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Research Article

Anti-inflammatory and analgesic effects of *Filipendula ulmaria* extract

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Abstract

The present study investigated the chemical content, analgesic, and anti-inflammatory properties of *Filipendula ulmaria* (L.) Maxim. (meadowsweet) aerial part extract. F. ulmaria is native to Bulgaria. Based on its phytochemicals, meadowsweet has a vast range of pharmacological activities, like antipyretic, analgesic, anti-inflammatory, antirheumatic, immunostimulating, anti-microbial, anti-allergic, and gastroprotective activity. In our experiment, *F. ulmaria* aerial part extract was practically non-toxic (oral LD50 > 2000 mg/kg). The performed experiments revealed that diclofenac possesses better anti-inflammatory and antinociceptive activity than *F. ulmaria* extracts (100 and 200 mg/kg). The results revealed a dose-dependent moderate inhibition of the inflammation and pain induced by 3 days of oral pretreatment with methanolic extract of *F. ulmaria* extracts. No additive effect of FUA extracts on the effects of diclofenac was noted. *F. ulmaria* extracts did not induce any alterations in blood biochemical analysis.

Keywords

Filipendula ulmaria, anti-inflammatory activity, antinociceptive activity, phytochemical constituents, secondary metabolites

Introduction

Filipendula ulmaria (L.) Maxim. (meadowsweet) is an herbaceous perennial plant belonging to the Rosaceae family. It is widespread in Europe and temperate regions of Asia (Markova 1973). In recent decades, the interest in this species has been increasing. Phytochemical data on *F. ulmaria* flowers revealed a significant content of polyphenolics, among which flavonol glycosides and ellagitannins are the most distinguished (Olennikov and Kruglova 2013). Elsewhere, the presence of spiraeoside in meadowsweet aerial part extract is reported (Katanić et al. 2015). Additionally, derivatives of salicylic acid, 2-pyrone 4,6-dicarboxylic acid, phenylpropanoids, flavonoids, tannins, and essential oils were reported (Okuda et al. 1992; Wilkes and Glasl 2001; Pemp et al. 2007; Fecka 2009; Blazics et al. 2010).

Based on its phytochemicals, meadowsweet has a vast range of pharmacological activities. The salicylate content determines significant antipyretic, analgesic, and antirheumatic effects (Blazics et al. 2010; Samardžić et al. 2016). Phenolic compounds are responsible for notable antioxidant and anti-inflammatory activity (Katanić et al. 2016; Gurita et al. 2018; Samardžić et al. 2018; Sukhikh et al. 2022; Andonova et al. 2024), as well as immunostimulating (Halkes et

al. 1998), anti-microbial (Barros et al. 2013), and anti-allergic (Nitta et al. 2013) effects of *F. ulmaria*. A gastroprotective property (Samardžić et al. 2018) and effect on gout, common cold, and fever were also established (Jarić et al. 2007; Corp and Pendry 2013). Meadowsweet tea is approved as a new functional beverage (Olennikov et al. 2016).

Notwithstanding the thorough characteristics of *F. ulmaria*, the phytochemical constituents and their anti-inflammatory and analgesic properties are not sufficiently defined. There are numerous animal models for pain research induced by chemical, thermal, or mechanical stimuli (Barrot 2012). Pain is divided into nociceptive, neuropathic, and nociplastic (Yoo and Kim 2024). In clinical settings, it is divided by onset into acute or chronic pain. The main distinction between nociceptive and neuropathic or nociplastic pain is the cessation of pain signaling when the noxious stimulus has either ceased or the tissues have healed. Unlike nociceptive and neuropathic pain, nociplastic pain usually occurs without the presence of disease or lesion and without clear evidence of tissue damage that would activate peripheral nociceptors. Nociceptive pain is triggered by an actual or threatening noxious stimulus that activates nociceptors. Inflammatory pain is a particular subset of nociceptive pain and is similar to acute clinical pain. It occurs secondary to inflammatory mediators release, such as prostaglandins, cytokines, nerve growth factor, lipids, lipoxygenase products, and ATP (Woolf 2010; Larson et al. 2019).

The current study aimed to examine the chemical content of *Filipendula ulmaria* (L.) Maxim. aerial parts extract (FUA) and its antinociceptive and anti-inflammatory properties.

Materials and methods

Chemicals

Carrageenan (CRG) (No. 22049) and diclofenac sodium (No. D6899) were purchased from Sigma-Aldrich. Acetonitrile (hypergrade for LC–MS), formic acid (for LC-MS), and methanol (analytical grade) were purchased from Chromasolv (Bulgaria). The reference standards used for compound identification were obtained from Extrasynthese (Genay, France) for protocatechuic, caffeic, gentisic, *p*-coumaric, *m*-coumaric, *o*-coumaric, and gallic acids, hyperoside, isoquercitrin, rutin, isorhamnetin 3-*O*-glucoside, kaempferol-3-*O*-glucoside quercetin, kaempferol, and catechin. Naringenin and ellagic acid were supplied from Phytolab (Vestenbergsgreuth, Germany).

Plant material

Filipendula ulmaria (L.) Maxim. plant material (aerial parts) was collected at the "Kamen del" hut locality, Vitosha Mt., Bulgaria, at 1,470 m a.s.l. (42.62°N, 23.26°E) at the time of the initial and full flowering stage in July 2022. The plant was identified by one of the authors (D. Zh.-D.) according to Markova (1973) and WFO (www.worldfloraonline.org). A voucher specimen was deposited at the Herbarium Academiae Scientiarum Bulgariae (SOM 179261). *F. ulmaria* aerial parts were dried in the laboratory in the shade for a week at room temperature (20–22 °C) and then grounded with a grinder (Rohnson, R-942, 220–240 V, 50/60 Hz, 200 W, Prague, Czech Republic). Afterward, the samples were stored in a dry and cool place till further analysis.

Sample extraction

Air-dried powdered plant material (100 g) was extracted with 80% MeOH (1:20 w/v) by sonication (100 kHz, ultrasound bath Biobase UC- 20C) for 15 min (×2) at room temperature. The extract was concentrated in vacuo and subsequently lyophilized (lyophilizer Biobase BK- FD10P) to yield crude extract of 14.39 g. Then, the sample was dissolved in 80% methanol (0.1 mg/mL), filtered through a 0.45 μm syringe filter (Polypure II, Alltech, Lokeren, Belgium), and an aliquot (2 mL) of each solution was subjected to further UHPLC–HRMS analyses. The lyophilized extract was used in the pharmacological experiments too.

UHPLC-HRMS dereplication/annotation

The UHPLC-HRMS was carried out as previously described (Gevrenova et al. 2023) on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific, Inc.) equipped with a heated electrospray ionization (HESI-II) probe (ThermoScientific). The equipment was operated in negative and positive ion modes within the m/z range from 130 to 2000. The chromatographic separation was achieved on a reversed-phase column, Kromasil EternityXT C18 (1.8 μm, 2.1 × 100 mm), at 40 °C. The UHPLC analyses with a mobile phase contain 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a 33 min run time and 0.3 mL/min flow rate. The gradient elution program was used as follows: 0–1 min, 0–5% B; 1–20 min, 5–30% B; 20–25 min, 30–50% B; 25–30 min, 50–70% B; 30–33 min, 70–95%; 33–34 min, 95–5% B. Equilibration time was 4 min. The injection volume and the flow rate were set to 1 μL and 300 μL/min, respectively. Data acquisition was performed using Xcalibur 4.2 (ThermoScientific, Waltham, MA, USA) instrument control/data handling software. MZmine 2 software was applied to the UHPLC–HRMS raw files of the studied *F. ulmaria* extract for the semi-quantitative analysis. Results are expressed as % peak area of the compound to the total peak areas of the corresponding group of secondary metabolites and all metabolites.

Experimental animals

Male mice, line H, weighing 30–40 g, purchased from the National Breeding Center, Sofia, Bulgaria, were used throughout the experiment. The animals were allowed 7 days of acclimatization before the study commenced. The mice were housed in plexiglass cages (4 mice/cage) in a 12/12 light/dark cycle, under standard laboratory conditions (ambient temperature 20 ± 2 °C and humidity 72 ± 4%) with free access to water and standard pelleted food.

The Institutional Animal Care Committee has approved anti-inflammatory and analgesic activity experiments (No. 364/08.11.2023). The principles stated in the EU Directive for the welfare of laboratory animals (86/609/EEC) and the guidelines of Good Laboratory Practice (GLP) were strictly followed throughout the experiments. The welfare of animals was monitored daily by a veterinary physician. Twelve hours before the experiments, the animals were deprived of food but had unrestricted access to water. Animals (n = 8/group) were randomly divided into the following groups:

I Group NC (negative control) was treated with distilled water (10 mL/kg b.w., p.o.)
II Group CRG (positive control) – carrageenan 0.5% 50 µL
III Group D25 – diclofenac 25 mg/kg
IV Group D50 – diclofenac 50 mg/kg
V Group FUA100 – *F. ulmaria* extract 100 mg/kg
VI Group FUA200 – *F. ulmaria* extract 200 mg/kg
VII Group FUA+D 100/25 – FUA extract 100 mg/kg + diclofenac 25 mg/kg
VIII Group FUA+D 200/50 – FUA extract 200 mg/kg + diclofenac 50 mg/kg

Groups V to VIII were pre-treated with 0.5 mL FUA extract for three days before CRG injection. Immediately after the FUA administration, the third CRG injection was performed. Diclofenac was given only once, orally, at a volume of 0.5 mL immediately after the CRG injection.

Acute toxicity

A single oral dosage (2000 mg/kg) of the *F. ulmaria* 80% methanolic extract or an equivalent of the vehicle's volume was given to the six mice (3 male and 3 female). Before administering the dosage, the animals were deprived of food for 24 hours but with free access to water. The animals were observed for 14 days for behavioral changes (piloerection, sensitivity to sound and touch, movement, tremors, aggression) and mortality. After the extract administration, the animals had food and water ad libidum.

Carrageenan-induced mice paw edema

Paw edema was induced by a subplantar injection of 0.5% (w/v) 50 µL λ-carrageenan, dissolved in sterile saline (0.9% NaCl) solution, into the right hind paw of each mouse (Winter et al. 1962). Immediately before and at 1 h (early phase), 3 h (late phase), and 24 h after CRG treatment, the volume of paw edema was quantified by measuring the volume of the paw (in mL) using a plethysmometer (Ugo Basile Co., Italy). The percentage of inhibition was evaluated for every mouse in each group by the following formula:

$$\% \text{ Paw edema increase} = (a - b)/b \times 100$$

where a – paw volume at different time points after injection (1 h, 3 h, and 24 h); b – paw volume before the carrageenan injection.

Hot plate test

At the 4th-hour post-CRG treatment, mice were subjected to the hot plate test (Melo et al. 2013). The animals were placed on the hot (55 ± 0.5 °C) plate surface until they lifted or licked a paw as the surface heat became uncomfortable (Mulder and Pritchett 2004; Savage and Ma 2015). The measurement of the reaction time of the first elicited behavior provides a good insight into the hyperalgesic state of the rodents (Yam et al. 2020). When those reactions were noted, the mice were immediately removed from the apparatus. Reaction latency to the thermal stimulation was recorded, and the cut-off time of 30 s was specified (Mulder and Pritchett 2004; Bannon and Malmberg 2007); however, none of the animals in our study achieved this time. The percentage of inhibition was determined for every mouse in each group using the following method:

$$\% \text{ Inhibition} = (a - b)/b \times 100$$

where a – latency to respond (test); b – latency to respond (control).

Hematological and biochemical analysis

Immediately after evaluating the paw edema at 24 h post-treatment, the animals were sacrificed. Blood was collected for further analysis of hematological (WBC, Lymph#, Mon#, Gran#, Lymph%, Mon%, Gran%, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW, PCT) and biochemical parameters (urea, creatinine, ASAT, ALAT, amylase, and uric acid). The obtained blood samples for the hematological analysis were collected in 200 µl tubes containing EDTA-K2 and were analyzed on a Mindray BC-2800Vet Auto Hematology Analyzer. The biochemical parameters were assessed using an automatic biochemistry analyzer (BS-120, Mindray, China). Within 30 min of collection, blood samples were centrifuged (Eppendorf MiniPlus) at 1500 rpm for 10 min at a temperature of 4 °C.

Statistical methods

Statistical analysis was performed using the MEDCALC program. The Kruskal-Wallis variance analysis test and a post-hoc analysis using the Mann-Whitney U test were performed. Data are expressed as a mean ± standard deviation (SD). Statistical significance was considered at $p \leq 0.05$.

Results

Acute toxicity

A single oral dosage (2000 mg/kg) of the *F. ulmaria* 80% methanolic extract did not alter the animal's behavior, and no mortality was noted during the observation period of 14 days. The animals gained weight as normal (male mice:

pre-treatment mean body weight was 37.4 g, and on the 14th day, it was 42.7 g; female mice: pre-treatment mean body weight was 32.7 g, and on the 14th day, it was 36.2 g).

Anti-inflammatory activity of *F. ulmaria* extracts in vivo

The test animals were injected with 0.1 mL of 0.5% CRG solution subplantarly into the right hind paw and developed a localized edema reaction shortly after injection that peaked after 3 hours. Only animals treated with diclofenac (either dose) or the combination of diclofenac + FUA extract (either dose) responded by a decrease in paw volume to almost normal levels at 24 h post-treatment. In group II CRG, paw edema increased by 47.80% at the 3rd h post-treatment. A similar pattern was observed with both FUA extracts; however, it was less pronounced in Group VI treated with FUA 200 mg/kg (Figs 1, 2). *F. ulmaria* 100 mg/kg extract did not significantly reduce the edema (Figs 1, 2). On the 3rd hour after the production of inflammation, diclofenac (25 and 50 mg/kg; p.o.) provided the antiedematous effect of 28.95% and 35.3%, respectively (Figs 1, 2), while 100 mg/kg and 200 mg/kg FUA extract reduced the edema by 12.71% and 18.87%, respectively. This demonstrates that the higher dose of FUA (200 mg/kg) was more efficient in reducing edema in the first phase of the inflammatory reaction. Both combinations of FUA/D (groups VII and VIII) do not differ from the corresponding diclofenac-treated groups, meaning

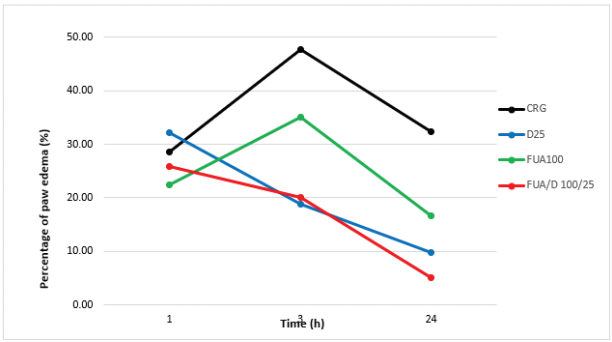

Figure 1. Anti-inflammatory effects of 100 mg/kg *F. ulmaria* extract (FUA100), 25 mg/kg diclofenac (D25), and combination (100/25 mg/kg) on carrageenan-induced (CRG) edema.

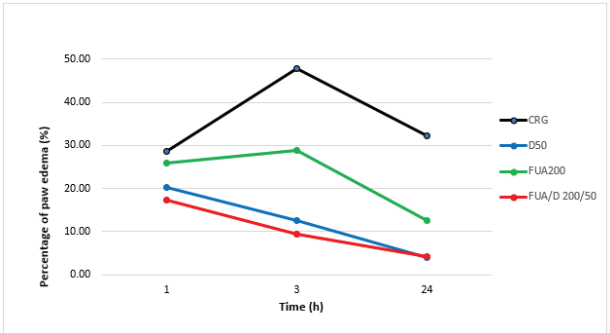

Figure 2. Anti-inflammatory effects of 200 mg/kg *F. ulmaria* extracts (FUA200), 50 mg/kg diclofenac (D50), and a combination (200/50 mg/kg) on carrageenan-induced (CRG) edema.

the effect is due mainly to diclofenac. Comparing treatment with *F. ulmaria* extracts at both dosages (100 and 200 mg/kg b.w.) to diclofenac (25 and 50 mg/kg), the reduction in paw edema at 24 hours was less pronounced in FUA-treated groups but significantly different from group II (CRG treatment).

Antinociceptive activity of *F. ulmaria* extracts in vivo

The effects of *F. ulmaria* extracts and diclofenac on animals' thermally induced discomfort, as measured by their latency to respond, are displayed in Table 1. A statistically significant extension of latency time was observed in all treatment groups except the FUA200 group. At 100 mg/kg b.w., the FUA extract exhibited a strong analgesic effect significantly different from Group II (CRG treated), despite latency reaction being reduced when 200 mg/kg of FUA extract was administered. The combination of FUA/D 200/50 also showed a reduced latency time despite remaining statistically significant compared to Group II. According to our research data, FUA extracts do not significantly interfere with the effect of diclofenac.

Table 1. Effects of *F. ulmaria* extracts and diclofenac on latency to respond to the hot plate test.

Group	Mean ± SD (sec)	Statistical significance
Positive control	6.07 ± 1.33	–
D25	8.6 ± 1.25	$p < 0.05$
D50	8.34 ± 1.31	$p < 0.05$
FUA100	7.6 ± 1.50	$p < 0.05$
FUA200	6.51 ± 1.25	NS
FUA/D 100/25	8.18 ± 1.53	$p < 0.05$
FUA/D 200/50	7.38 ± 0.91	$p < 0.05$

$p < 0.05$ compared to the control group;
NS: not significant.

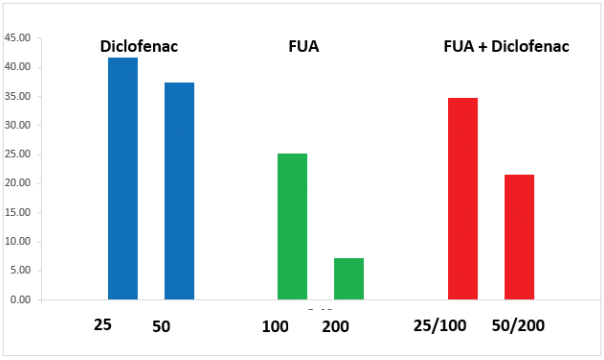

Figure 3. Percentage inhibition compared to the negative control (Group I).

Hematological and biochemical evaluation

Biochemical evaluation revealed that FUA extract and diclofenac possess no effect on WBC, PLT, MCV, MCHC, RDW, MPV, lymphocytes, monocytes, granulocytes, creatinine, ASAT, ALAT, and amylase (data not shown).

However, diclofenac and the combination of diclofenac+-FUA induced a statistically significant decrease in RBC, HGB, HTC, and uric acid while urea increased (Table 2).

UHPLC-HRMS dereplication/annotation

Based on the comparison with reference standards and literature data (Bijttebier et al. 2016; Katanic et al. 2017; Pannakal et al. 2023) of the retention times, MS and MS/MS accurate masses, fragmentation patterns in MS/MS spectra, and relative ion abundance, a total of 118 secondary metabolites were identified or tentatively annotated in *F. ulmaria* extract (Table 3, Suppl. material 1: table S1). The total ion chromatogram (TIC) in the negative ion mode of the studied extract is depicted in Suppl. material 1: fig S1. Herein, 37 phenolic acids and derivatives, 49 gallic and ellagic acid derivatives, 25 flavonoids, and 7 other compounds were reported in *F. ulmaria* extract (Table 3, Suppl. material 1: table S1). The extracted ion chromatograms showed that the *F. ulmaria* aerial part profile was dominated by gaultherin (27) (13.94%), quercetin-3-*O*-hexuronide (93) (13.69%), caffeoylthreonic isomer II (13) (5.37%), kaempferol-3-O-glucoside (105) (5.05%), epicatechin and catechin (90 and 88) (4.93% and 4.14%), quercetin (109) (3.76%), and digalloyl-HHDP-hexoside 2 and 1 (60 and 53) (2.87% and 2.43%). Extracted ion chromatograms of ellagitannins and gallotannins were presented in Suppl. material 1: figs S2, S3, while the extracted ion chromatogram and MS/MS spectra of rugosin A (71), casuarinin/casuarictin (78), and rugosin B2 (59) were presented in Suppl. material 1: figs S4–S6, respectively (Suppl. material 1: figs S2–S6).

Table 2. Hematological and biochemical evaluation.

	RBC (10^{12}/L)	HGB (g/L)	HTC (%)	Urea (mg/dl)	Uric acid (mg/dL)
Control	8.05 ± 0.4	137 ± 8.22	38.62 ± 2.40	8.33 ± 0.97	90.25 ± 6.57
CRG	7.57 ± 0.36	131 ± 4.86	38.33 ± 1.41	8.67 ± 0.46	121.25 ± 54.67
D25	4.98 ± 0.89 *	76.5 ± 10.5*	24.05 ± 3.25*	15.1 ± 1.11*	59.67 ± 15.97*
D50	3.92 ± 0.85*	56.5 ± 12.74*	18.75 ± 3.97*	15.7 ± 2.92*	38.4 ± 28.47*
FUA100	8.60 ± 0.16	148.33 ± 6.60	44.07 ± 2.57	7.41 ± 0.88	136 ± 44.66
FUA200	8.45 ± 0.37	145 ± 8.94	41.26 ± 2.55	7.93 ± 0.77	99 ± 17.42
FUA/D 100/25	5.51 ± 1.46 *	87.5 ± 25.07*	26.53 ± 6.53*	10.37 ± 3.39*	78 ± 23.18*
FUA/D 200/50	3.96 ± 1.22*	58.75 ± 20.10*	19.08 ± 6.25*	12.34 ± 3.48*	72.5 ± 23.90*

*$p < 0.05$ compared to the control group; CRG: carrageenan.

Table 3. Secondary metabolites in *Filipendula* methanol-aqueous extract.

№	Identified/tentatively annotated compound	Molecular formula	Exact mass $[M-H]^-$	t_R (min)	Level of confidence (Sumner et al. 2007)
	Phenolic acids and their derivatives				
1	citric/isocitric acid	$C_6H_8O_7$	191.0197	0.83	2
2	citric/isocitric acid	$C_6H_8O_7$	191.0197	1.06	2
3	dihydroxybenzoic acid-*O*-hexoside	$C_{13}H_{16}O_9$	315.0722	1.87	2
4	galloyl-threonic acid	$C_{10}H_{12}O_9$	287.0409	2.38	2
5	dihydroxybenzoic acid-*O*-hexoside isomer I	$C_{13}H_{16}O_9$	315.0722	2.39	2
6	hydroxycaffeoyl threonic acid	$C_{13}H_{14}O_9$	313.0565	2.54	2
7	vanillic acid-*O*-hex	$C_{14}H_{18}O_9$	329.0878	2.69	2
8	dihydroxybenzoic acid-*O*-hexoside isomer II	$C_{13}H_{16}O_9$	315.0722	2.81	2
9	hydroxybenzoic acid-*O*-hexoside	$C_{13}H_{16}O_8$	299.0772	2.82	2
10	protocatechic acid*	$C_7H_6O_4$	153.0193	2.87	2
11	dihydroxybenzoic acid-*O*-hexoside isomer III	$C_{13}H_{16}O_9$	315.0722	3.12	2
12	caffeoylthreonic acid	$C_{13}H_{14}O_6$	297.0616	3.39	2
13	caffeoylthreonic acid	$C_{13}H_{14}O_8$	297.0616	3.59	2
14	caffeoyl-*O*-pentoside	$C_{14}H_{16}O_8$	311.0772	3.75	2
15	*p*-coumaroyl-hydroxyglutaric acid	$C_{14}H_{16}O_8$	311.0772	2.537	2
16	gentisic acid*	$C_7H_6O_4$	153.0193	3.89	1
17	caffeic acid-*O*-hexoside	$C_{15}H_{18}O_9$	341.0878	4.05	2
18	*p*-coumaric acid*	$C_9H_8O_3$	163.0401	4.23	1
19	diOH-benzoic acid	$C_7H_6O_4$	153.0193	4.40	2
20	*m*-coumaric acid*	$C_9H_8O_3$	163.0401	4.40	1
21	coumaroyl-threonic acid	$C_{13}H_{14}O_7$	281.0667	4.41	2
22	caffeoylthreonic acid isomer	$C_{13}H_{14}O_8$	297.0616	4.58	2
23	caffeic acid*	$C_9H_8O_4$	179.0350	4.57	1
24	*p*-coumaroyl-hexonic acid	$C_{13}H_{14}O_8$	341.00878	4.63	2
25	feruloyl-*O*-threonic acid	$C_{14}H_{16}O_8$	311.0772	4.85	2

№	Identified/tentatively annotated compound	Molecular formula	Exact mass [M-H]$^-$	t_R (min)	Level of confidence (Sumner et al. 2007)
26	coumaric acid -O-hexoside	$C_{15}H_{18}O_8$	325.0929	4.85	2
27	monotropitin	$C_{19}H_{26}O_{12}$	445.1351	4.94	2
28	methyl salicylate	$C_8H_8O_3$	151.0401	4.97	2
29	caffeoyl-*O*-gluconolactone isomer	$C_{15}H_{16}O_9$	339.0722	4.82	2
30	caffeoyl-O-gluconolactone	$C_{15}H_{16}O_9$	339.0722	5.15	2
31	coumaroyl-threonic acid isomer	$C_{13}H_{14}O_7$	281.0667	5.49	2
32	*o*-coumaric acid*	$C_9H_8O_3$	163.0401	5.50	1
33	galloyl-caffeoyl-threonic acid	$C_{20}H_{18}O_{12}$	449.0726	6.07	2
34	galloyl-*p*-coumaroyl-threonic acid	$C_{20}H_{18}O_{11}$	433.0776	6.96	2
35	caffeoyl-digalloyl-threonic acid	$C_{27}H_{22}O_{16}$	601.0835	7.12	2
36	methylcafeate	$C_{10}H_{10}O_4$	193.0506	7.57	2
37	digalloyl-coumaroyl-threonic acid	$C_{27}H_{22}O_{15}$	585.0886	7.94	2
	Gallic and ellagic acids derivatives				
38	HHDP-hexoside	$C_{20}H_{18}O_{14}$	481.0624	1.08	2
39	gallic acid hexoside1	$C_{13}H_{16}O_{10}$	331.0671	1.22	2
40	galloyl hexose	$C_{13}H_{16}O_{10}$	331.0671	1.36	2
41	galloyl-HHDP-hexoside1	$C_{27}H_{22}O_{18}$	633.0733	1.65	2
42	gallic acid*	$C_7H_6O_5$	169.0142	1.66	1
43	gallic acid hexoside2	$C_{13}H_{16}O_{10}$	331.0671	1.66	2
44	digalloyl hexoside1	$C_{20}H_{20}O_{14}$	483.0780	1.78	2
45	galloyl-HHDP-hexoside2	$C_{27}H_{22}O_{18}$	633.0733	2.31	2
46	gallic acid hexoside3	$C_{13}H_{16}O_{10}$	331.0671	2.36	2
47	ethylgalate	$C_9H_{10}O_5$	197.0455	2.45	2
48	digalloyl hexoside2	$C_{20}H_{20}O_{14}$	483.0780	2.55	2
49	di-HHDP-hexoside1	$C_{34}H_{24}O_{22}$	783.0686	2.64	2
50	galloyl-HHDP-hexoside3	$C_{27}H_{22}O_{18}$	633.0733	2.88	2
51	galloyl-HHDP-hexoside4	$C_{27}H_{22}O_{18}$	633.0733	3.30	2
52	di-HHDP-hexoside2	$C_{34}H_{24}O_{22}$	783.0686	3.39	2
53	digalloyl-HHDP-hexoside1	$C_{34}H_{26}O_{22}$	785.0843	3.75	2
54	digalloyl hexoside3	$C_{20}H_{20}O_{14}$	483.0780	3.80	2
55	methylgalate	$C_8H_8O_5$	183.0299	4.00	2
56	trigalloyl hexoside1	$C_{27}H_{24}O_{18}$	635.0890	4.07	2
57	galloyl-diHHDP- hexoside (Rugosin B1)	$C_{41}H_{30}O_{27}$	953.0902	4.24	2
58	trigalloyl hexoside2	$C_{27}H_{24}O_{18}$	635.0890	4.42	2
59	galloyl-diHHDP- hexoside (Rugosin B2)	$C_{41}H_{30}O_{27}$	953.0902	4.45	2
60	digalloyl-HHDP-hexoside2	$C_{34}H_{26}O_{22}$	785.0843	4.48	2
61	tuberonic acid hexoside	$C_{18}H_{28}O_9$	387.1661	4.62	2
62	trigalloyl hexoside3	$C_{27}H_{24}O_{18}$	635.0890	4.74	2
63	galloyl-diHHDP- hexoside	$C_{41}H_{30}O_{27}$	953.0902	4.82	2
64	ellagitannin B1	$C_{42}H_{32}O_{27}$	967.1058	4.95	2
65	digalloyl-HHDP-hexoside3	$C_{34}H_{26}O_{22}$	785.0843	5.04	2
66	rugosin E1	$C_{75}H_{54}O_{48}$ (1722.1785)	[M-2H]$^{2-}$ 860.0819	5.22	2
67	casuarinin/casuarictin	$C_{41}H_{28}O_{26}$	935.0796	5.27	2
68	ellagitannin B2	$C_{42}H_{32}O_{27}$	967.1058	5.34	2
69	rugosin E2	$C_{75}H_{54}O_{48}$ (1722.1785)	[M-2H]$^{2-}$ 860.0819	5.40	2
70	methyl brevifolin carboxylate	$C_{14}H_{10}O_8$	305.0304	5.53	2
71	rugosin A	$C_{48}H_{32}O_{31}$ (1106.1084)	[M-2H]$^{2-}$ 552.0469	5.54	2
72	tellimagrandin	$C_{41}H_{30}O_{26}$	937.0953	5.59	2
73	ellagitannin A1	$C_{83}H_{60}O_{53}$ 1904.2000	[M-2H]$^{2-}$ 951.0927	5.59	2
74	tetragalloyl hexoside1	$C_{34}H_{28}O_{22}$	787.0999	5.64	2
75	ellagitannin A2	$C_{83}H_{60}O_{53}$ 1904.2000	[M-2H]$^{2-}$ 951.0927	5.74	2
76	tetragalloyl hexoside2	$C_{34}H_{28}O_{22}$	787.0999	5.78	2
77	ellagic-acid-*O*-pentoside	$C_{19}H_{14}O_{12}$	433.0412	5.80	2

№	Identified/tentatively annotated compound	Molecular formula	Exact mass $[M-H]^-$	t_R (min)	Level of confidence (Sumner et al. 2007)
78	casuarinin/casuarictin	$C_{41}H_{28}O_{26}$	935.0796	5.81	2
79	tetragalloyl hexoside3	$C_{34}H_{28}O_{22}$	787.0999	5.89	2
80	rugosin D	$C_{82}H_{56}O_{52}$ (1874.1894)	$[M-2H]^{2-}$ 936.0874	5.95	2
81	galloyl-caffeoyl-threonic acid	$C_{20}H_{18}O_{12}$	449.0726	6.07	2
82	rogosin A methyl ester	$C_{49}H_{36}O_{31}$	1119.1168	6.16	2
83	pentagalloyl hexose	$C_{41}H_{32}O_{26}$	939.1109	6.35	2
84	ellagic acid*	$C_{14}H_6O_8$	300.9991	6.40	1
85	galloyl-*p*-coumaroyl-threonic acid	$C_{20}H_{18}O_{11}$	433.0776	6.96	2
86	casuarinin/casuarictin/	$C_{41}H_{28}O_{26}$	935.0796	7.02	2
	Flavonoids				
87	kaempferol -3,7-O-diglucoside*	$C_{27}H_{30}O_{16}$	609.1461	2.57	1
88	catechin*	$C_{15}H_{14}O_6$	289.0718	4.02	1
89	quercetin-*O*-dihexoside isomer	$C_{27}H_{30}O_{16}$	625.1410	4.26	2
90	epicatechin	$C_{15}H_{14}O_6$	289.0718	4.78	2
91	quercetin-*O*-dihexoside isomer	$C_{27}H_{30}O_{16}$	625.1410	5.35	2
92	rutin*	$C_{27}H_{30}O_{16}$	609.1461	6.07	1
93	quercetin-3-*O*-hexuronide	$C_{27}H_{18}O_{13}$	477.0675	6.23	2
94	hyperoside*	$C_{21}H_{20}O_{12}$	463.0882	6.34	1
95	quercetin-*O*-galloyl-dihexoside	$C_{34}H_{34}O_{20}$	761.1571	6.35	2
96	isoquercitrin*	$C_{21}H_{20}O_{12}$	463.0882	6.45	1
97	quercetin-3-*O*-galloyl-hexoside	$C_{28}H_{24}O_{16}$	615.0992	6.53	2
98	quercetin-3-*O*-pentoside	$C_{20}H_{18}O_{11}$	433.0776	6.61	2
99	kaempferol 7-O-rutinoside*	$C_{27}H_{30}O_{15}$	593.1512	6.67	1
100	isorhamnetin-3-*O*-glucoside*	$C_{22}H_{22}O_{12}$	477.1038	6.77	1
101	cinchonain Ia/Ib	$C_{24}H_{20}O_9$	451.1035	6.83	2
102	quercetin-4'-*O*-hexoside	$C_{21}H_{20}O_{12}$	353.165	7.10	2
103	deoxycinchonain Ia/Ib	$C_{24}H_{20}O_8$	435.1085	7.69	2
104	quercetin-3-*O*-galloyl-hexoside	$C_{28}H_{24}O_{16}$	615.0992	7.78	2
105	kaempferol-3-*O*-glucoside*	$C_{21}H_{20}O_{11}$	447.0933	7.30	1
106	cinchonain Ia/Ib	$C_{24}H_{20}O_9$	451.1035	7.76	2
107	kaempferol-*O*-galloyl-hexoside	$C_{28}H_{24}O_{15}$	599.1042	8.13	2
108	deoxycinchonain Ia/Ib	$C_{24}H_{20}O_8$	435.1085	8.42	2
109	quercetin*	$C_{15}H_{10}O_7$	301.0354	8.92	1
110	naringenin*	$C_{15}H_{12}O_5$	271.0612	9.81	1
111	kaempferol*	$C_{15}H_{10}O_6$	285.0405	10.28	1
	Others				
112	gluconic acid	$C_6H_{12}O_7$	195.0510	0.74	2
113	hexose	$C_6H_{12}O_6$	179.0561	0.74	2
114	malyl-sucrose	$C_{16}H_{24}O_{14}$	441.1250	1.24	2
115	phenylalanin	$C_{16}H_{24}O_{14}$	164.0717	2.06	2
116	glucolactone	$C_6H_{10}O_6$	177.0405	6.15	2
117	azelaic acid	$C_9H_{16}O_4$	187.0976	7.16	2
118	sebacic acid	$C_{10}H_{18}O_4$	201.1132	8.57	2

*Compare to reference standards.

Discussion and conclusion

The present study investigated the chemical content, analgesic, and anti-inflammatory properties of *Filipendula ulmaria* (L.) Maxim. aerial part extract. *F. ulmaria* is widespread in Europe and temperate regions of Asia. In recent years, interest in this species has been growing (Samardžić et al. 2018; Bespalov et al. 2018; Stawarczyk et al. 2021; Savina et al. 2023; Van der Auwera et al. 2023). The extract contains various phenolic acids, gallic and ellagic acid derivatives, flavonoids, and other compounds (Katanić et al. 2015; Pukalskiene et al. 2015; Bijttebier et al. 2016; Katanić et al. 2016; Farzaneh et al. 2022).

The current study identified or tentatively annotated 118 secondary metabolites in the *F. ulmaria* extract (Bijttebier et al. 2016; Pannakal et al. 2023). In line with the LC-PDA-MS analysis of *F. ulmaria* aerial parts done by Bijttebier et al. (2016), a series of monomeric (57, 59, 71, 72, 78, and 86) and dimeric (66, 69, and 80) ellagitannins was described (Table 3 and Suppl. material 1: table S1). It is worth noting

that the monomeric (64, 68, and 82) and dimeric (73 and 75) ellagitannins were elucidated for the first time in the assayed species. In addition, several ellagitannins were not previously reported in the literature: digalloyl-HHDP-hexoside (isomers 53, 60, and 65), di-HHDP-hexoside (isomers 49 and 52), along with ethylgalate (47) and methylbrevifolin carboxylate (70). Among the phenolic acid derivatives, caffeic acid-pentoside (14), caffeic acid-hexoside (17), caffeoyl-O-gluconolactone (30), and p-coumaroyl-hydroxyglutaric acid (15) were evidenced for the first time along with the flavonoid kaempferol 3, 7-diglucoside. Regarding the recent study of Pannakal et al. (2023), an LC-MS analysis performed in full MS mode without higher energy collisional dissociation (HCD) fragmentation allowed for the assessment of the accurate masses and molecular formulas of numerous secondary metabolites.

F. ulmaria has a wide range of pharmacological activities due to its phytochemical composition. Anti-inflammatory, antipyretic, analgesic, and antirheumatic effects, among others, have been described (Katanić et al. 2016; Samardžić et al. 2016; Samardžić et al. 2018; Farzaneh et al. 2022). A review of the scientific literature shows that *F. ulmaria* extracts have anti-inflammatory potential, associated with the inhibition of pro-inflammatory mediators (Drummond et al. 2013; Nitta et al. 2013). Despite the thorough characterization of *F. ulmaria*, the specific phytochemical constituents and their role as anti-inflammatory and analgesic agents have not been sufficiently defined.

Carrageenan-induced inflammation represents a widely utilized model of inflammatory pain, whereby noxious stimuli are administered (NRC 2009). Carrageenan, a water-soluble polysaccharide extracted from certain red seaweed species (Das and Bal 2024), induces localized inflammation, allodynia, and pain hypersensitivity upon intraplantar injection in a hind paw of rodents (Winter et al. 1962; Meller and Gebhart 1997; Fehrenbacher et al. 2012; Mert et al. 2018). Carrageenan-derived pain hypersensitivity typically peaks 3–4 h after injection and has been used to study peripheral and spinal mechanisms of tonic inflammatory pain sensitivity (NRC 2009; Fehrenbacher et al. 2012; Karimi et al. 2024). The induction of CRG-mediated inflammation serves as a reliable model for the evaluation of novel anti-inflammatory pharmacological interventions, as it effectively elicits inflammatory pain associated with prostaglandin-mediated processes (Le Bars et al. 2001). The injection of CRG triggers an acute inflammation associated with hyperalgesia, which is characterized by edema and inflammation, including the release of mediators like reactive oxygen species, nitric oxide, prostaglandins, and cytokines that have a role in the formation of this edema (Posadas et al. 2004). In our experiment, the standard diclofenac showed better inhibitory activity than FUA extracts. The animals treated with diclofenac (25 or 50 mg/kg) or both combinations of FUA with diclofenac responded by decreasing paw volume to near-normal levels at 24 h post-treatment. According to the data obtained, FUA extract did not affect the antiedematous effect of diclofenac. The FUA extract exhibited significantly less pronounced anti-inflammatory activity than diclofenac at the 3rd hour post-treatment. The nociceptive response produced by CRG is biphasic, and the two phases can be distinguished pharmacologically. In the early phase (i.e., during the first hour), the acute inflammation involves the release of histamine and serotonin and cyclooxygenase products in the second phase (>1 hour) (Vinegar et al. 1969; Di Rosa et al. 1971; Halici et al. 2007). However, the edema of the group treated with FUA 200 mg/kg was reduced to a greater extent at the onset of inflammation, i.e., by the 3rd hour. Katanić et al. (2016) showed that the *F. ulmaria* extracts (aerial part and root) significantly inhibited COX enzymes; however, the root extract was less effective. The authors also reported that 200 mg/kg FUA was more effective in reducing edema in the first phase of the inflammatory response, which is in line with our data, as we also observed that FUA 200 mg/kg induced a significant decrease in edema in the 3rd hour after inflammation induction compared to the CRG-only treated group.

Increased sensitivity to thermal stimulus was measured on the 4th hour post-CRG-induced inflammation. Various tests can be used to quantify changes in thermal sensitivity. Pain assessment in animals typically involves measuring changes in paw withdrawal latencies, which are used to evaluate heat hyperalgesia. The effect of *F. ulmaria* extract was investigated for analgesic effects in a hot plate test by measuring the latency to reflex responses (paw lift or paw licking) of rodents placed in contact with a hot surface (in seconds) (Ankier 1974; Modi et al. 2023). The hot plate test is a reliable method for assessing thermal nocifensive responses (Menéndez et al. 2002; Lavich et al. 2005; Yam et al. 2020). It evaluates supraspinally organized responses to a noxious stimulus. The administration of FUA extracts 3 days before the CRG injection was associated with a statistically significant prolongation of latency time in all treatment groups except the FUA 200 mg/kg group. Similar results were obtained by Katanić et al. (2016) as the authors noticed that the 100 mg/kg FUA induced a greater paw withdrawal threshold compared to 200 mg/kg FUA. These confirmatory results are interesting, but we have no explanation for why this is so. As expected, a distinct and significant reduction in tactile hyperalgesia was observed in the diclofenac-treated groups. Once, no change in the effect of diclofenac was observed when given in combination with FUA (either dose).

Nonsteroidal anti-inflammatory drugs are widely used in pain, inflammation, and fever treatment. However, their side effects are also well known. In our experiment, biochemical analysis revealed that diclofenac and the combination of FUA+diclofenac induced a statistically significant decrease in RBC, HGB, HTC, and uric acid while urea increased. This is most likely attributed to diclofenac, whose gastrointestinal and renal toxicity are well described (Sánchez et al. 2002). No such alterations were observed in the groups treated with the FUA extracts, nor did the FUA extract contribute to more pronounced changes in the combination group (FUA+D).

In acute toxicity testing, a single oral dose of 2000 mg/kg of methanolic extract of *F. ulmaria* aerial parts did not alter animal behavior, and no mortality was observed during the 14-day observation period. Samardžić et al. (2016) also found a good safety profile of *F. ulmaria* flower extract (oral LD50 > 2000 mg/kg).

Conclusion

In our experiment, the oral LD50 was over 2000 mg/kg. The standard NSAID, diclofenac, showed better anti-inflammatory and antinociceptive activity than FUA extracts. The results revealed a dose-dependent moderate inhibition of the inflammation and pain induced by 3 days of oral pretreatment with methanolic extract of *F. ulmaria* aerial parts. No additive effect of FUA extracts on diclofenac was noted.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: 364/08.11.2023

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary data

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