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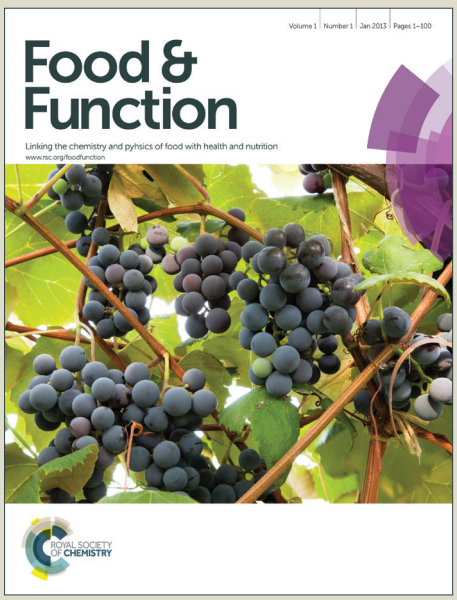

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Bioactivity, stability and phenolic characterization of *Filipendula ulmaria* (L.) Maxim.

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Abstract

The purpose of the present study was to evaluate the antioxidant and the antimicrobial potential of *Filipendula ulmaria* (L.) Maxim. methanolic extracts, their stabilities in different pH and thermal conditions and *in vitro* digestibility. The results showed considerable content of phenolic compounds in the extracts, especially total phenolic acids (47.47 mg CAE/g) and flavonoids (45.47 mg RUE/g) in aerial parts. HPLC analysis indicated presence of spiraeoside in the aerial part extract. The extracts revealed an interesting antimicrobial effect against the tested microorganisms, especially bacteria *E. coli* and *E. faecalis* (MIC 0.156–0.625 mg/mL), and fungi *P. cyclopium* and *F. oxysporum* (MIC 2.5–5 mg/mL). The extracts exerted high antioxidant activities, particularly the roots, paralleled by their considerable activities against lipid oxidation process. The results of this study suggest that both extracts potentially could be functional food ingredients considering their good antioxidant and antimicrobial activities, and stability in different conditions.

Keywords: *Filipendula ulmaria*; phenolic compounds; antimicrobial activity; antioxidant activity; stability; *in vitro* digestion

1. Introduction

Filipendula ulmaria L. Maxim. (Rosaceae, syn. *Spiraea ulmaria* L. - meadowsweet, queen of the meadow) is a perennial herb with creamy-white flowers, a short, pink rhizome and stems 50-120 cm high. It can be found in meadows, swamps, and areas with higher humidity in Europe and Asia. Dried flowers, leaves, the whole plant herba and the rhizomes are used medicinally as drugs.[1] The medicinal parts of the plant contain a number of phenolic compounds including phenolic acids (gallic acid, salicylic acid, ellagic acid, caffeic acid derivatives), flavonoids (catechin, kaempferol, astragalin, quercetin, hyperoside, quercitrin, rutin, spiraeoside), hydrolysable and condensed tannins, and salicylate aglycons and glycosides.[2-5] The phenolic content of *F. ulmaria* is highly related to its pharmacological effect, including antipyretic, anti-inflammatory, anti-rheumatic, analgesic, astringent, stomachic and diuretic effect.[2] The herb and the flowers are traditionally used for fevers, gout, infections and urinary problems or minor painful joint conditions. The flowers are a component of some mixed herbal teas as remedies for influenza, rheumatism and kidney-bladder teas.[6,7]

Reactive oxygen and nitrogen species (ROS, RNS) are well recognized as deleterious because their overproduction results in oxidative stress, a process which leads to destruction of biomolecules like lipids, proteins, and DNA. The ROS are associated with the induction of the diseases such as the cancer, cardiovascular disease, atherosclerosis, hypertension, diabetes mellitus, neurodegenerative diseases (Alzheimer's disease and Parkinson's disease), rheumatoid arthritis, and aging.[8,9] The existence of defensive compounds called antioxidants is essential, because they poses significant role to alleviate or prevent the oxidation of other molecules that are more easily oxidized. Antioxidants are also added to a variety of foods to prevent or deter free radical-induced lipid oxidation and to extend a shelf-life of food products, mainly diary and meat products.[10] On the other hand, decrease in the quality of food could also cause a wide range of microorganisms. The increased use of natural antimicrobial compounds for food preservation is justified by the number of positive results that have been published in the past several decades. Plant extracts and essential oils, as well as pure natural substances isolated from the plants, are in usage as additives for food preservation due to their excellent antimicrobial properties.[11,12] There is always growing interest in the investigation of natural products for the discovery of compounds which possess both antioxidant and antimicrobial activities and have none harmful repercussion on the human health.

Some investigations have shown the antimicrobial, antioxidant and anti-inflammatory activities of the extracts from meadowsweet flowers.[3,5,13-15] Also, it has been known that *F. ulmaria* possess effects on gastric ulcers, anticancer and hepatoprotective activities.[16-18] Antioxidant properties of meadowsweet were studied by using various methods, mainly $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ scavenging assays and TPC measured with Folin-Ciocalteu reagent.[14,19] However, in many cases the results are difficult to compare due to different extraction process, sample preparation and antioxidant activity evaluation procedures. Literature survey shows that there is a need for more systematic studies of antioxidant properties of meadowsweet extracts in order to comprehensively evaluate their beneficial properties in human nutrition and their role as potential functional food ingredients.[20] In that sense, the purpose of our study was to evaluate antioxidant properties of aerial parts (FUA) and roots (FUR) extracts of *Filipendula ulmaria* (L.) Maxim. by applying different methods and model-systems. The contents of phenolic compounds in these two extracts were estimated and they were characterized by HPLC analysis. pH and thermal stability studies, *in vitro* digestion, antibacterial and antifungal activities of the extracts were also determined.

2. Materials and methods

2.1. Chemicals

All spectrophotometric measurements were performed on UV–VIS double beam spectrophotometer Halo DB-20S (Dynamica GmbH, Switzerland). Gallic acid, vanillic acid, kaempferol and quercetin were purchased from Sigma–Aldrich (Deisenhofen, Germany), caffeic acid was purchased from Merck KGaA (Darmstadt, Germany), (+)-catechin and ellagic acid from Serva (Heidelberg, Germany), hyperoside and rutin from Carl Roth (Karlsruhe, Germany), epicatechin from Thermo Fisher Scientific (Geel, Belgium) and spiraeoside from Extrasynthese (Genay, France). HPLC-grade acetonitrile, water and trifluoroacetic acid (Merck, Darmstadt, Germany) were used in HPLC analyses. All chemicals and reagents used for analyses of total phenolic compounds, antioxidant and antimicrobial activities and *in vitro* digestion study were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). Nutrient agar (NA), Sabouraud dextrose agar (SDA), Müller–Hinton broth (MHB) and Sabouraud dextrose broth (SDB) were purchased from Torlak Institute of Virology, Vaccines and Sera (Belgrade, Serbia).

2.2. Plant material and preparation of the extracts

The aerial parts and roots of *Filipendula ulmaria* (L.) Maxim. were collected during flowering season at the Goč Mountain, Serbia, in July 2013. Voucher specimen (No. 112/013) was prepared and deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia, after the identification of species. The air-dried aerial parts (94 g) and roots (113 g) of *F. ulmaria* were fine powdered and separately macerated with methanol at the room temperature (25-27 °C) for 24 h for three times (300 mL each). After filtration, the solvent was entirely removed in the rotary evaporator under low pressure to obtain the dry extracts. The final weights of *F. ulmaria* dry extracts were 7.52 g for FUA and 31.38 g for FUR. The percentage yields of FUA and FUR extracts were found to be 8% (w/w) and 27.8% (w/w), respectively. The concentrations used in the experiments were based on the dry weight of the extracts.

For HPLC analysis of phenolic acids and flavonoid glycosides the purified extracts were hydrolyzed to obtain the free phenolic acids and flavonoid aglycons by modifying a method described by Engida et al. (2013).[21] The dried extracts were reconstituted and heated at 80 °C for 1 h (to hydrolyze glycosides to aglycons) in a solution of 40 mL 65% aqueous methanol in which 10 mL 6 N HCl was added.

2.3. Determination of phenolic compounds

2.3.1. Total phenolic content (TPC)

The total phenolic content was estimated according to Singleton et al. (1999).[22] Briefly, in 0.5 mL of the extracts (1 mg/mL) was added 2.5 mL of Folin–Ciocalteu reagent (diluted 10-fold) and 2 mL of $NaHCO_3$ (7.5%). Absorbance was measured at 765 nm after 15 min of incubation. TPC values were expressed as gallic acid equivalents (mg GAE/g dry extract).

2.3.2. Total phenolic acids

Procedure for estimating of total phenolic (hydroxycinnamic) acids was adopted from Polish Pharmacopoeia (2005).[23] 5 mL of water was added to 1 mL extract (1 mg/mL). Then, in the mixture were added HCl (1 mL, 0.1 M), Arnow reagent (1 mL, 10% w/v of sodium molybdate and 10% w/v sodium nitrite), NaOH (1 mL, 1 M), and filled up to 10 mL and the absorbance was read immediately at 490 nm. The results were expressed as caffeic acid equivalents (mg CAE/g extract).

2.3.3. Flavonoid content

The total flavonoid content was determined using the $AlCl_3$-method.[24] The aluminum trichloride solution (0.5 mL, 2%) and the same volume of methanol solution of plant extracts were incubated for 1 h at room temperature. Then, the absorbance was measured at 415 nm. The total flavonoid content was expressed as rutin equivalents (mg RUE/g extract).

2.3.4. Flavonols content

The content of flavonols was determined by Yermakov et al. (1987) method.[25] 2 mL of plant extracts (1 mg/mL) were mixed with 2 mL (20 mg/mL) $AlCl_3$ and 6 mL (50 mg/mL) sodium acetate. The absorbance was read after 2.5 h at 440 nm. The content of flavonols was calculated as rutin equivalents (mg RUE/g extract).

2.3.5. Condensed tannins content

The condensed tannins content were estimated using the method described by Scalbert et al. (1989).[26] Formaldehyde was used for the precipitation of proanthocyanidins. The first step was measuring of the total phenolic content using the Folin-Ciocalteu reagent. Then, 0.5 mol-equivalent of phloroglucinol was added for every gallic acid equivalent in the extracts. In 2 mL of the extracts and phloroglucinol was added 1 mL HCl /H_2O solution (2:5) and 1 mL of formaldehyde solution (13 mL of 37% formaldehyde diluted to 100 mL in water). After an overnight incubation at room temperature, the unprecipitated phenols are estimated in the supernatant by the Folin-Ciocalteu method. The precipitate contains the proanthocyanidins and the known amount of phloroglucinol, which is always quantitatively precipitated. Content of condensed tannins was estimated as gallic acid equivalents (mg GAE/g extract).

2.3.7. Gallotannins content

The content of gallotannins was determined by Haslam (1965) method.[27] The potassium iodate (KIO_3) reacts with galloyl esters, form a red intermediate and ultimately a yellow compound. The reaction was performed by adding 1.5 mL of a saturated potassium iodate solution to 3.5 mL of methanol solution of plant extracts, followed by incubation at 15 °C until a maximum absorbance was reached (regardless of the time). The concentration of the red intermediate was measured spectrophotometrically at 550 nm. Gallotannins content was determined as gallic acid equivalents (mg GAE/g extract).

2.4. Individual phenolic compound determination with HPLC analysis

The HPLC system (Shimadzu Prominence) consisted of a system controller (CBM-20A), a column oven CPO-20AC and a solvent delivery pump with a degasser (DGU-20A5) with a Photo Diode Array detector (SPD-M20A) that monitored the wavelengths 190-800 nm. The responses of the detectors were recorded using LC Solution software version 1.24 SP1. The chromatography was performed at 40 °C and a flow rate of 2 mL/min using a Phenomenex Kinetex® C18 column (10 cm x 4.6 mm I.D., 2.7 µm particle size). The following gradient method using water (solvent A) and acetonitrile (solvent B), both containing 0.1 % of trifluoroacetic acid, was utilized: 0-1 min 5% B, 1-10 min 5-30% B, 10-15 min 100% B. The injection volume of all samples was 5 µL. Chromatograms were monitored at 260, 280, 325 and 360 nm. The identification of phenolic acids and flavonoids was performed by comparing retention times and absorption spectra of unknown peaks with reference standards as well as co-chromatography with added standards. For quantification of phenolic acids in the extracts, calibration curves were prepared for gallic acid, caffeic acid, vanillic acid, ellagic acid, (+)-catechin, epicatechin, quercetin, hyperoside, rutin, spiraeoside and kaempferol. Eight mass concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.78125 µg/mL of standard solution were prepared. For further identification of phenolic acids and flavonoid glycosides, the purified extracts were hydrolyzed as described in plant extracts preparation section 2.2.[21] After cooling, the solution was sonicated for 5 min and made to a final volume of 100 ml by adding methanol, then filtered through a 0.2 µm syringe filter for HPLC analysis.

2.5. Antimicrobial activity

2.5.1. Test microorganisms

The tests with *F. ulmaria* extracts were performed on the ATCC cultures and the clinically isolated strains of bacteria and fungi. The extracts were individually tested against a panel of 17 microorganisms from which six were bacterial strains: *Klebsiella pneumoniae* ATCC 70063, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* FSB 24, *Escherichia coli* FSB 41, *Pseudomonas aeruginosa* FSB 37; and 11 were fungal strains: *Candida albicans* ATCC 10259, *Trichoderma harzianum* FSB 12, *Trichoderma longibrachiatum* FSB 13, *Penicillium cyclopium* FSB 23, *Penicillium canescens* FSB 24, *Aspergillus niger* FSB 31, *Aspergillus glaucus* FSB 32, *Fusarium oxysporum* FSB 91, *Alternaria alternata* FSB 51, *Doratomyces stemonitis* FSB 41, *Phialophora fastigiata*

FSB 81. All test microbial strains were obtained from the Faculty of Chemistry, University of Belgrade and Laboratory for Microbiology, Department of Biology, Faculty of Science, University of Kragujevac, Serbia. The bacteria and fungi cultures were stored at +4 °C and subcultured once a month. Bacterial strains were cultured overnight at 37 °C in nutrient agar (NA) and fungi were cultured on Sabouraud dextrose agar (SDA) and potato glucose agar (PDA) at 28 °C for 3 days.

2.5.2. Antibacterial activity

The minimum inhibitory concentrations (MIC) of the extracts of *F. ulmaria* against tested microorganisms were determined based by the microdilution method in 96 multi-well microtiter plates.[28] All tests with bacterial strains were performed in Müller–Hinton broth (MHB). In brief, a fresh overnight culture of bacteria was suspended in sterile water and adjusted by the colorimeter to a concentration of 1.0×10^6 CFU/mL. Different solvent dilutions of plant extracts and standard phenolic compounds (50 µL) which were dissolved in sterile water were carried out over the wells containing 50 µL of MHB, and then 10 µL of resazurin indicator solution (270 mg resazurin in 40 mL of sterile distilled water) and 30 µL of MHB were added to each well. Finally, 10 µL of bacterial spore suspension was added to all the wells. For each strain, the growth conditions and the sterility of the medium were checked. Standard antibiotic amracin (tetracycline) was used to control the sensitivity of the tested bacteria. The microplates were incubated for 24 h at 37 °C. Any color change of the indicator from purple to pink or colorless was recorded as positive. The lowest concentration that produced a significant inhibition of the growth of the bacteria in comparison with the positive control was identified as the MIC. All tests were done in triplicate.

2.5.3. Antifungal activity

The fungal spores were washed from the surface of agar plates with sterile distilled water. The spore suspension was adjusted with sterile water to a concentration of 1×10^4 CFU/mL. Identically, the 2-fold serial microdilution method was used for determination of MICs. The test was performed on Sabouraud dextrose broth (SDB). The extracts and phenolic compounds (50 µL) dissolved in sterile water were added into the first row of the plate and double dilutions were made in all the other rows that were filled with 50 µL of SDB. Thereafter, 10 µL of SDB was added in all wells instead of the resazurin indicator solution followed by addition of fungal spore suspension. For each strain, the growth conditions and the sterility of the medium were checked. Ketoconazole and klotrimazole

were used as controls against the tested fungi. Plates were placed in an incubator at 28 °C for 48 h. The lowest concentrations without visible growth of fungi were defined as MICs. All tests were repeated in triplicate.

2.6. Antioxidant activity

2.6.1. Determination of total antioxidant capacity

To determine total antioxidant capacity of extracts the method of Prieto et al. (1999) was employed.[29] The total antioxidant activity of the extracts was monitored by the formation of a green phosphate/Mo (V) complex at acid pH. In 0.3 mL of extract solution were added 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then, mixtures were incubated at 95 °C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm. The results were evaluated through the standard curve of ascorbic acid (AA) obtained by the same procedure. The total antioxidant capacity is expressed as ascorbic acid equivalents (mg AA/g).

2.6.2. DPPH free-radical scavenging activity

The methodology described by Kumarasamy et al. (2007)[30] was used to determine scavenging DPPH radical activity of the extracts. Different concentrations of extracts in methanol (2 mL) were mixed with the same volume of DPPH solution (80 µg/mL). After 30 min of incubation at room temperature, the absorbance was measured at 517 nm. Ellagic acid (EA), ascorbic acid (AA), quercetin (QU) and butylated hydroxytoluene (BHT) were used as reference standards. The DPPH free-radical scavenging activity (%) was calculated with the following equation: *% radical scavenging activity* = $[(A_{control} - A_{sample}) / A_{control}] \times 100$; where $A_{control}$ is the absorbance of the DPPH radical in methanol and A_{sample} is the absorbance of the samples. The IC_{50} value, which is the concentration of the test material that reduces 50% of the free-radical concentration, was calculated as µg/mL through sigmoidal doseresponse curve.

2.6.3. Superoxide radical scavenging activity

The alkaline DMSO method was used to evaluate the superoxide radical scavenging activity.[31] Method is based on generating superoxide radical by the addition of sodium hydroxide to the air saturated dimethyl sulfoxide (DMSO). The generated superoxide remains stable in solution and reduces nitroblue tetrazolium (NBT) in to formazan dye at room temperature which can be measured at 560 nm. Briefly, NBT (0.1 mL, 1 mg/mL) was added

into the reaction mixture containing 1 mL of alkaline DMSO (1 mL DMSO with 5 mM NaOH in 100 mL water) and 0.3 mL of the extract in DMSO at various concentrations, to give a final volume of 1.4 mL. The absorbance was measured at 560 nm. EA, AA, QU and BHT were used as standards.

2.6.4. ABTS radical-cation scavenging activity

The $ABTS^{\cdot+}$ radical scavenging activity was estimated by the method described by Re et al. (1999).[32] The radical cation ($ABTS^{\cdot+}$) was generated by reacting 7 mM stock solution of ABTS [2,2′-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) diammonium salt] with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 16 h before use. The $ABTS^{\cdot+}$ solution was diluted with 5 mM phosphate-buffered saline (pH 7.4) to rich the absorbance of 0.70±0.02 at 734 nm. After 30 min of the addition 100 μL of sample to 900 μL of $ABTS^{\cdot+}$ solution, the absorbance was measured at 734 nm. EA, AA, QU and BHT were used as reference antioxidants. A control sample was prepared containing the same volume without test compounds or reference antioxidants. The $ABTS^{\cdot+}$ radical-cation scavenging activity of the samples was expressed as: *% radical scavenging activity* = $[(A_{control} - A_{sample}) / A_{control}] \times 100$, where $A_{control}$ is the absorbance of the blank control ($ABTS^{\cdot+}$ solution without test sample) and A_{sample} is the absorbance of the test sample.

2.6.5. Measurement of reducing power

The determination of reducing power was performed by the method of Oyaizu (1986).[33] The plant extracts (2.5 mL, different concentrations) prepared in distilled water were mixed with the same volume of 0.2 M sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then the reaction mixture was acidified with 2.5 mL of 10% trichloroacetic acid (w/v). The mixture was centrifuged at 1000 rpm for 8 min and the upper layer (5 mL) was mixed with 1 mL of $FeCl_3$ (0.1%). The absorbance was measured at 700 nm. BHT, quercetin, EA and AA were used as standards. A higher absorbance of this mixture indicates a higher reducing activity.

2.7. Antioxidant activity in various model systems

2.7.1. Inhibitory activity toward lipid peroxidation (oil-in-water emulsion)

The inhibitory activity of the extracts toward lipid peroxidation was determined using the thiocyanate method.[34] The linoleic acid emulsion was prepared by homogenizing 0.2804 g of linoleic acid, 0.2804 g of Tween-20 as emulsifier and 50 mL 40 mM phosphate buffer

(pH 7.0). Reaction mixture was composed of 2.5 mL linoleic acid emulsion and 0.5 mL of extract solution in different concentrations. The final volume was adjusted to 5 mL with 40 mM phosphate buffer (pH 7.0) and samples were incubated at 37 °C in the dark. After 72 h of incubation, 4.7 mL of ethanol (75%) was mixed with 0.1 mL of the reaction mixture and 0.1 mL $FeSO_4$ (20 mM). Finally, 0.1 mL ammonium thiocyanate (30%) was added to this solution and the absorbance was measured at 500 nm, after it was stirred for 3 min. GA, AA, RU, α-tocopherol and BHT were used as the reference compounds. Inhibition percent of linoleic acid peroxidation was calculated using the following formula: *% inhibition* = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

2.7.2. β-Carotene-linoleic acid model system

The β-carotene bleaching method described by Wu et al. (2010) was used.[35] The β-carotene-chloroform solution (2 mL, 0.2 mg/mL) was pipetted into a round-bottomed flask and chloroform was removed using a rotary evaporator. 40 mg of purified linoleic acid, 400 mg of Tween 40 emulsifier, and 100 mL of distilled water were added to the flask with stirring to form an emulsion. Aliquots (4.8 mL) of this emulsion were added into test tubes with sample solutions (0.2 mL, different concentrations), and the absorbance was immediately measured at 470 nm against a blank (without β-carotene). The tubes were incubated in a water bath at 50 °C, and the absorbance of emulsion was measured over a 60 minute period. EA, QU and α-tocopherol were used as the reference compounds. The antioxidant activity was expressed as an inhibition percentage with reference to the control after a 60 min incubation using the following equation: AA = [(DRC – DRS) / DRC] × 100; where AA - antioxidant activity, DRC - degradation rate of the control = [ln (a/b)/60], DRS-degradation rate in the presence of the sample = [ln (a/b)/60], a - absorbance at time 0, and b - absorbance at 60 min.

2.7.3. Meat model system

The method described by Wettasinghe and Shahidi (1999),[36] with slight modification, was used for determining inhibition of lipid peroxidation of plant extracts in meat model system. Freshly cut pork meat samples were obtained from the local supermarket and most of visible fat was removed. The meat was ground in a meat grinder, divided into six equal parts (500 g each) and every part was mixed with 20% by weight of deionized water. Four parts of ground meat were fully mixed with plant extracts, two parts with FUA and FUR at a concentration of 100 mg/kg and two parts with 500 mg/kg of FUA and FUR. BHT (50

mg/kg) was used for comparative purposes, as standard synthetic antioxidant. One part of meat, containing no additive, was prepared as a control. Then, all systems were homogenized in a blender for 30 s, transferred into plastic pans and stored for 14 days at 4 °C. After designated time (0, 1, 2, 3, 7, 9, 11 and 14 days), the samples were randomly taken for TBARS determination according to the method of Siu and Draper (1978).[37] Meat samples (2 g) were mixed with trichloroacetic acid (5 mL, 10% TCA) and vortexed for 2 min. TBA reagent (5 mL) was added to the mixture and vortexed again for 0.5 min. Samples were centrifuged (4000 rpm, 10 min) and the supernatants were filtered. The tubes with supernatants were placed in a boiling water bath for 45 min, cooled to room temperature in ice, and the absorbance value of TBA-malonaldehyde adduct was read at 532 nm. The TBARS values were then calculated using the standard curve of malondialdehyde (MDA) and expressed as mg MDA equivalents/kg sample.

2.8. pH and thermal stability studies

The pH and thermal stability studies were determined according to Kittiphattanabawon et al. (2012).[38] pH stability was designed as follows. The plant extracts were dissolved in distilled water to obtain a final concentration of 5 mg/mL. The pH of the extract solutions (10 mL) were adjusted to different pH values (1, 3, 5, 7 and 9) with 1 M HCl or 1 M NaOH. The volumes of solutions were made up to 25 mL by distilled water previously adjusted to the same pH values. Then, the mixtures were incubated at room temperature for 1 h. After that, the pH of the mixtures was adjusted to 7.0 and their volumes were made up to 50 mL with distilled water. Thermal stability of the plant extracts was evaluated following the procedure. Briefly, the extracts were dissolved in distilled water to obtain a final concentration of 5 mg/mL. The pH of the plant extract solution (10 mL) was adjusted to 7 and the volume of the solution was made up to 50 mL with distilled water. 10 mL of the each solution was transferred to the test tubes. The tubes were placed in a boiling water bath (100 °C) for 0, 15, 30, 60, 120, 180 and 240 min. After designated heating times, samples were immediately cooled in ice water. The residual antioxidant activity of the extracts was determined using the measurement of total phenolic content (TPC) and DPPH scavenger assay expressed as the activity (%) relative to that obtained without pH adjustment and without heat treatment, using the following equation: *% relative antioxidant activity* = (A × 100) / B, where A - % radical scavenging activity of sample with pH or heat treatment, B - % radical scavenging activity of sample without any treatment.

2.9. In vitro gastrointestinal digestion

The gastrointestinal digestion of plant extracts was simulated *in vitro* as reported by Enari et al. (2008),[39] with slight changes. The process could be illustrated as shown in **Fig. 1**. To simulate oral conditions the extracts solutions (1 mg/ml; 100 ml) were mixed with 10 ml of 10 mM phosphate buffer (pH 6.8) and the mixtures were incubated for 2 min at 37 °C. Stomach conditions were simulated by adding 0.5 mL of 1 M HCl–KCl buffer (pH 1.5), and 5 mL porcine pepsin solution (32 U/mL in HCl–KCl buffer). Incubation was performed for 60 min at 37 °C. The third phase of digestion, intestinal digestion, was made by neutralization of conditions with $NaHCO_3$ (1 M) to pH 6.8. Thereafter, the enzyme mixture of bile and pancreatic juice (1 mL) that contained pancreatin (10 mg/mL), trypsin (14 600 U/mL) and bile extract (13.5 mg/mL) in 10 mM phosphate buffer (pH 8.2), was added to the solutions, followed by incubation at 37 °C for 3 h. During this process of *in vitro* digestion the aliquots of extracts mixtures were taken at 0, 0.5, 1, 2, 3 and 4 h, test tubes were immediately placed in boiling water for 10 min. The residual antioxidant activity of the FUA and FUR extracts were determined using the measurement of total phenolic content (TPC) and DPPH scavenger assay and expressed as the activity (%) relative to those without any treatment using equation above.

2.10. Statistical analysis

The data are expressed as mean ± standard deviation (SD). The IC_{50} for *in vitro* antioxidant potential was calculated using nonlinear regression analysis from the sigmoidal dose-response inhibition curve. Statistical analyses of the data were analyzed using analysis of variance (ANOVA) and the group means were compared with the least significant difference test (LSD). The results were considered statistically significant if the $p < 0.05$.

3. Results and discussion

3.1. The phenolic contents of plant extracts

The results in **Table 1** show the presence of phenolic compounds in extracts of the aerial parts (FUA) and roots (FUR) of *F. ulmaria*. It is obvious from presented results that roots of *F. ulmaria* were richer in the total phenolic compounds (287.77 mg GAE/g) than aerial parts (249.53 mg GAE/g). The root extract is distinguished by its higher content of condensed tannins (250.83 mg GAE/g) and gallotannins (60.91 mg GAE/g). On the other hand, the aerial part extract possesses much higher amounts of flavonoids and flavonols

compared to the root extract values, 45.47 and 37.05 mg RUE/g, respectively. The examined plant contains large amount of condensed tannins as the most abundant polyphenols, either in the roots as well as the aerial parts, considering TPC contents. Harbourne et al. (2009)[40] found that in the meadowsweet herba TPC values varied from 110 to 119 mg GAE/g dry extract, depending on drying conditions. The same work confirmed high level of flavonoids and hydrolyzable tannins in the aerial parts of plant (30-35 % each). Barros et al. (2011)[14] reported much higher content of total phenolic compounds (228 mg GAE/g) in flowers of meadowsweet. Piwowarski et al. (2011)[41] found that the total polyphenol content in the meadowsweet herba was 30.6% and total tannin content was 21%. These results are more approximated to our findings. Amounts of the total and the monomeric anthocyanins, expressed as cyanidin-3-glucoside equivalents, were very low as it was expected considering that the roots, herba and flowers of meadowsweet are not known for being rich in anthocyanins.

The compositions of extracts obtained from *F. ulmaria* aerial parts and roots were evaluated by high performance liquid chromatography (HPLC) analysis. HPLC chromatograms for the *F. ulmaria* extracts before and after hydrolysis are presented on **Fig. 2** and individual quantification of presented compounds are presented in **Table 2**. In the aerial part extract (FUA) five polyphenolic compounds were identified: gallic acid, catechin, epicatechin, rutin and spiraeoside. In the root extract (FUR) catechin and epicatechin were identified and quantified. The major components are the epicatechin in FUA and the catechin in FUR. The quercetin glycoside spiraeoside, characteristic for meadowsweet, were identified only in the aerial part extract. After hydrolysis of both extracts, the contents of gallic acid and ellagic acid increased, so it can be concluded that some derivatives of this two phenolic acids are presented in crude extracts of *F. ulmaria*. In the hydrolyzed aerial part extract significant amount of the flavonoids quercetin and kaempferol are quantified. That indicates the presence of some glycoside derivatives of these flavonoids in the meadowsweet herba. By comparing the amount of quercetin in hydrolyzed FUA extract and amount of quercetin glycosides rutin and spiraeoside before hydrolysis, it is evidently that quantity of quercetin is much higher than the summary amount of rutin and spiraeoside, so we can conclude that in FUA are present quercetin glycosides which are not identified by HPLC. The identification of flavonoid kaempferol in the aerial part extract after hydrolysis also indicates that some of kaempferol derivatives are presented in crude extract. Papp et al. (2004)[3] confirmed that the leaves of meadowsweet contain several kaempferol glycosides. Also, the recent findings of Gniewosz et al. (2014)[5] demonstrated that ethanol and water-ethanol extracts from

meadowsweet flowers contain most of the phenolic components that we quantified in aerial parts extract, except hyperoside which was not quantified in our study. Quantitative determination of individual flavonoid glycosides in plant materials is difficult, due to their large number. Therefore, the glycosides are normally hydrolysed and the resulting aglycones are identified and quantified. The amount of quercetin, kaempferol, gallic and ellagic acid in the mixture increased due to its formation from its glycosides. It is interesting to note that ellagic acid, which was not present in the extracts, and gallic acid which present in low concentration in FUA and not present in FUR, were identified in the hydrolysates of FUA and FUR. Thus, it may be postulated that ellagic and gallic acid may belong to hydrolyzable tannins (ellagitannins and gallotannins) in which the hydroxyl groups of the carbohydrate are partially or totally esterified with phenolic groups of ellagic or gallic acid. However, the hydrolysis conditions which result in optimal breakdown of glycosides are too harsh for some of the other phenolic compounds present in the same plant material. Compared to the original extracts the mass concentrations of catechin and epicatechin were lower in the hydrolysates, revealing the degradation these compounds in acid condition and high temperature. Degradation of catechins, due to acid hydrolysis, has also been reported by Häkkinen et al. (1998).[42]

3.2. In vitro antimicrobial activity

The results of antibacterial activity of *F. ulmaria* aerial parts and roots metanolic extracts evaluated by microdilution method are reported in **Table 3**. Minimal inhibitory concentrations (MIC) of the extracts are in the range of 0.156–5 mg/mL. The antibacterial potential of FUA and FUR were similar against the majority of the tested bacteria. The FUA extract activity in the tested bacteria, decreased in the order: *E. coli* ATCC 25922 > *E. faecalis* FSB 24 > *P. aeruginosa* ATCC 10145 and FSB 37 = *E. coli* FSB 41 = *K. pneumoniae* ATCC 70063. For FUR order of decrease was a little different: *E. faecalis* FSB 24 > *E. coli* ATCC 25922 > *P. aeruginosa* ATCC 10145 and FSB 37 = *E. coli* FSB 41 = *K. pneumoniae* ATCC 70063. The most sensitive bacterial spices on tested extracts were *E. coli* ATCC 25922 and *E. faecalis* FSB 24. In case of *E. coli*, FUA showed lower MIC value (MIC 0.156 mg/mL) than gallic acid (MIC 0.25 mg/mL). Similar results were reported by Rauha et al. (2000),[13] where *F. ulmaria* herba extract exerted excellent antimicrobial activity against *Escherichia coli*.

The results of antifungal activity of the extracts, phenolic compounds and standard antifungal compounds against eleven fungi are presented in **Table 3**. FUA showed the lowest

MIC values (MIC 2.5 mg/mL) against *T. harzianum*, *P. cyclopium* and *F. oxysporum*. The same MIC values exerted FUR against *P. cyclopium* and *F. oxysporum*. Therefore, the most sensitive fungi were *F. oxysporum* and *P. cyclopium*. On the other hand, *A. niger*, *A. alternata*, *D. stemonitis* and *C. albicans* were the most resistant to the tested extracts (MIC >10 mg/mL). Commercial antifungal agents, ketoconazole (MIC 0.156–10 µg/mL) and klotrimazole (MIC 0.156–1.25 µg/mL) were in general more active than FUA, FUR and tested phenolic compounds, although gallic acid and quercetin showed good antifungal activity against some fungi. For most fungal spices FUA showed better MICs than FUR. Gniewosz et al. (2014)[5] confirmed that meadowsweet flower extracts possess very good antibacterial activity with MIC values between 0.2–6 mg/mL and that they exerted certain antifungal activity against tested fungal species, but with no effect on *A. niger* which is consistent with our results.

3.3. Antioxidant activity and potential against lipid peroxidation

The ability to possess antioxidant activity plants owes to their composition, a mixture of different antioxidants, mainly polyphenolic compounds with different action mechanisms. Because of their synergistic interactions, it is indispensable to use several methods in order to determine *in vitro* antioxidant capacity of plant extracts.[43] Therefore, the antioxidant activity of the *F. ulmaria* aerial parts (FUA) and roots extracts (FUR) was estimated using the following methods: total antioxidant capacity assay; methods for $DPPH^{\cdot}$, superoxide radical and $ABTS^{\cdot+}$ scavenging activities; measurement of reducing power and methods for evaluating lipid peroxidation inhibition (oil-in-water emulsion, β-carotene-linoleic acid model system and meat model system). The total antioxidant capacity values (**Table 1**) for both extracts were very high, with higher values for root extract, 494.67 and 419.56 mg AA/g, respectively. Bearing in mind these results, it can be assumed that all the others results for antioxidant potential would be similarly high. The results of antioxidant activity compared to the standard pure natural and synthetic antioxidant compounds are summarized in **Table 4** The *F. ulmaria* extracts possess very good IC_{50} values for scavenging (antiradical) activities on the DPPH radical, superoxide radical and ABTS radical-cation, compared to the natural phenolic antioxidant compounds and synthetic antioxidant BHT. For example, in the DPPH assay, IC_{50} value for FUA was not significantly different ($p > 0.05$) from quercetin and BHT, and value for FUR was not significantly different ($p > 0.05$) from ellagic acid. The best results for DPPH scavenging activity showed ascorbic acid (IC_{50} 6.05 µg/mL). In the determination of activity against superoxide radical, the results were somewhat different. The

ascorbic acid showed the lowest activity, while quercetin demonstrated the best activity (IC_{50} 250.24 μg/mL). The results for *F. ulmaria* extracts were not that good, but the root extract showed a better scavenging capacity than the aerial parts (IC_{50} 603.47 μg/mL). The same characteristic was observed in ABTS assay, FUR reveal much better activity in regard to the FUA. Both extracts showed much better and significantly different results ($p < 0.05$) compared to the ellagic acid and quercetin in ABTS assay. Reducing power of FUA and FUR in different concentrations (**Fig. 3**) was remarkable, compared to the pure antioxidant compounds. It is important to note that the results were similar to the quercetin activity, wherein the activity of FUR was better than quercetin and more alike to BHT activity in lower concentrations (concentration less than 0.05 mg/mL). Although FUR extract showed better reducing power and scavenging activity, the FUA extract was more effective in inhibitory activity toward lipid peroxidation (IC_{50} 50.06 μg/mL) and β-carotene-linoleic acid assays (IC_{50} 69.47 μg/mL). In previous research *F. ulmaria* flower extract showed good antioxidant activity examined by β-carotene bleaching and TBARS inhibition assays with EC_{50} values lower than 0.1 mg/mL.[14]

In all the assays (DPPH-scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS inhibition) FUA and FUR extract demonstrated better values than mentioned flower extract, probably due to higher level of total phenolic compounds. Also, very good antioxidant activity of meadowsweet flowers (DPPH and FRAP methods) was reported by Proestos et al. (2013).[44] However, there were no reported results of antioxidant activities for aerial parts and roots of *F. ulmaria* so it could not be easily compared.

Lipid oxidation is one of the major causes of quality deterioration in food products which also could have influence on consumers' health. Both lipid oxidation and the microbial growth are undesirable, so the food industry uses many additives to slow or inhibit these processes.[10] The most commonly used antioxidant additives are the butylated hydroxyanisole (BHA) and the butylated hydroxytoluene (BHT), however, it has been shown that these additives can cause DNA damage and carcinogenesis.[45] Since *F. ulmaria* extracts exerted good antioxidant activity, we applied them in the meet model system in two concentrations, to evaluate their protective activity on lipid oxidation in meet, compared to the BHT. Dependence of TBARS levels on storage days are presented in **Fig. 4**. As expected, BHT (50 mg/mL) inhibited formation of TBARS in the highest percentage during hole storage time period compared to the control sample without any antioxidant. Interestingly, FUA and FUR both in concentration of 500 mg/mL had TBARS values very similar to the BHT, but insignificantly higher ($p > 0.05$). Values for groups treated with FUA and FUR (100 mg/mL)

were not significantly different until the third day of storage, thereafter FUR at 100 mg/mL exerted lower TBARS values ($p < 0.05$). All values decreasing from day 7 onwards, because of instability of MDA and other short-chain products and formation of products that could not react with TBA as we reported earlier.[46] From presented diagram it could be concluded that applied extracts possess good activity against lipid oxidation in meet until seventh day of storage, the extracts in higher concentration revealed better activity (500 mg/mL), and the root extract had better properties against lipid oxidation than extract from aerial parts of *F. ulmaria*.

3.4. Thermostability and pH stability of plant extracts

The stability studies of meadowsweet extracts were employed to determine the possibility and justifiability of application in different food formulations. Therefore, the FUA and FUR were subjected for different pH values (pH 1-9) and 240 min at boiling temperature, and then total phenolic content and DPPH scavenging activity were evaluated. The results of stability for both extracts are shown at **Fig. 5**. The impact of incubation time on extracts is presented in **Fig. 5A** and **5B**. TPC values for both samples were not significantly different ($p > 0.05$) during the boiling process in first 30 min, compared to those without any treatment (zero time). However, starting at 60 min until the end of treatment TPC values were increased. Im et al. (2011)[47] found that bioactive compounds in some vegetable extracts, like polyphenols, flavanols, flavonoids, anthocyanins and tannins, have high thermostability and high antioxidant properties, so we can presume that meadowsweet extracts with their high content of polyphenols followed a similar trend. Also, Harbourne et al. (2009)[20] showed that meadowsweet herba extraction at temperatures at or above 90 °C for 15 min yields extracts, which may be included in beverages. The relative antioxidant activity of FUA and FUR were around 100% until 60 min, and then was observed a weak decrease of values to 180 min, but still with a very good antioxidant capacity of extracts. In various pH conditions, TPC values were inconsistent (**Fig. 5C** and **5D**). The lowest TPC of FUA was observed at pH 9, and increased in order pH 9 > 1 > 5 > 3 > 7. Meanwhile, TPC values of FUR decreased in order pH 1 > 9 > 7 > 5 > 3. Antioxidant activity based on DPPH radical assay was very good at all pH values except pH 9. Also, there was no significant difference between FUA and FUR activities and compared to untreated extracts ($p > 0.05$), except for the values on pH 9. Similar results of pH stability during meadowsweet herba storage were reported by Harbourne et al. (2013),[48] where in the pH range of 5 to 7 showed good stability.

3.5. In vitro gastrointestinal digestion

In order to provide more information about antioxidant activity and behavior of meadowsweet extracts in the gastrointestinal tract, the extracts were subjected to *in vitro* simulation of gastrointestinal digestion. The results of total phenolic content and relative antioxidant activity (DPPH) in different times of digestion are presented in **Fig. 6A** and **6B**. TPC values for both extracts (FUA and FUR) follow a similar trend (**Fig. 6A**). After 30 min of pepsin digestion values were not significantly different ($p > 0.05$) compared to the values at the beginning of the process. Then, TPC values increased to 120 min of digestion ($p < 0.05$), and after that values were decreased and remained constant to the end of digestion time. Generally, both extracts had high DPPH relative activities (**Fig. 6B**) in the stomach phase of the process. DPPH values in the first two hours of duodenal phase of digestion were significantly different ($p < 0.05$) compared to the samples without any treatment. Compared with the stomach phase of digestion, the DPPH values of *F. ulmaria* extracts significantly ($p < 0.05$) decreased in duodenal conditions. At the end of digestion time relative antioxidant values were slightly increased. Chen et al. (2014)[49] in their study confirmed that selected fruit extracts exhibited much higher DPPH values after gastric phase than after duodenal phase of digestion, probably due to acidic pH conditions in the gastric phase, and they were less effective in duodenal pH because increase of pH values could provoke racemization of phenolic compounds. They also showed that TPC values increased after *in vitro* digestion in stomach conditions, and values decreased after the pancreatic digestion phase because polyphenols are extremely sensitive to alkaline pH and could be degraded in high pH conditions. These results are in correlation with our findings, with exception of TPC values at 60 min of digestion where values start to increase, and they could predict and elucidate good antioxidant activity of *F. ulmaria* in *in vivo* conditions.

4. Conclusion

The phytochemical composition of *F. ulmaria* extracts showed that the aerial parts are predominantly rich in flavonoids, and roots contain a large amount of condensed tannins. Meadowsweet aerial parts and roots showed good antimicrobial activity against most of the selected bacteria and fungi species. Also, *F. ulmaria* exhibit very good antioxidant activity and antiradical properties. Most of the antioxidant capacity values of the extracts were better or very similar compared to the natural phenolic compounds like quercetin, ellagic and ascorbic acid, and synthetic antioxidant BHT. Stability studies suggested that tested extracts

could be incorporated into different food formulations and thermally treated without any significant loss of activity. This research also provided the useful information about the behavior of the *F. ulmaria* extracts in a simulated digestion process. Since the synthetics compounds exerted many harmful effects, there has been growing interest for investigation of compounds from natural sources that possess good antimicrobial and antioxidant traits with no negative effects on the human health so they could be implemented in the food industry. In that sense, this study indicates that the meadowsweet aerial parts and roots potentially could be used for incorporation in various functional foods, to improve food properties and to enhance the health benefits of human diet. However, there is necessity for further toxicity studies of *F. ulmaria* extracts, as well as their activity in *in vivo* conditions.

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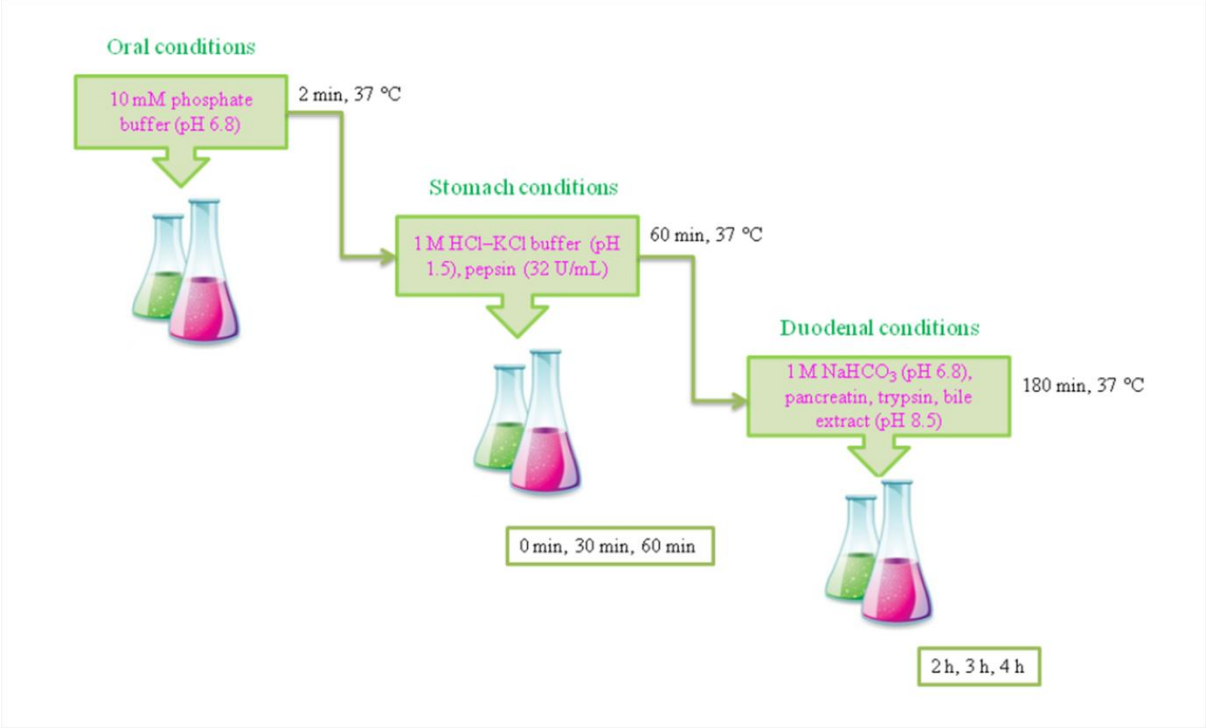


Fig. 1. Scheme of *in vitro* digestion protocol.

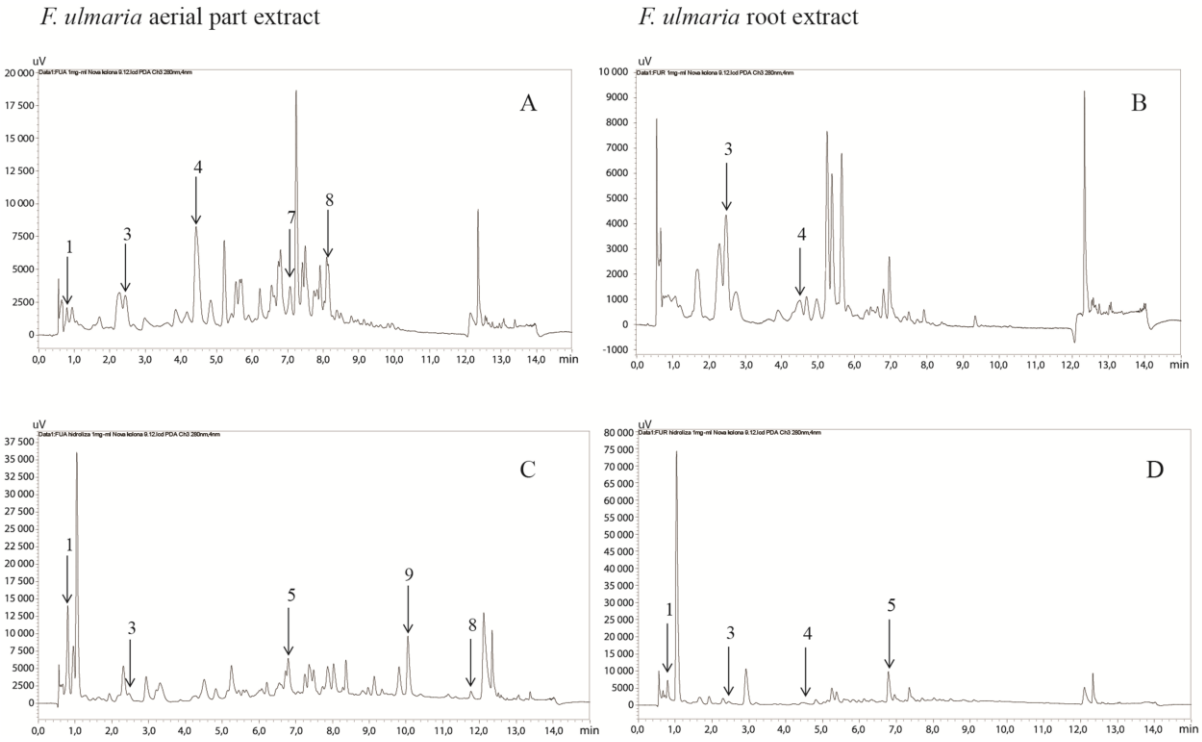
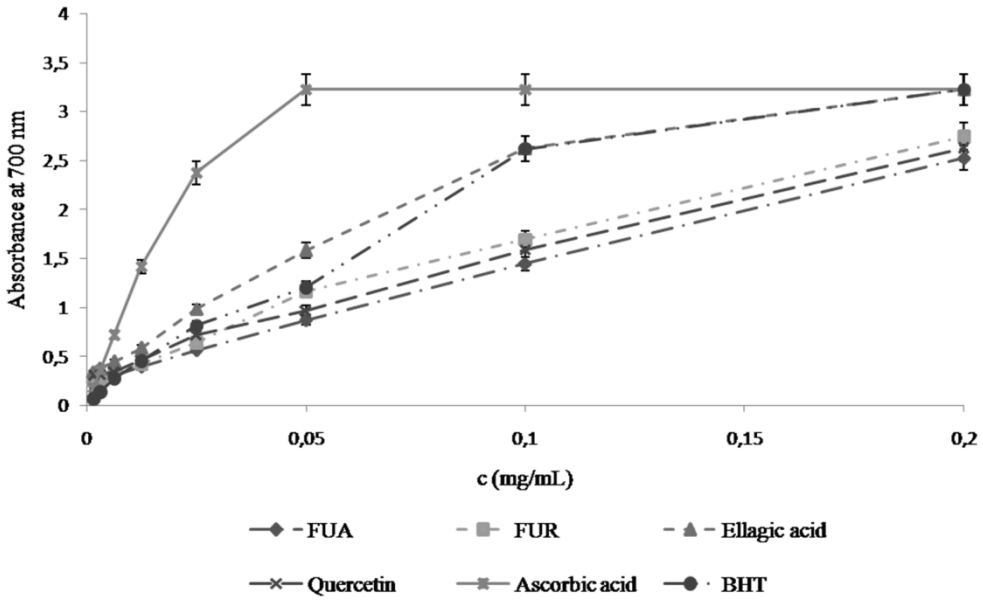


Fig. 2. HPLC profiles of *Filipendula ulmaria* methanol extracts before (A, B) and after hydrolysation (C, D). Detection was performed at 280 nm. Peaks identification: 1 - gallic acid; 2 - caffeic acid; 3 – catechin; 4 – epicatechin; 5 – ellagic acid; 6 – hyperoside; 7 – rutin; 8 – spireoside; 9 – quercetin; 10 – kaempferol.



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809 **Fig. 3.** Reducing power of *F. ulmaria* extracts compared to the reducing power of standards
810 at different concentrations. Each value is the average of three measurements with error bars
811 representing SD.
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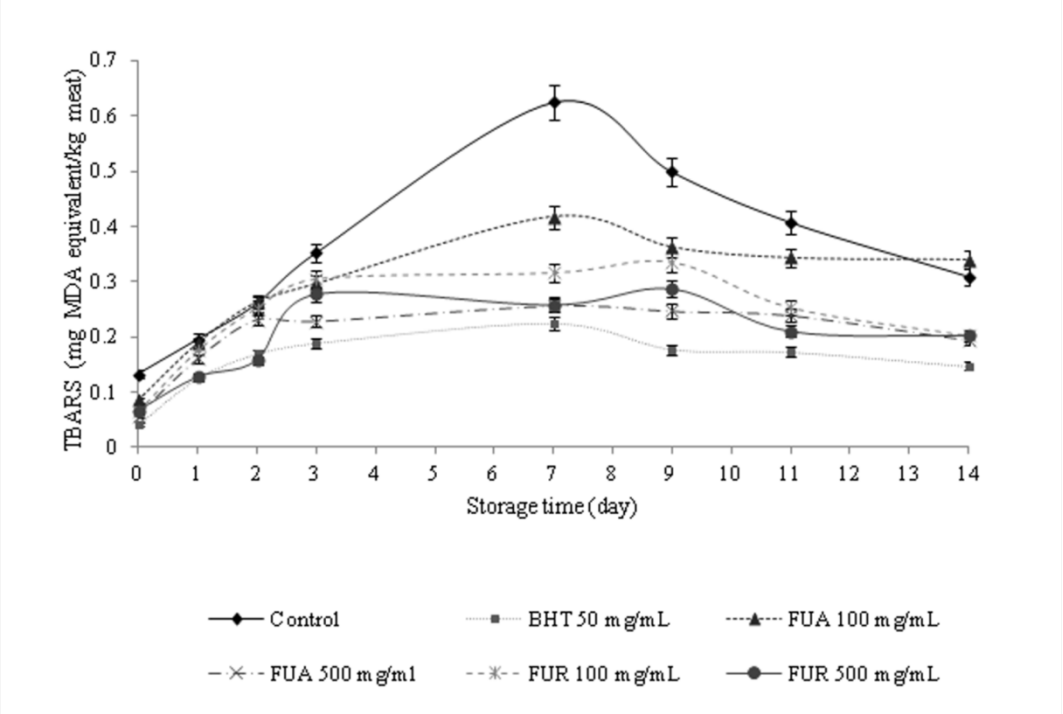

Fig. 4. Changes in lipid oxidation of meat model system added with methanolic extracts of *F. ulmaria* aerial parts (FUA) and roots (FUR) at different concentrations. Butylated hydroxytoluene (BHT) was used as referent synthetic antioxidant. Bars represent standard deviation (n = 3).

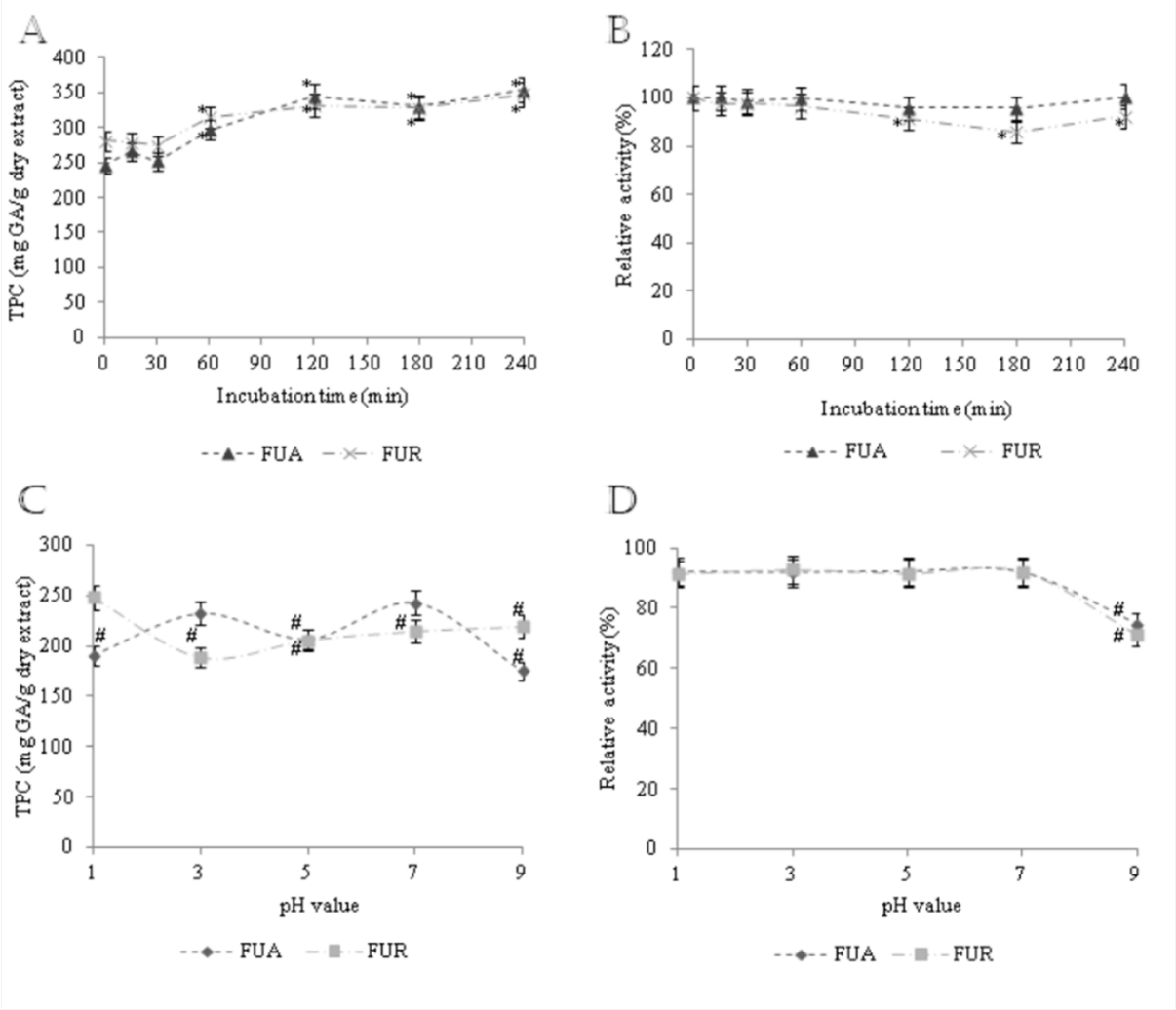

Fig. 5. Thermal (A, B) and pH (C, D) stabilities of *F. ulmaria* aerial parts and roots (FUA and FUR) extracts monitored by the total phenolic content (TPC) and DPPH scavenger activity. Bars represent standard deviation (n = 3). $^{*}p < 0.05$ when compared to the zero time (without any treatment). $^{\#}p < 0.05$ when compared with the untreated extracts.

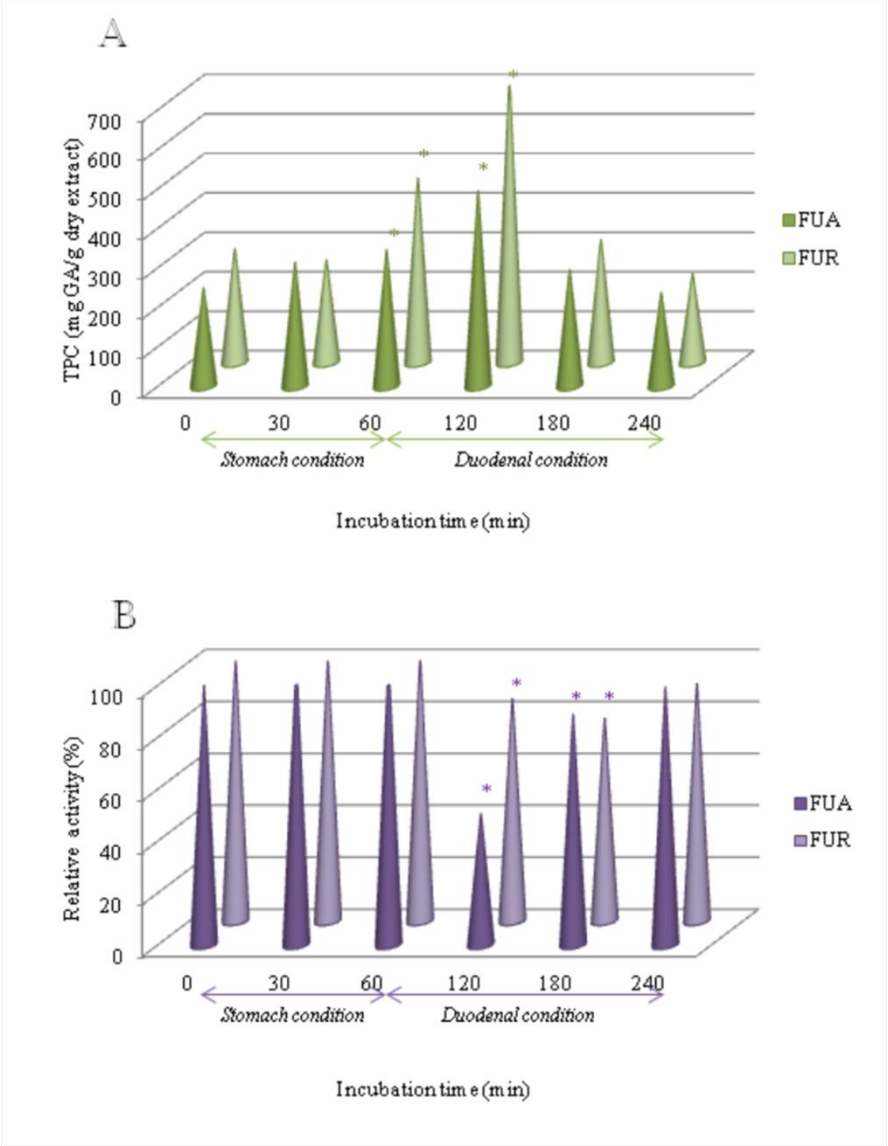

Fig. 6. *In vitro* digestibility of *F. ulmaria* extracts (FUA and FUR) monitored by the total phenolic content (A) and DPPH scavenger activity (B). $^{*}p < 0.05$ when compared to the zero time (without any treatment).

Table 1

The phenolic compounds contents and total antioxidant capacity of *F. ulmaria* aerial parts (FUA) and roots (FUR) methanolic extracts.

Plant extract	Total phenolic content (mg GAE/g)	Total phenolic acids (mg CAE/g)	Flavonoid content (mg RUE/g)	Flavonol content (mg RUE/g)	Condensed tannins content (mg GAE/g)	Gallotannins content (mg GAE/g)	Total antioxidant capacity (mg AAE/g)
FUA	249.53 ± 10.48	47.47 ± 1.31	45.47 ± 3.43	37.05 ± 2.38	183.49 ± 5.11	33.86 ± 2.16	419.56 ± 10.07
FUR	287.77 ± 11.57	57.35 ± 2.06	15.50 ± 1.76	0.10 ± 0.01	250.83 ± 8.32	60.91 ± 3.22	494.67 ± 11.56

Data represented as means ± SD (n = 3). GAE – gallic acid equivalents; CAE – caffeic acid equivalents; RUE – rutin equivalents; AAE – ascorbic acid equivalents.

Table 2

Phenolic components (mg/g) of methanolic extracts from *Filipendula ulmaria* aerial parts and roots before and after hydrolization (mean±SD).

Compounds	*F. ulmaria* extracts		Hydrolyzed extracts	
	FUA	FUR	FUA	FUR
Gallic acid	0.74 ± 0.003	-	7.02 ± 0.031	3.05 ± 0.024
Ellagic acid	-	-	8.87 ± 0.025	12.16 ± 0.037
Vanillic acid	-	-	-	-
Catechin	11.30 ± 0.106	17.17 ± 0.098	4.15 ± 0.016	2.50 ± 0.013
Epicatechin	39.24 ± 0.141	3.12 ± 0.014	-	2.83 ± 0.011
Hyperoside	-	-	-	-
Rutin	6.22 ± 0.032	-	-	-
Spiraeoside	5.94 ± 0.027	-	-	-
Quercetin	-	-	15.49 ± 0.074	-
Kaempferol	-	-	1.24 ± 0.009	-

- : not determined

926 **Table 3**

927 Antibacterial and antifungal activity of *F. ulmaria* extracts, gallic acid and quercetin.

	MIC* values					
Bacterial species	FUA	FUR	Gallic acid	Quercetin	Amracin	
P. aeruginosa ATCC 10145	5	5	>1	>1	5	
P. aeruginosa FSB 37	5	5	>1	>1	5	
E. coli ATCC 25922	0.156	0.625	0.25	>1	0.3125	
E. coli FSB 41	5	5	>1	>1	0.625	
E. faecalis FSB 24	0.3125	0.3125	0.125	>1	1.25	
K. pneumoniae ATCC 70063	5	5	>1	>1	0.625	
Fungal species	FUA	FUR	Gallic acid	Quercetin	Ketoconazole	Klotrimazole
C. albicans ATCC 10259	>10	>10	>1	>1	-	-
T. harzianum FSB 12	2.5	10	>1	>1	5	-
T. longibrachiatum FSB 13	5	10	>1	>1	1.25	-
P. cyclopium FSB 23	2.5	5	1	1	0.156	-
P. canescens FSB 24	10	>10	1	1	1.25	1.25
A. niger FSB 31	>10	>10	>1	>1	0.625	-
A. glaucus FSB 32	5	>10	0.5	1	2.5	1.25
F. oxysporum FSB 91	2.5	2.5	1	0.5	0.3125	-
A. alternata FSB 51	>10	>10	1	1	5	0.156
D. stemonitis FSB 41	>10	>10	>1	1	5	0.156
P. fastigiata FSB 81	5	10	0.0156	0.5	10	0.3125

928 * MIC - minimum inhibitory concentration values given as mg/mL for plant extracts, gallic
929 acid and quercetin, and as µg/mL for antibiotic (amracin) and antimycotics (ketoconazole and
930 klotrimazole); - not tested.
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Table 4

IC_{50} values of antioxidant activities of the methanolic extracts from aerial parts (FUA) and roots (FUR) of *F. ulmaria* compared to the standard antioxidant compounds.

Plant extracts and standards	IC_{50} (µg/mL)				
	$DPPH^{\cdot}$ scavenging activity	Superoxide radical scavenging activity	$ABTS^{\cdot+}$ scavenging activity	Inhibitory activity toward lipid peroxidation	β-Carotene-linoleic acid model system
FUA	16.41 ± 1.74^{a}	611.80 ± 16.72^{a}	36.75 ± 1.79^{a}	50.06 ± 2.15^{a}	69.47 ± 2.56^{a}
FUR	10.58 ± 0.86^{b}	603.47 ± 15.83^{a}	28.73 ± 1.64^{a}	77.23 ± 2.94^{b}	138.36 ± 7.12^{b}
Ellagic acid	8.84 ± 0.41^{b}	285.95 ± 10.06^{b}	767.80 ± 21.72^{b}	1.37 ± 0.16^{c}	12.68 ± 0.94^{c}
Ascorbic acid	6.05 ± 0.34^{c}	778.89 ± 13.56^{c}	10.94 ± 0.95^{d}	>1000	-
Quercetin	17.49 ± 1.12^{a}	250.24 ± 11.02^{d}	228.84 ± 10.35^{c}	24.60 ± 1.23^{d}	>200
BHT	15.61 ± 1.26^{a}	> 2000	7.00 ± 0.87^{e}	1.00 ± 0.23^{c}	-
α-Tocoferol	-	-	-	0.51 ± 0.03^{c}	>1000

IC_{50} values were determined by nonlinear regression analysis.
Results are mean values ± SD from three independent experiments; -, Not tested.
Means in the same column with superscript with different letters are significantly different at $p < 0.05$.

966 **Highlights**

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968 Meadowsweet extracts possess excellent antioxidant and antimicrobial properties, as well as
969 good stability in *in vitro* conditions.

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