https://doi.org/10.31925/farmacia.2025.1.11 **ORIGINAL ARTICLE**

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY AND CHEMICAL COMPOSITION OF *FILIPENDULA VULGARIS* MOENCH AND *FILIPENDULA ULMARIA* (L.) MAXIM EXTRACTS OBTAINED BY ULTRASONIC METHOD

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Abstract

Scientific interest in herbal medicines can be explained by their numerous advantages, including lower toxicity compared to synthetic analogues. Plants contain a wide variety of biologically active substances, which contributes to the diverse pharmacological effects of herbal medicines. In this context, the search for new types of medicinal plant materials is recognised as a crucial task. Additionally, developing new methods or enhancing existing technologies for intensifying the extraction of biologically valuable active substances from plants is a highly relevant goal in pharmaceutical science. In the composition of thick extracts, 16 phenolic compounds were identified and quantified using HPLC-MS, including 6 phenolic acids and 10 flavonoids. Our study investigated the anti-inflammatory and regenerative effects of herbal extracts derived from *Filipendula vulgaris* Moench and *Filipendula ulmaria* (L.) Maxim, collected in Central Kazakhstan, and obtained *via* ultrasound-assisted extraction. The anti-inflammatory effects of these thick extracts were evaluated using 2 models: the "formalin oedema" model to simulate acute exudative reactions and the "felt granuloma" model to assess chronic proliferative inflammation. The results, along with histological analyses of the samples, indicate that extracts of *Filipendula vulgaris* and *Filipendula ulmaria* exhibit significant anti-inflammatory and wound-healing properties. These findings highlight their potential for the development of domestic anti-inflammatory and wound-healing drugs.

Rezumat

Studiul a investigat efectele antiinflamatorii și regenerative ale extractelor derivate din *Filipendula vulgaris* Moench și *Filipendula ulmaria* (L.) Maxim, colectate în Kazahstan și obținute prin extracție cu ultrasunete. În compoziția extractelor, 16 compuși fenolici au fost identificați și cuantificați utilizând HPLC-MS, inclusiv 6 acizi fenolici și 10 flavonoide. Efectele antiinflamatorii ale acestor extracte au fost evaluate cu ajutorul a 2 modele: modelul „edem indus de formol" pentru a simula reacțiile exudative acute și modelul „granulom" pentru a evalua inflamația proliferativă cronică. Rezultatele, împreună cu analizele histologice ale probelor, indică faptul că extractele de *Filipendula vulgaris* și *Filipendula ulmaria* prezintă proprietăți antiinflamatorii și cicatrizante semnificative. Astfel, a fost evidențiat potențialul acestor extracte pentru dezvoltarea de noi candidați terapeutici cu efect antiinflamator și cicatrizant.

Keywords: *Filipendula vulgaris*, *Filipendula ulmaria*, anti-inflammatory activity, ultrasound

Introduction

Particular interest in herbal medicines is justified by their low toxicity and other advantages compared to synthetic drugs. The diverse array of biologically active substances in plants ensures a broad spectrum of pharmacological effects in herbal medicines.

In Kazakhstan, approximately 196 medicinal plant species from 74 genera have been identified, representing one-fourth of the entire family of plants in the republic, which comprises 885 species. This highlights the importance of the search for new types of medicinal plant raw materials, making it a highly relevant task [1-6]. It is well known that many plants possess medicinal properties and are widely used in both traditional and modern medical practices. The main criteria for selecting plant raw materials as sources of biologically active substances include: a high quantitative content of key components, the availability of raw materials in nature and the simplicity of cultivation techniques. Among such plants are *Filipendula vulgaris* Moench and

Filipendula ulmaria (L.) Maxim, which are prevalent in Kazakhstan [3].

The genus *Filipendula* L. (meadowsweet) comprises perennial herbaceous plants belonging to the family *Rosaceae* Juss. According to foreign studies, plants of the genus *Filipendula* L. are known to contain biologically active substances with valuable properties, including flavonoids, glycosides, triterpenoids, tannins, catechins, essential oils and small amounts of ascorbic acid [3, 4].

In the recent years, interest in plants of the genus *Filipendula* L. has continued to grow due to their significant resource potential and wide range of pharmacological activities. Advances in modern research methodologies have further expanded opportunities for their study and application.

Extracts from the leaves and flowers of *Filipendula ulmaria* and *Filipendula vulgaris*, collected in Serbia (near Belgrade) and Romania (near Basia Lokva and the Aninej Mountains), have demonstrated antioxidant properties. Furthermore, methanol extracts from the aerial parts of these plants exhibit gastroprotective, anti-ulcer, anti-inflammatory (Samardzic S *et al.*), antigenotoxic (Matic S *et al.*), hepatoprotective (Cebovic T. *et al.*) and antioxidant (Gurita VG *et al.*) effects [7-11].

Researchers from various countries have proposed dry extracts of *Filipendula ulmaria* as active ingredients for nootropic, adaptogenic (Shilova IV *et al.*), hepato-protective (Neagu E *et al.*) and immunotropic (Pukalskiene M *et al.*) agents [12-14].

This underscores the relevance of not only identifying promising natural sources of biologically active substances but also developing more efficient methods for extracting these substances from plant raw materials. In this context, one of the most promising physical methods for intensifying technological processes involves the application of mechanical vibrations in the ultrasonic range. This method facilitates the extraction process by disrupting the intracellular structure and releasing cellular contents to accelerate mass transfer. Additionally, it enhances the conversion rate of ultrasonic wave oscillation energy into thermal energy, thereby accelerating the extraction process. This effect occurs regardless of whether dry or pre-soaked raw materials are used and is explained by the mechanical action of translucent acoustic solvent cavitation at the cellular and sub-cellular levels.

The analysis and systematization of literature on the genus *Filipendula* L. and its pharmacological activity demonstrate that research on these plants, as well as the development of analytical and processing methods, continues abroad. However, there are currently no pharmaceutical products based on these raw materials in the Republic of Kazakhstan. The development of such products, including the determination of their qualitative and quantitative composition, biological activity, standardization and the creation of regulatory documentation in the form of monographs for inclusion in the State Pharmacopoeia of the Republic of Kazakhstan, is of significant interest to the domestic pharmaceutical industry. This forms the objective of our research, the relevance of which is confirmed by an analysis of available scientific sources [15, 16].

The purpose of our study was to investigate the anti-inflammatory activity of thick extracts of *Filipendula vulgaris* Moench and *Filipendula ulmaria* L. obtained through ultrasonic extraction.

Materials and Methods

The starting raw materials consisted of *Filipendula vulgaris* and *Filipendula ulmaria* herbs, collected in Central Kazakhstan (Karaganda region, Abay district, coordinates N 49°50'553", E 73°25'366" and N 49°53'375", E 73°20'678", during the flowering phase, 2019). All studies related to the identification and description of the raw materials at macroscopic and microscopic levels, as well as their collection and preparation, were conducted in compliance with the State Pharmacopoeia of the Republic of Kazakhstan (SP RK), the Eurasian Economic Union (EAEU) Pharmacopoeia and the principles of Good Agricultural and Collection Practices (GACP) [16-19].

Plant extracts of the 2 types of meadowsweet were prepared at the pharmacotechnological laboratory of the School of Pharmacy, NCJSC "Karaganda Medical University", Kazakhstan. The extracts were obtained using a double-extraction method in an ultrasonic bath (VGT-1200, China) operating at an ultrasonic frequency of 40 kHz for 30 min *per* cycle (total extraction duration: 60 min) with a raw material-to-extractant ratio of 1:20. The extractant used was 70% ethanol, as described in previous research [16]. Following ultrasonic extraction, the extracts were filtered and concentrated using a Labtech IR-1LT vacuum rotary evaporator (DLab Scientific Inc, USA) at 50°C. The resulting viscous extracts (moisture content ≤ 25%) of the 2 types of meadowsweet were obtained.

Study of chemical composition using HPLC-MS

HPLC coupled with ultraviolet (UV) detection and first-time tandem mass spectrometry (ESI-MS/MS) was used to analyse the phenolic compounds of the extracts. The following reagents were used in the process: HPLC grade acetonitrile (ACN) (≥ 99.9%, Sigma-Aldrich, France), formic acid (99 - 100%, AnalaR NORMAPUR®, VWR Chemicals, France), highly purified prepared water, obtained using the Milli-Q water purification system (Millipore, France). Standards 20 phenolic compounds: catechin, epicatechin, naringin, rutin, luteolin-7-O-glucoside, quercetin 3-glucoside, dihydro-quercetin, myricetin, quercetin, naringenin, apigenin, luteolin, kaempferol, plant acid, gallic acid, chlorogenic acid, cinnamic acid ferulic acid, p-coumaric acid, rosmarinic acid, (Sigma – Aldrich, USA).

Analysis of the results on a liquid chromatograph Agilent 1260 Infinity HPLC system (Agilent Technologies,

USA), equipped with a 4-channel pump G1311C 1260, a VL pump, an autosampler G1329B 1260 ALS, a thermostat-column G1316A 1260 TCC; G1314C 1260 VWD VL+ variable wavelength detector and G6130A quadrupole LC-MS/MS mass spectrometer, ChemStation software running Windows NT was used [20].

Chromatographic separation was carried out on a column with a reverse-phase sorbent Zorbax Eclipse Plus C18 (150 mm × 4.6 mm, 3.5 μm, Agilent Technologies, USA). For separation, a gradient of mobile phase A (2.5% solution of formic acid in water) and mobile phase B (2.5% solution of formic acid in acetonitrile) was used. The gradient profile was set as follows: 0.00 min 3% eluent B, 7.00 min 20% eluent B, 7.10 min 30% eluent B, 27.00 min 40% eluent B, 35.00 min 50% eluent B, 35.10 min 20% eluent B and 40.00 min 3% eluent B. Flow rate was set at 0.4 mL/min, column temperature 30ºC. Ultrasonic extracts and standards were dissolved in a solvent mixture of acetonitrile:water = 1:1 (v/v). The injection volume was 20 μL for extract and standard solutions. The column effluent passed through a UV detector before reaching the MS interface. The UV detection wavelengths were 280 nm and 360 nm. Electrospray ionization mass spectrometry detection was performed in negative mode with the following optimised parameters: capillary temperature, 350°C; drying gas N_2 8 L/min; spray pressure 45 pounds *per* square inch. Data collection was carried out using a multiple reaction monitoring (MRM) method, which monitors only specific mass transitions within a given retention time. The identification of each compound was made by comparing their retention times with authentic standards and was also confirmed by an Agilent G6130A LC-MS/MS spectrometer equipped with an electrospray ionization source [20].

The content of compounds in the extracts was calculated using the external standard method according to formula (1):

$$X = \frac{S_1 \times m_0 \times 25 \times P \times 100}{S_0 \times m \times 25 \times 100}, \quad (1)$$

where, S_1 is the peak area of the compound in the chromatogram of the test solution; S_0 is the peak area value of the compound in the chromatogram of the standard sample; m_0 is the sample weight of the standard compound, in grams; m_1 - extract weight, in grams; P is the content of the compound in the standard sample of the compound, in %; 25 and 25 - dilutions.

Method for Studying the Anti-inflammatory Effect

The anti-inflammatory effect of thick extracts of the *Filipendula vulgaris* and *Filipendula ulmaria*, obtained *via* ultrasound, was studied using 2 experimental models: "formalin-induced oedema" to simulate an acute exudative reaction and "felt granuloma" to simulate chronic proliferative inflammation. The methodology followed the protocols described in the "Experimental (Preclinical) Guide for Studying New Pharmacological Substances" (Khabriev RU, 2005) and the work of Voronkov AV *et al.* [21, 22].

The experimental study complied with the Minister of Health of the Republic of Kazakhstan's Order No. KR DSM-248/2020, dated on 11st of December 2020, which outlines rules for conducting clinical trials of medicinal products and medical devices outside a living organism (*in vitro*) and requirements for clinical sites. It also adhered to Good Laboratory Practice (GLP) standards, the European Convention for the Protection of Experimental Animals (ETS 123), the Guide for the Care and Use of Laboratory Animals (8th edition), ARRIVE guidelines (2.0) and Directive 2010/63/EU of the European Parliament and of the Council [23-27]. Permission for conducting medical and biological experiments involving animals was obtained from the Bioethics Committee of NCJSC "Karaganda Medical University" (Protocol No. 18, dated on 16th May 2019, with assigned No. 50). This study formed part of the dissertation research of PhD student Tulebayev Ye.A., entitled "Pharmacognostic study and prospects for the use in medicine of *Filipendula vulgaris* and *Filipendula ulmaria* growing in Central Kazakhstan" under specialty 6D110400 - Pharmacy [16].

To evaluate the anti-inflammatory effect in the two models, 80 outbred white rats of both sexes, weighing 230 - 250 g, were used (40 animals *per* model). The rats were divided into 4 groups, each containing 10 animals *per* model.

Group I (Control): Animals received an equivalent volume of purified water (n = 10).

Group II (Comparison): Animals received the comparison drug diclofenac sodium at a dose of 25 mg/kg (n = 10).

Group III (Experimental): Animals received a thick extract of *Filipendula vulgaris* (FV extract) at a dose of 25 mg/kg (n = 10).

Group IV (Experimental): Animals received a thick extract of *Filipendula ulmaria* (FU extract) at a dose of 25 mg/kg (n = 10).

In the formalin-induced paw oedema model, acute exudative inflammation was induced by injecting 0.1 mL of a 2% formalin solution into the back of each rat's paw. The volume of the animals' limbs was measured using the oncometric method before and 3 h after the phlogogen injection. The increase in limb volume and the inflammation inhibition rate served as key criteria for evaluating the anti-inflammatory effectiveness. The increase in oedema was calculated using the formula used in the work of Voronkov AV *et al.* [22]:

$$P = (O-I)/I \times 100, \quad (2)$$

where, P is the increase in oedema; O is the volume of the paw after administration of the inflammation inducer; I – the volume of the paw before the injection of the inflammation inducer.

The degree of inflammation inhibition was calculated using the formula:

$$100\% - [((O-I)/I \times O)/((O-I)/I \times K)] \times 100, \quad (3)$$

where, O is the increase in oedema in experimental animals; K – increase in oedema in the control group.

Assessment of the exudative phase and proliferation process

To evaluate the intensity of the exudative phase and the quality of the proliferation process, the "felt granuloma" model was used. Under chloral hydrate anaesthesia (350 mg/kg), the rats' hair was clipped from the back area. Under aseptic conditions, a 1 cm incision was made in the skin and subcutaneous tissue using scissors. A cavity was created in the subcutaneous tissue with tweezers, into which a sterile cotton ball weighing 10 mg was implanted. The wound was then closed with 2 sutures.

On the 8th day, the cotton balls surrounded by the granulation tissue were removed, weighed on an electronic scale and dried in an oven to a constant weight at 60°C. The proliferative response was evaluated as the difference between the weight of the dried granuloma and the initial weight of the cotton ball. The exudative reaction was assessed as the difference between the weight of the wet and dried granuloma. The anti-inflammatory effect was expressed as a percentage relative to the control group.

Histological examination of samples

Preparation and processing of research objects. After the experimental phase, samples were prepared and processed following the protocols described in a previous research [28]. The histological examination focused on the area of the surgical wound containing the foreign body (sterile cotton wool) and a 2% formaldehyde solution.

After the animals were euthanised, a skin fragment was resected from the surgical intervention area. The sample included the wound bottom, its border and adjacent intact tissue. The collected material was fixed in 10% neutral buffered formalin. The samples, no thicker than 5 mm, were placed in histological cassettes for processing. Paraffin blocks were prepared using a carousel-type tissue processor. From each paraffin block, representative histological sections 5 μm thick were obtained and stained with haematoxylin and eosin. For histopathological examination, 5 samples of large intestine tissue, measuring 0.5 - 1.0 cm in length, were taken from each section of the intestine in both cross-sectional and longitudinal planes.

Histological and morphometric analysis

To achieve the study objectives, a comparative morphometric analysis of the inflammatory response (acute and chronic inflammation), granulation tissue, re-epithelialization and neovascularization was conducted. Basic morphometric measurements and imaging were performed using a Leica DM1000 microscope (Leica Microsystems GmbH, Germany) at magnifications of 40x, 100x and 400x. Digital microphotographs were captured, and measurements were analysed using Leica Image software (version 6).

Statistical analysis

The statistical processing of the results was carried out using the Statistica software (StatSoft, trial version) and Microsoft Excel. The data are presented as the mean ± standard error of the mean (SEM). For the comparisons between 2 groups, the nonparametric Mann-Whitney U test was used. For comparisons among 3 or more independent groups, the Kruskal-Wallis test was applied. Differences were considered statistically significant at a p-value of ≤ 0.05.

Results and Discussion

The data obtained indicate that extracts of *Filipendula vulgaris* and *Filipendula ulmaria* exhibit anti-inflammatory effects, which can be attributed to the presence of phenolic compounds such as cynaroside, apigenin, quercetin and gallic acid [29]. The ability of luteolin-7-glucoside to regulate proliferative responses has also been investigated. It was found that local administration of cynaroside significantly reduced acanthosis. The cynaroside treatment led to a decrease in the expression of proliferation markers, an increase in the production of differentiation markers and overall phenotypic improvement [30-32]. Furthermore, apigenin, quercetin and gallic acid were shown to downregulate inflammatory cytokine expression and protect cells from oxidative stress-induced cell death [33, 34]. These findings suggest that these phenolic compounds may play a beneficial role in the therapeutic management of inflammatory diseases.

Phenolic compounds identified in *Filipendula vulgaris* and *Filipendula ulmaria* extracts by HPLC have significant anti-inflammatory potential through different mechanisms of action. The results of the study suggest that they are able to inhibit the activity of inflammatory enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), and regulate the synthesis of proinflammatory and anti-inflammatory cytokines (IL-1β, IL-6, TNF-α and IL-10). Another possible mechanism may be their antioxidant activity, reducing oxidative stress through neutralization of free radicals. It is possible that these compounds modify signalling pathways, such as NF-κB and MAPK, and stabilize cell membranes, preventing the release of inflammatory mediators. Further studies are needed to clarify this. The ultrasonic extraction method probably contributes to an increase in the content of biologically active substances during the extraction of these types of meadowsweet raw materials, which potentially enhances their therapeutic effect [35-39].

The composition of phenolic compounds in the extracts of *Filipendula vulgaris* and *Filipendula ulmaria* obtained *via* the ultrasonic method, along with the mass spectra of the identified compounds in negative ionization mode, are presented in Table I.

Table I

Identification and content of phenolic compounds in extracts of the *Filipendula vulgaris* and *Filipendula ulmaria*

Peak No.	Retention time, min	Experimental mas M-H$^-$ (m/z)	Identified components	Content (mg/g *per* extract weight)	
				Extract of *Filipendula vulgaris*	Extract of *Filipendula ulmaria*
1	8.887	169	gallic acid	4.85 ± 0.15	4.94 ± 0.11
2	13.186	353	chlorogenic acid	3.13 ± 0.08	3.62 ± 0.19
3	13.226	289	catechin	2.58 ± 0.05	3.81 ± 0.13
4	13.946	289	epicatechin	2.27 ± 0.07	0.11 ± 0.10
5	14.128	609	routine	4.38 ± 0.17	4.23 ± 0.11
6	14.584	447	luteolin-7-o-glucoside (cynaroside)	46.31 ± 0.28	37.42 ± 0.19
7	14.651	463	quercetin-3'-glucoside (isoquercetin)	1.40 ± 0.10	1.35 ± 0.09
8	16.12	303	dihydroquercetin	3.43 ± 0.21	2.50 ± 0.17
9	16.315	193	ferulic acid	2.55 ± 0.06	2.34 ± 0.04
10	16.829	163	m-coumaric acid	1.51 ± 0.02	1.12 ± 0.01
11	16.858	359	rosmarinic acid	2.23 ± 0.08	3.07 ± 0.15
12	18.412	163	o-coumaric acid	4.29 ± 0.10	2.05 ± 0.08
13	22.289	301	quercetin	7.92 ± 0.16	7.94 ± 0.13
14	27.06	271	naringenin	1.11 ± 0.02	1.14 ± 0.05
15	27.218	269	apigenin	14.15 ± 0.18	16.57 ± 0.20
16	28.656	285	kaempferol	3.26 ± 0.07	3.44 ± 0.11

As shown in Table I, a total of 16 phenolic compounds were identified and quantified in the extracts of the *Filipendula vulgaris* and *Filipendula ulmaria*, including 6 phenolic acids and 10 flavonoids. While the extracts demonstrated similarities in their qualitative composition, significant differences were observed in the quantitative content of phenolic acids and flavonoids.

The dominant phenolic compounds in the extracts were cynaroside, with contents of 46.31 mg/g and 37.42 mg/g, apigenin (14.15 mg/g and 16.57 mg/g), quercetin (7.92 mg/g and 7.94 mg/g) and gallic acid (4.85 mg/g and 4.94 mg/g) for *F. vulgaris* and *F. ulmaria*, respectively.

Studies revealed that *Filipendula* sp. extracts comprise several classes of the phenolic compounds, primarily flavonoids, hydrolysable tannins, procyanidins, derivatives of gallic and caffeic acids and gaulterin (a derivative of salicylic acid). Additionally, flavonoid glycosides such as isoquercitrin, hyperoside and spireoside, along with hydrolysable tannins, were identified as the main components of the aerial parts of *Filipendula* sp. [11, 16].

Results of anti-inflammatory activity studies

The results of anti-inflammatory activity of *Filipendula vulgaris* and *Filipendula ulmaria* extracts in the formalin-induced paw edema model are presented in Table II.

Table II

Indicators of anti-inflammatory activity of extracts of 2 types of meadowsweet in acute exudative inflammation in model formalin-induced paw oedema

Groups, n = 10, dose 25 mg/kg	Paw thickness, mm		Increase in oedema, %	Inhibition of inflammation, %
	Before injection	3 h after injection		
Group I	1.29 ± 0.27	1.94 ± 0.23	50.38 ± 0.25	-
Group II	1.23 ± 0.17	1.34 ± 0.13	8.94 ± 0.05	82.25 ± 0.17
Group III	1.23 ± 0.11	1.33 ± 0.09	8.13 ± 0.07*	83.86 ± 0.09*
Group IV	1.26 ± 0.02	1.37 ± 0.05	8.73 ± 0.08**	82.67 ± 0.10**

$p \leq 0.01$ relative to the values in the corresponding groups before the introduction of formalin; * – significance of differences with the comparison group $p < 0.05$; ** – significance of differences with the comparison group $p < 0.01$

The tested extracts of the *Filipendula vulgaris* and *Filipendula ulmaria*, when administered orally, demonstrated a pronounced inhibitory effect on the development of formaldehyde-induced oedema, and comparable to that of the reference drug diclofenac sodium. In rats treated with *Filipendula vulgaris* and *Filipendula ulmaria* extracts at a dose of 25 mg/kg, oedema inhibition after 3 h reached 83.86% and 82.67%, respectively, closely matching the effect of diclofenac sodium, which was 82.25%.

In the case of chronic proliferative inflammation, assessed using the felt granuloma model, the impact of the *Filipendula vulgaris* and *Filipendula ulmaria* extracts on the exudation and proliferation phases was evaluated. Sterile cotton balls weighing 10 mg were implanted subcutaneously into the backs of rats and removed on the 8th day. Granulation tissue formed around the cotton balls was weighed using an electronic scale and dried in an oven at 60^{o}C to a constant weight. The proliferative response was determined as the difference between the weight of the dried granuloma and the initial weight of the cotton ball. The exudative reaction was calculated as the difference between the wet and dried granuloma weights. The anti-inflammatory effect, expressed as a percentage relative to the control, is presented in Table III.

Table III

The influence of extracts of 2 types of meadowsweet on the indicators of the exudation and proliferation phases in chronic proliferative inflammation in the felt granuloma model

Groups, n = 10, dose 25 mg/kg	Exudation, mg	Exudation (relative to control), %	Proliferation, mg	Proliferation (relative to control), %
Group I	0.257 ± 0.015	-	0.072 ± 0.003	-
Group II	0.168 ± 0.010*	65.37 ± 0.05	0.024 ± 0.004*	33.33 ± 0.34
Group III	0.159 ± 0.012**	61.87 ± 0.01	0.019 ± 0.001**	26.39 ± 0.25
Group IV	0.156 ± 0.011	60.71 ± 0.08	0.026 ± 0.002	36.11 ± 0.18

$p \leq 0.01$ relative to the indicators in the corresponding groups; * – significance of differences with the comparison group $p < 0.05$;
** – significance of differences with the comparison group $p < 0.01$

According to the data presented in Table III (from the felt granuloma model), the administration of the *Filipendula vulgaris* and *Filipendula ulmaria* extracts, as well as the reference drug diclofenac sodium, significantly reduced the exudation phase compared to the control group. The degree of exudation observed with *Filipendula vulgaris* and *Filipendula ulmaria* extracts was statistically comparable to that seen with diclofenac sodium, indicating similar efficacy in reducing exudation.

In terms of proliferative activity, the *Filipendula vulgaris* extract resulted in the lowest intensity of proliferation (26%) among the experimental groups, while the effect of the *Filipendula ulmaria* extract on proliferation was comparable to that of diclofenac sodium.

Results of comparative microscopic analysis

A comparative microscopic analysis of tissue samples from the "Formalin-induced paw oedema" model revealed a small number of collagen fibres in all groups. However, in Groups III (treated with *Filipendula vulgaris*) and IV (treated with *Filipendula ulmaria*), the collagen fibres were more organised, evenly crimped and parallel, with uniform spacing between fibres. Additionally, flattened cells were observed in a linear arrangement. These observations are illustrated in Figure 1.

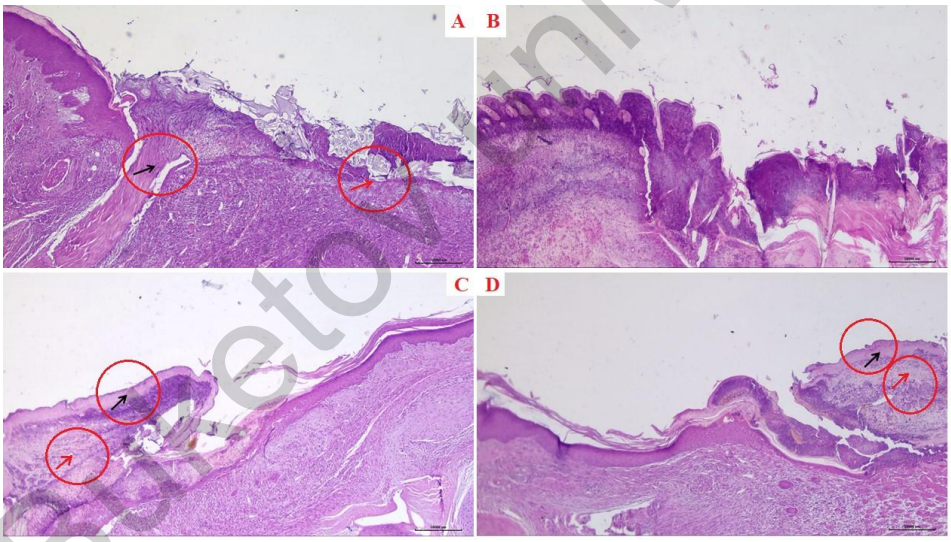

Figure 1.

Areas of the wound of the limb, formed surgically using a 2% formaldehyde solution, following the formalin-induced paw oedema model. Characterizations of formulations: A – microscopy of group I (control); B – group II (diclofenac sodium); C – group III (FV extract); D – group IV (FU extract). Staining: haematoxylin and eosin. Magnification: x40

In Figure 1, the microscopic analysis showed the following: Group I (A - control): At the edge of the wound (indicated by the black arrow), the growth of connective tissue delimiting the affected area is visible. The bottom of the wound (marked by the red arrow) shows granulation tissue with scattered granulocytic and lymphohistiocytic infiltration. The surface epithelium is absent. Group II (B - diclofenac sodium): A large area of granulation tissue is observed, and re-epithelialization of the wound defect is evident. Group III (C - FV extract): The wound area is completely re-epithelialized (black arrow), and a substantial amount of mature granulation tissue is present at the bottom of the wound (red arrow). Group IV (D - FU extract): Similar to Group III, the defect area is fully re-epithelialized (black arrow), and a significant amount of mature granulation tissue is found in the wound bottom (red arrow).

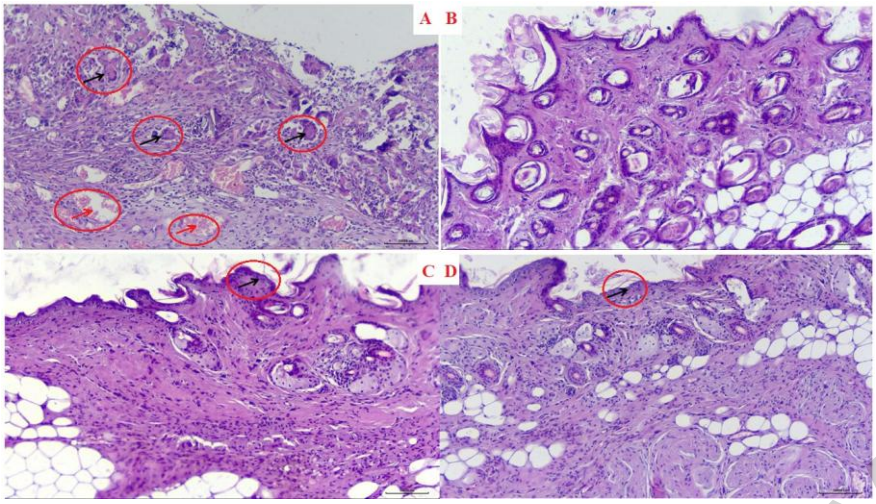

Figure 2.
The area of the wound of the limb, formed surgically using sterile cotton wool (the centre of the lesion), following the felt granuloma model. Characterizations of formulations: A – microscopy of group I (control; magnification: x100); B – group II (diclofenac sodium; magnification: x40); C – group III (FV extract; magnification: x40), D – group IV (FU extract; magnification: x40). Staining: haematoxylin and eosin

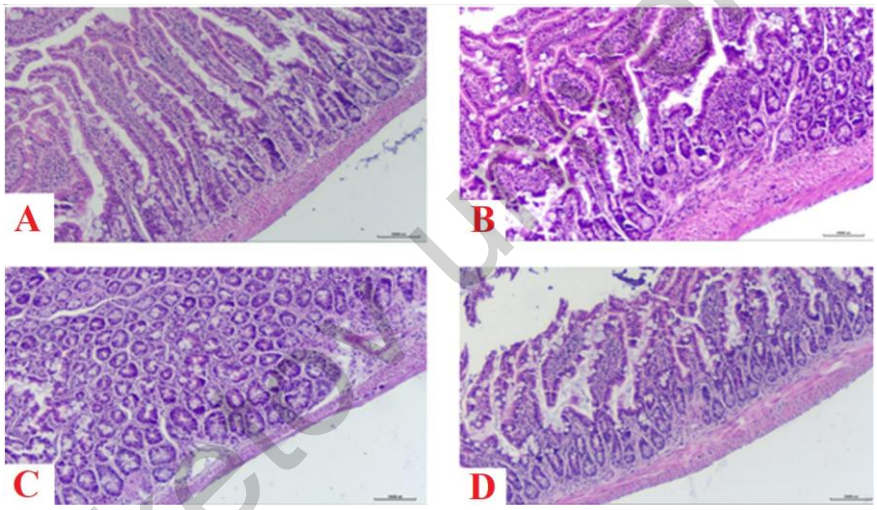

Figure 3.
Intestinal tissue. Characterizations of formulations: A – group I (control), B – group II (diclofenac sodium), C – group III (FV extract), D – group IV (FU extract). Staining: haematoxylin and eosin. Magnification: x40.

Regarding the results of the "felt granuloma" model, Figure 2 illustrates the microscopic analysis, where a small number of collagen fibres of uniform width are observed. These fibres are uniformly crimped, arranged parallel to one another with even spacing between them. Flattened cells are also noted, arranged in a linear order. Figure 2 shows the following observations.

Group I (A - control): Immature granulation tissue is observed with scattered lymphocytic infiltration and focal accumulation of granulocytes. At the bottom of the wound, a large number of giant multinucleated foreign body cells (black arrows) and newly formed, full-blooded vessels (red arrow) are visible. The surface of the wound lacks an epithelial layer.

Group II (B - diclofenac sodium): Mature granulation tissue is observed, and the defect area is fully epithelialized.

Group III (C - FV extract): Mature granulation tissue is present, and the defect area is completely epithelialized (black arrow).

Group IV (D - FU extract): Similar to Group III, mature granulation tissue is seen, and the defect area is fully epithelialized (black arrow).

Figure 3 presents the results of microscopic examination of intestinal sections. All groups exhibited the preservation of the normal morphological structure of the intestine, with no visible differences in tissue and cellular structures.

As can be seen in Figure 3, the morphological pattern of the intestine was preserved; no differences were

found in groups III (FV extract) and IV (FU extract) from the control group and the group using diclofenac.

Conclusions

In the thick extracts of the *Filipendula vulgaris* and *Filipendula ulmaria*, 16 phenolic compounds were identified and quantified using HPLC-MS, with 6 phenolic acids and 10 flavonoids. The dominant compounds were cynaroside (46.31 mg/g for *F. vulgaris* and 37.42 mg/g for *F. ulmaria*), apigenin (14.15 mg/g and 16.57 mg/g, respectively), quercetin (7.92 mg/g and 7.94 mg/g) and gallic acid (4.85 mg/g and 4.94 mg/g).

The results of the study on anti-inflammatory and wound-healing activity demonstrate that the tested extracts of the *Filipendula vulgaris* and *Filipendula ulmaria* have a significant inhibitory effect on the development of formaldehyde-induced oedema when administered orally, showing effects comparable to the reference drug diclofenac sodium. In the felt granuloma model, both extracts, next to diclofenac sodium, significantly reduced the exudation phase. The intensity of proliferative processes was particularly pronounced in the *Filipendula vulgaris* extract, with results comparable to those of diclofenac sodium.

Histological evaluation revealed that both meadowsweet extracts reduced the activity of acute inflammation and diminished the chronic inflammatory response. They promoted increased cell proliferation, as evidenced by the active formation of granulation tissue and the accelerated re-epithelialization. Moreover, both extracts facilitated collagen formation and angiogenesis.

Overall, the data suggest that *Filipendula vulgaris* and *Filipendula ulmaria* extracts possess significant anti-inflammatory and wound-healing potential, positioning them as promising candidates for the development of the domestic anti-inflammatory and wound-healing medications.

Conflict of interest

The authors declare no conflict of interest.

References

1. Tulebayev YeA, Losseva IV, Ishmuratova My, Current state of scientific research on *Filipendula ulmaria* and *Filipendula vulgaris* as sources of biologically active substances. Bulletin KazNMU, 2020; 1: 555-561.
2. Badekova KZh, Atazhanova GA, Ishmuratova MYu, Akhmetova SB, Brazhanova AK, Tazhina AM, Morpho-anatomical study of *Origanum vulgare* L. growing in the Akmola Region of Kazakhstan. *Res J Pharm Tech.*, 2024; 17(7): 3013-3018.
3. Tulebayev YeA, Ishmuratova MYu, Losseva IV, Kukuła-Koch W, Poleszak E, Nadirbek SK, Comparative histochemical analysis of above-ground parts of *Filipendula vulgaris* and *Filipendula ulmaria* growing in Central Kazakhstan. *Res J Pharm Tech.*, 2021; 14(9): 4863-4867.
4. Medeshova A, Orazbayeva P, Romanova A, Dildabekova N, Orazbayev B, Ashirbekova B, Ashirbekova A, Development and Rheological studies of gels with Essential oil of *Chamomile* (*Chamomillae recutita* L.). *Res J Pharm Tech.*, 2023; 16(11): 5161-5166.
5. Itzhanova HI, Remetova NS, Ishmuratova MYu, Nogayeva AN, Abdrakhmanova GM, Medeshova AT, Samorodov AV, Histochemical analysis of *Xanthium strumarium* raw materials, growing on the territory of Kazakhstan. *Res J Pharm Tech.*, 2023; 16(12): 5796-5801.
6. Medeshova A, Orazbayeva P, Tulebayev Y, Kaiyrbekova K, Dildabekova N, Romanova A, Ashirbekova B, Orazbayev B, Development of composition and technology of suppositories based on substance from medicinal plant raw materials *Rosmarinus officinalis* L. *Res J Pharm Tech.*, 2024; 17(11): 5452-5456.
7. Samardžić S, Arsenijević J, Božić D, Milenković M, Tešević V, Maksimović Z, Antioxidant, anti-inflammatory and gastroprotective activity of *Filipendula ulmaria* (L.) Maxim and *Filipendula vulgaris* Moench. *J Ethnopharmacol.*, 2018; 213: 132-137.
8. Matić S, Katanić J, Stanić S, Mladenović M, Stanković N, Mihailović V, *In vitro* and *in vivo* assessment of the genotoxicity and antigenotoxicity of the *Filipendula hexapetala* and *Filipendula ulmaria* methanol extracts. *J Ethnopharmacol.*, 2015; 174: 287-292.
9. Cebovic T, Maksimovic Z, Hepatoprotective effect of *Filipendula hexapetala* Gilib. (*Rosaceae*) in carbon tetrachloride-induced hepatotoxicity in rats. *Phyto Research.*, 2012; 26(7): 1088-1091.
10. Ciobotaru LGG, Pavel AZ, Poenaru R, Moaca E, Florescu R, Danciu R, Dumitrascu C, Imbrea I, Pop O, Assessment of the antioxidant effect of ethanolic extracts obtained from *Agrimonia eupatoria* L., *Filipendula ulmaria* (L.) Maxim and *Filipendula vulgaris* Moench collected from the eastern part of Romania. *Rev Chim (Bucharest).*, 2018; 69(9): 2385-2390.
11. Katanić J, Pferschy-Wenzig EM, Mihailović V, Boroja T, Pan SP, Nikles S, Kretschmer N, Rosić G, Selaković D, Joksimović J, Bauer R, Phytochemical analysis and anti-inflammatory effects of *Filipendula vulgaris* Moench extracts. *Food Chem Toxicol.*, 2018; 122: 151-162.
12. Shilova IV, Samylina IA, Suslov NI, Development of nootropic drugs based on Siberian plants. Tomsk: Print manuf., 2013: 268.
13. Neagu E, Paun G, Albu C, Radu G, Assessment of acetylcholinesterase and tyrosinase inhibitory and antioxidant activity of *Alchemilla vulgaris* and *Filipendula ulmaria* extracts. *J Taiwan Inst Chem Eng.*, 2014; 52: 1-7.
14. Pukalskiene M, Venskutonis PR, Pukalskas A, Phytochemical characterization of *Filipendula ulmaria* by UPLC/Q-TOF-MS and evaluation of antioxidant activity. *Rec Nat Prod.*, 2015; 9(3): 451-455.
15. Tulebayev YeA, Nadirbek SK, Akhmetzhan GB, Losseva IV, Prospects for the use of ultrasonic influence for the extraction of flavonoids from *Filipendula vulgaris* and *Filipendula ulmaria*. *Coll Sci Pap.*, 2020: 236-244.
16. Tulebayev YeA, Pharmacognostic study and prospects for use in medicine of *Filipendula vulgaris*, *Filipendula ulmaria*, growing in the territory of Central Kazakhstan.

NJSC "KazNMU named after S. D. Asfendiyarov", 2023: 163.
17. Decision of the Board of the Eurasian Economic Commission No. 100 of 08/15/2020 "Pharmacopeia of the Eurasian Economic Union", https://docs.eaeunion.org/docs/ru-ru/01426917/err_13082020_100.
18. State Pharmacopoeia of the Republic of Kazakhstan. Publ hous "Zhibek Zholy", 2008; 1: 592.
19. Decision of the Council of the Eurasian Economic Commission No. 15 of 01/26/2018 "On approval of the Rules for good practice in the cultivation, collection, processing and storage of raw materials of plant origin", https://adilet.zan.kz/rus/docs/H18EV000015.
20. Tulebayev YeA, Pharmacognostic study and prospects for use in medicine of *Filipendula vulgaris*, *Filipendula ulmaria*, growing in the territory of Central Kazakhstan: diss. doc. philosopher. (PhD): 6D110400 - Pharmacy / NJSC "KazNMU". Almaty, 2023; 145 p.
21. Khabriyev RU, Guide to experimental (preclinical) study of new pharmacological substances. *Publ House Med*., 2005; 2: 832.
22. Voronkov AV, Luzhnova SA, Comparative analysis of the anti-inflammatory and analgesic effects of the acyclic precursor 1,3-diazinon-4 compound PYATd1 and the drug Dapsone. *Bulletin VolgSMU.*, 2020; 2(74): 109-113.
23. Order of the Minister of Health of the Republic of Kazakhstan No. KR DSM-248/2020 dated 12/11/2020 "On approval of the rules for conducting clinical trials of medicines and medical devices for diagnostics outside a living organism (in vitro) and the requirements for clinical sites and the provision of public services "Issue of permission for conducting clinical research and (or) testing of pharmacological and medicinal products, medical devices" (as amended and supplemented on 05/26/2023), https://adilet.zan.kz/rus/docs/V2000021772.
24. Acting order Minister of Health of the Republic of Kazakhstan No. KR DSM-15 dated 02/04/2021 "On approval of good pharmaceutical practices" (as amended and additionally dated 04/03/2023), https://adilet.zan.kz/rus/docs/V2100022167.
25. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Hurst V, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H, Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.*, 2020; 18(7): e3000411.
26. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Nat Acad Press US., 2011.
27. Directive 2010/63/EU of the European Parliament and of the Council of 09/22/2010 on the protection of animals used for scientific purposes Text with EEA relevance. *Spec ed Croat*, 2010; 15(028): 82-128 (version: 26/06/2019), http://data.europa.eu/eli/dir/2010/63/oj.
28. Kittel B, Ruehl-Fehlert C, Morawietz G, Klapwijk J, Elwell MR, Lenz B, O'Sullivan MG, Roth DR, Wadsworth PF, RITA Group; NACAD Group. Revised guides for organ sampling and trimming in rats and mice-Part 2. A joint publication of the RITA and NACAD groups. *Exp Toxicol Pathol.*, 2004; 55(6): 413-431.
29. Zou Y, Zhang M, Zhang T, Wu J, Wang J, Liu K, Zhan N, Antioxidant and anti-inflammatory activities of cynaroside from *Elsholtiza bodinieri*. *Nat Prod Commun.*, 2018; 13(12): 1501-1504.
30. Palombo R, Savini I, Avigliano L, Madonna S, Cavani A, Albanesi C, Mauriello A, Melino G, Terrinoni A, Luteolin-7-glucoside inhibits IL-22/STAT3 pathway, reducing proliferation, acanthosis, and inflammation in keratinocytes and in mouse psoriatic model. *Cell Death Dis.*, 2016; 7(8): e2344.
31. Gayibova S, Ivanisova E, Herbs and Spices - New Perspectives in Human Health and Food Industry 2024; https://doi.org/10.5772/intechopen.1005623
32. Rozirwan, Khotimah NN, Putri WAE, Fauziyah, Apri R, Isnaini, Nugroho RY, Investigating the antioxidant activity, total phenolics, and phytochemical profile in *Avicennia alba* and *Excoecaria agallocha* root extracts as a defense mechanism against pollutants. *Farmacia*, 2024; 72(5): 1216-1226.
33. Yoon JH, Kim MY, Cho JY, Apigenin: A Therapeutic Agent for Treatment of Skin Inflammatory Diseases and Cancer. *Int J Mol Sci.*, 2023; 24(2): 1498.
34. Zhao X, Zhang L, Wu N, Liu Y, Xie J, Su L, Zhu Q, Unger BH, Altaf F, Hu Y, Ye W, Qiao Z, Cheng Y, Zhang W, Wang Y, Xia XQ, Gallic acid acts as an anti-inflammatory agent *via* PPARγ-mediated immunomodulation and antioxidation in fish gut-liver axis. *Aquaculture*, 2024; 578: 740142.
35. Ivanov AA, Petrov BB, Biological activity of phenolic compounds in plant extracts. *J Exp Clin Med.*, 2020; 59(3): 45-52.
36. Smirnov SV, Kuznetsova OM, Antioxidant properties of flavonoids and their effect on inflammatory processes. *Q Biochem.*, 2019; 45(2): 123-134.
37. Kovalenko II, Lebedev AN, Free radicals and their neutralization: The role of plant antioxidants. *Russ J Bioorg Chem.*, 2021; 47(4): 567-578.
38. Sergeeva EA, Mikhaylov KD, Influence of plant phenolic compounds on the expression of inflammation-related genes. *Mol Med.*, 2020; 8(1): 27-35.
39. Fyodorov PV, Pavlova LA, Advantages of ultrasonic extraction of biologically active compounds from medicinal plants. *Pharm J.*, 2022; 58(2): 85-92.