation of such technologies is particularly relevant for Kazakhstan, where the development of the pharmaceutical industry is a strategic priority. According to the directive of the President of the Republic of Kazakhstan, Kassym-Jomart Tokayev, dated September 1, 2020, the share of domestically produced medicines should reach 50% by 2025. Achieving this goal requires the active use of local plant resources and the introduction of innovative technologies, making *Rheum cordatum* a unique object for comprehensive study and practical application.

Aim of the study – to investigate the pharmacological properties of a new polyphenolic complex based on *Rheum cordatum* Losinsk.

Objectives of the study:

1. To conduct a comparative phytochemical analysis of extracts obtained using supercritical carbon dioxide extraction ($ScCO_2$), subcritical ethanol extraction (sbcEtOH), and ultrasound-assisted extraction (UAE);
2. To evaluate the antioxidant activity of the extracts using DPPH, ABTS, and CUPRAC assays, as well as total phenolic content (TPC);
3. To investigate and compare the antimicrobial properties of extracts obtained by $ScCO_2$, sbcEtOH, and UAE methods;
4. To determine the acute and chronic toxicity of the polyphenolic complex based on *Rheum cordatum* Losinsk;
5. To assess the effects of oral administration of *Rheum cordatum* Losinsk extracts in laboratory animals under experimental models of hepatosteatosis.

Research methods. The study employed phytochemical, antioxidant, antimicrobial, pharmacological, biochemical, and histological methods.

Scientific novelty and significance

For the first time, a comprehensive investigation of extracts from the roots and leaves of *Rheum cordatum* Losinsk obtained using green extraction technologies $ScCO_2$, sbcEtOH, and UAE was carried out.

A comparative phytochemical analysis using UHPLC-MS/MS was performed for the first time, identifying key phenolic compounds and flavonoids, including gallic, protocatechuic, caffeic, p-coumaric, ferulic, and ellagic acids, catechin, epicatechin, epigallocatechin gallate, epicatechin gallate, rutin, isoquercitrin, hesperidin, quercetin, kaempferol, apigenin, luteolin, and chrysin.

A comparative analysis of antioxidant and antimicrobial activities of the obtained extracts was conducted depending on the extraction method and phenolic content using DPPH, ABTS, CUPRAC, and TPC assays, as well as MIC, MBC, and BIC90 parameters.

For the first time, the toxicological safety of the polyphenolic extracts was evaluated in vivo, with acute and chronic toxicity parameters established and the pharmacological safety of the studied samples confirmed.

The hepatoprotective effect of the $ScCO_2$ extract of *Rheum cordatum* Losinsk was investigated for the first time using a diet-induced hepatosteatosis model in laboratory animals.

The theoretical significance of the research lies in the fact that the obtained results provide new scientific data on modern methods for the extraction of plant raw materials from *Rheum cordatum* Losinsk. The present study demonstrates the pharmacological properties of extracts derived from the Kazakhstan's species *Rheum cordatum* Losinsk. For the first time, a relationship between the quantitative content of polyphenolic complexes and their antioxidant and antimicrobial activities has been established.

Under conditions of experimental hepatosteatosis in laboratory animals, oral administration of the $ScCO_2$ extract of *Rheum cordatum* Losinsk. for 35 days resulted in a reduction of hepatic enzyme levels, normalization of cholesterol and triglyceride concentrations in blood serum, as well as a decrease in the severity of fatty infiltration and dystrophic changes in hepatocytes, as confirmed by histostructural analysis.

This work forms a basis for the further development of theoretical approaches to the application of modern extraction technologies in pharmacology, pharmacognosy, and biotechnology. Toxicopharmacological studies of the efficacy and safety of *Rheum cordatum* extracts will contribute to further research on the highly selective extraction of biologically active compounds, ensuring the possibility of comprehensive processing of medicinal plant raw materials in Kazakhstan. This, in turn, creates prerequisites for expanding the range of domestic pharmaceutical products and enhancing the international competitiveness of the pharmaceutical industry of the Republic of Kazakhstan.

Main provisions submitted for defense:

1. The *Rheum cordatum* Losinsk. extract obtained using UAE-EtOH-1h, UAE-EtOH-4h, sbcEtOH-E (140°C–60 bar), sbcEtOH-E (140°C–80 bar), $ScCO_2$ (100 bar–60°C), and $ScCO_2$ (150 bar–60°C) methods contains 27 compounds in the roots and 28 phenolic compounds in the leaves, with the predominance of gallic acid, catechins, and flavonoid glycosides (rutin and hesperidin).
2. The leaf extract of *Rheum cordatum* Losinsk. obtained under ScCO2-150 conditions was characterized by high antioxidant activity, as confirmed by the lowest IC50 value in the DPPH assay (0.0132 mg/mL) and the highest total phenolic content (TPC = 140 mg GAE/g) ($p < 0.05$).
3. The leaf-based extract of *Rheum cordatum* Losinsk. obtained by the ScCO2-150 method exhibited antimicrobial activity, with MIC values of 125–250 μg/mL against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus spizizenii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.
4. No pharmacotoxic effects of the polyphenolic complex obtained from leaves using the $ScCO_2$-150 method were detected, as evidenced by the absence of toxic effects at doses up to 50 mg/kg, based on the open field test, relative organ weights, and all biochemical parameters ($p > 0.05$).
5. Oral administration of the *Rheum cordatum* Losinsk. extract obtained using the $ScCO_2$-150 method at a dose of 50 mg/kg for 28 and 35 days contributed to the normalization of blood biochemical parameters and a reduction in the severity of hepatic fatty infiltration.

The practical significance of the study lies in substantiating the feasibility of using *Rheum cordatum* Losinsk. as a promising source of natural antioxidants and hepatoprotective agents obtained by environmentally friendly methods, which is consistent with the development priorities of the pharmaceutical industry in Kazakhstan.

The personal contribution of the doctoral candidate to the preparation of each publication included data collection on the research subject, plant material collection, performance of laboratory studies on plant and animal models, analysis, interpretation, and presentation of the obtained results, as well as preparation of publication manuscripts.

Relation to the main research plan:

The dissertation was carried out within the framework of planned research under state scientific and technical projects: “Development of innovative approaches to increasing the productivity of agricultural animals using physiological and genetic methods” (IRN BR24993004, 2024–2026), project supervisor: Candidate of Biological Sciences, Associate Professor A.S. Musayeva; “Development of a program for the conservation and development of the resource base of plants promising for medicine and veterinary science under climate change conditions” (IRN BR21882180, Contract No. 417-PCF-23-25 dated November 15,

2023), project supervisor: Candidate of Biological Sciences, Professor N.V. Terletskaya.

Review and approval of research results

The main results of the dissertation were annually reviewed at meetings of the Scientific and Technical Council of the Faculty of Biology and Biotechnology, at sessions of the Department of Biodiversity and Bioresources of Al-Farabi Kazakh National University, and at the Academic Council of the Institute of Genetics and Physiology. The dissertation results constitute a component of the planned research conducted within the above-mentioned state scientific and technical projects.

Research results:

1. A comparative phytochemical analysis of Rheum cordatum root and leaf extracts obtained by $ScCO_2$, sbcEtOH, and UAE methods and investigated using LC–MS/MS revealed the presence of 30 compounds in the roots, whereas 28 compounds belonging to various classes of secondary metabolites were identified in the leaves. It was established that the roots were characterized by a higher content of catechins and their gallates (epicatechin gallate up to 83.53 μg/mg), whereas the leaves were distinguished by an increased content of flavonoid glycosides and phenolic acids, including rutin (up to 21,932 μg/mg) and hesperidin (up to 14,975 μg/mg).;

2. *In vitro* studies (DPPH, ABTS, CUPRAC, TPC) revealed high antioxidant activity of all extracts ($p < 0.05$), particularly $ScCO_2$-150 (leaves): DPPH IC_{50} = 0.0132 mg/mL, ABTS = 0.0462 mg/mL, TPC = 140 mg GAE/g; for sbcEtOH-60: DPPH = 0.0379 mg/mL, ABTS = 0.0521 mg/mL, TPC = 125 mg GAE/g; and for UAE-M-4h: DPPH = 0.0472 mg/mL, ABTS = 0.0612 mg/mL, TPC = 100 mg GAE/g;

3. The antimicrobial activity of root extracts of *Rheum cordatum* Losinsk. was confirmed against *Enterococcus faecalis*. The extract obtained by supercritical CO_2 extraction ($ScCO_2$ 100-60) demonstrated the lowest MIC value of 31.25 μg/mL, which is comparable to the activity of standard natural antimicrobial agents. Among the leaf samples, the best results were shown by the $ScCO_2$-150 extract, for which MIC values ranged from 125 to 250 μg/mL against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus spizizenii*.;

4. Acute and chronic toxicity studies showed that the supercritical CO_2 extract of *Rheum cordatum*, when administered orally at doses up to 50 mg/kg, did not exert toxic effects on animals: liver and kidney function parameters, lipid metabolism indices, and relative organ weights remained within physiological limits;

5. In a hepatosteatosis model, the polyphenolic complex of *Rheum cordatum* exhibited pronounced hepatoprotective activity, as evidenced by normalization of hepatic enzyme activities (ALT, AST, GGT, ALP), reduction of triglyceride and cholesterol levels ($p < 0.05$), and histologically confirmed regression of hepatic fatty infiltration.

Publications and approbation of the work.

The main results of the dissertation have been published in 6 scientific papers, including 3 articles in international peer-reviewed journals with an impact factor (Q1), indexed in the Scopus and Web of Science databases, and 3 conference abstracts published in the proceedings of international and national scientific conferences.

Structure and volume of the dissertation

The dissertation is presented on 116 pages of printed text and includes an introduction, literature review, materials and research methods, results and discussion, conclusion, list of references, and appendices. The work is illustrated with 20 tables and 14 figures. The reference list comprises 184 sources.