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Comparative study of biological activities and phytochemical composition of two rose hips and their preserves: *Rosa canina* L. and *Rosa arvensis* Huds.

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ABSTRACT

The aim of this study was to compare phenolic profile, vitamin C content, antioxidant, anti-inflammatory and cytotoxic activity of rose hips and the preserves (purée and jam) of two *Rosa* species: renowned *Rosa canina* L. and unexplored *Rosa arvensis* Huds. The liquid chromatography–tandem mass spectrometry analysis of 45 phenolics resulted in quantification of 14 compounds, with quercitrin, gallic and protocatechuic acids as the most dominant. High antioxidant potential of *R. canina* and a moderate activity of *R. arvensis* extracts were determined through several assays. Purée of both species and methanol extract of air-dried *R. canina* hips showed some anti-inflammatory (cyclooxygenase-1 and 12-lipooxygense inhibition potency) activity. Purée of *R. canina* exerted cytotoxic activity only against the HeLa cell line among several others (HeLa, MCF7, HT-29 and MRC-5). The presented results support traditional use of rose hips and their fruit preserves as food with health and nutritional benefits.

1. Introduction

Numerous epidemiological and clinical studies have clearly demonstrated the relationship between a diet and health. These findings increased the global interest towards proposing new nutraceuticals and functional foods, which are not only a source of nutrients, but can also work against numerous diseases, such as cancer, cardiovascular disorders and diabetes or enhance the immune response and slow down the process of aging, leading to general physical and mental well-being (Shahidi, 2004). Therefore, there is a great need for an estimation of potential health benefits of plant-based foodstuffs as a rich source of bioactive compounds, through a detailed characterization of their phytonutrients and biological activities.

The genus *Rosa* (family *Rosaceae*) includes about 200 species spread in the temperate and subtropical zones of the Northern hemisphere. *Rosa* species produce rose hips, a pseudocarp or a false fruit, which have one seed covered with achenes and surrounded by fleshy outer layer (Cheikh-Affene et al., 2013). This wild fruit is extensively used worldwide in food preparation and traditional medicine. Usually, it has been consumed as a tea, jelly, jam and beverages and, nowadays, it is also used as an ingredient in probiotic drinks, yoghurts and soups (Demir, Yildiz, Alpaslan, & Hayaloglu, 2014). Known as vitamin C (ascorbic acid) enriched fruit, rose hips are used in ethnomedicine in a prevention and treatment of a wide range of illnesses including cold, flu, vitamin C deficiency, diabetes, arthritis, sciatica, poor peripheral circulation, gastrointestinal, kidney and a lower urinary tract disorders (Chrubasik, Roufogalis, Müller-Ladner, & Chrubasik, 2008). *Rosa canina* L., the best known and mostly utilized *Rosa* species, was extensively investigated by means of phytochemical profile and

Abbreviations: 12-HETE, 12(*S*)-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid; 12-HHT, 12(*S*)-hydroxy(5*Z*,8*E*,10*E*)-heptadecatrienoic acid; 12-LOX, 12-lipoxygenase; AAE, ascorbic acid equivalents; BHT, butylated hydroxytoluene; COX-1, cyclooxygenase-1; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; dw, dry weight; FRAP, reducing power assay; GAE, gallic acid equivalents; HeLa, cervix epithelioid carcinoma cell line; HT-29, colon adenocarcinoma cell line; J, jam extract; loq, limit of quantification; LP, lipid peroxidation; MCF7, breast adenocarcinoma cell line; MD, methanol extract of air-dried rose hips; MDA, malondialdehyde; MF, methanol extract of fresh rose hips; MRC-5, human fetal lung cell line; P, purée extract; PG, propyl gallate; PGB_2, prostaglandin B_2; PGE_2, prostaglandin E_2; QE, quercetin equivalents; SRB, sulforhodamine B; TXB_2, thromboxane B_2; WD, water extract of air-dried rose hips; WF, water extract of fresh rose hips.

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biological potential. It was confirmed that the rose hips of *R. canina* is a valuable source of phytonutrients, such as vitamin C, tocopherols, phenolics, carotenoids, sugars, organic acids and essential fatty acids (Barros, Carvalho, & Ferreira, 2011; Demir et al., 2014; Ercisli, 2007). Also, other studies have shown that the rose hips of this species exhibit a wide range of bioactivities, such as anti-inflammatory (Deliorman Orhan, Hartevioğlu, Küpeli, & Yesilada, 2007; Jäger, Eldeen, & van Staden, 2007; Wenzig et al., 2008), antioxidant (Barros, Carvalho, Morais, & Ferreira, 2010; Barros et al., 2011; Egea, Sánchez-Bel, Romojaro, & Pretel, 2010), antiproliferative (Guimarães et al., 2014; Tumbas et al., 2012), anti-obesity and anti-diabetic activity (Chrubasik et al., 2008). On the other hand, chemical composition and biological activity of other *Rosa* species rose hips have been poorly investigated (Demir et al., 2014; Ercisli, 2007; Guimarães et al., 2013). These studies suggest that rose hips of *R. micrantha*, *R. dumalis*, *R. galica*, *R. hirtissima*, *R. pulverulenta*, *R. vilosa* and *R. pisiformis*, also contain a wide range of bioactive compounds and could be used for the same purposes as the recognized *R. canina*. However, there are no previous reports about the chemical composition or biological activity of *R. arvensis* Huds. rose hips. Namely, *R. arvensis* (field rosa) is a wild *Rosa* species, widespread in Europe, which widely grows on woodland margins, hedgerow and scrub habitats, sharing this habit with other wild roses (Hammond, 2002). *R. arvensis* rose hips are often collected in rural areas, where plant collection is not supervised by professionals, for food preparation and as a remedy instead of *R. canina*. Consequently, the aim of the present study was to examine and compare phenolic profile, vitamin C content and antioxidant, anti-inflammatory and cytotoxic activities of rose hips of two autochthonous *Rosa* species, the renowned *R. canina* and the unexplored *R. arvensis*. Rose hips are soft and tasty during a relatively short period of time, from the first frost until snowing. Therefore, only small amounts of rose hips are consumed fresh, while generally they are preserved by air-drying or processed to purée, jam, juice, or related preserves. Thus, water and methanol extracts of fresh and air-dried rose hips, as well as purée and jam made according to traditional recipes of *R. canina* and *R. arvensis* rose hips were subject of this study.

2. Materials and methods

2.1. Chemicals and reagents

All standards of phenolic compounds, as well as all other chemicals, were purchased from Sigma–Aldrich Chem (Steinheim, Germany), Fluka Chemie GmbH (Buchs, Switzerland) or from ChromaDex (Santa Ana, USA). All reagents used in this study were of analytical grade.

2.2. Plant material and extract preparation

Plant material of *R. canina* L. 1753 was collected in October 2013, in Sajan (Republic of Serbia), while rose hips of *R. arvensis* Huds. 1762 were collected in December 2013, in Ada (Republic of Serbia). The specimen vouchers (*R. canina*, No. 2-1583; *R. arvensis*, No. 2-1596) were prepared, identified and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), University of Novi Sad Faculty of Sciences, Republic of Serbia.

Six extracts of each species were prepared: water extracts of fresh (WF) and air-dried (WD) rose hips with seeds, methanol extracts of fresh (MF) and air-dried (MD) rose hips with seeds, traditionally prepared purée extract (P) and jam extract (J). For preparation of water extracts, 30 g of fresh or air-dried rose hips with seeds were extracted by maceration with boiling distilled water (1 mL of water/0.1 g of plant material), constantly shaken at 120 rpm/min during 1 h at room temperature. For preparation of methanol extracts, 30 g of fresh or air-dried rose hips with seeds were extracted by maceration with 80% aqueous methanol (1 mL of solvent/0.1 g of plant material), constantly shaken at 120 rpm/min during 72 h at room temperature.

After filtration, the water or solvent were evaporated *in vacuo* at 40 °C and crude residues were dissolved in hot, distilled water (10 mL/1 g). In order to remove the non-polar compounds, the extracts were washed exhaustively with petroleum ether (fraction 40–60 °C) and concentrated to dryness under vacuum, yielding 16.21%, 15.51%, 19.20% and 18.08% for water extracts of fresh and air-dried and methanol extracts of fresh and air-dried rose hips of *R. canina*, respectively, or 26.69%, 39.24%, 24.50% and 30.96% for water extracts of fresh and air-dried and methanol extracts of fresh and air-dried rose hips of *R. arvensis*, respectively. Dried extracts were dissolved in distilled water to obtain 300 mg/mL stock solutions for evaluation of the total phenolics and flavonoid and vitamin C contents, as well as antioxidant, anti-inflammatory and cytotoxic activity.

Purée and jam were prepared according to Serbian traditional recipes. In the first step, after washing rose hips, steams and tails were removed. Rose hips were boiled in water and crushed during stirring. Afterwards, cooked rose hips were sieved in order to remove seeds and the irritant hairs of seeds (achene). According to Council Directive 2001/113/EC (2002), the obtained product corresponds to definition of fruit purée. Further, in order to prepare jam, the purée was boiled and 350 g of sugar per 1 L of purée was added (Council Directive 2001/113/EC, 2002). The mixture was stirred until appropriate consistency of jam was obtained. In order to prepare extracts of purée and jam, aliquots of purée and jam (40 mL and 10 g, respectively) of *R. canina* and *R. arvensis* were evaporated *in vacuo* at 40 °C. To remove insoluble particles, crude residue was dissolved in hot, distilled water (10 mL of water/1 g of crude residue), filtered and evaporated *in vacuo* at 40 °C, yielding 7.46% and 36.48% for purée and jam of *R. canina*, respectively, and 14.82% and 27.44% for purée and jam of *R. arvensis*, respectively. Dried extracts of purée and jam were dissolved in distilled water to obtain 200 mg/mL and 300 mg/mL stock solutions, respectively, for evaluation of the total phenolics and flavonoid and vitamin C contents, as well as antioxidant, anti-inflammatory and cytotoxic activity. Additionally, 20 mg/mL and 2 mg/mL stock solutions of all *R. canina* and *R. arvensis* extracts, respectively, were prepared for LC–MS/MS analysis.

2.3. LC-MS/MS analysis of the selected phenolics

Determination of selected phenolics in extracts of *R. canina* and *R. arvensis* rose hips was conducted according to previously published procedure (Orčić et al., 2014). Briefly, samples and 45 standards (prepared in serial dilutions, ranging from 1.53 ng/mL to 25.0×10^3 ng/mL, dissolved in a mixture of 0.5% formic acid and methanol (in 1:1 ratio)) were analyzed using Agilent Technologies 1200 Series HPLC coupled with Agilent Technologies 6410A QqQ mass spectrometer with electrospray ion source and controlled by Agilent Technologies MassHunter Workstation software (ver. B.03.01). Injection volume was 5 μL. Separation was performed using Zorbax Eclipse XDB-C18 (Agilent Technologies) column, 50 mm × 4.6 mm, 1.8 μm, held at 50 °C. Mobile phase, consisting of 0.05% aqueous formic acid (phase A) and methanol (B) was delivered at flow rate of 1 mL/min in gradient mode (0 min 30% B, 6 min 70% B, 9 min 100% B, 12 min 100% B, post time 3 min). Ion source parameters were: nebulization gas pressure 40 psi, drying gas flow 9 L/min and temperature 350 °C, capillary voltage 4000 V. All compounds were detected in negative mode, using dynamic selected reaction monitoring with optimized compound-specific parameters (retention time, precursor ion,

product ion, fragmentor voltage, collision voltage). Concentrations of standard compounds in extracts were determined from the peak areas by using the equation for linear regression obtained from the calibration curves.

2.4. Determination of total phenolic and flavonoid content

Determination of total phenolic and total flavonoid content was performed by previously reported procedure (Beara et al., 2014). Each experimental procedure is briefly explained in text below.

2.4.1. Determination of the total phenolic content

Extracts were used in concentration of 0.125, 0.25 and 0.5 mg/mL. Gallic acid, prepared in eleven concentrations ranging from 1.25 to 100 μg/mL, was used as a standard. Thirty microlitre of each extract or standard solution, except in blank probe, where only the solvent was used, was added to 150 μL of 0.1 mol/dm^3 Folin–Ciocalteu reagent and mixed with 120 μL of sodium carbonate (7.5%) after 10 min. Absorbance at 760 nm was read after 2 h. The phenolics concentration was determined by comparison with the standard calibration curve of gallic acid and results were presented as a mean value of triplicate tests. The total phenol content was expressed as mg of gallic acid equivalents (GAE) per g of dry weight (dw).

2.4.2. Determination of the total flavonoid content

Aluminium chloride colorimetric method was used to determine the total content of flavonoids. Extracts were prepared in concentrations of 20.0, 40.0 and 80.0 mg/mL, while quercetin solutions were prepared ranging from 1.25 to 100 μg/mL and used as a standard. Thirty microlitre of extract or standard solution was diluted with 90 μL of methanol and 6 μL of 10% aluminium chloride (substituted with distilled water in blank probe), 6 μL of 1 mol/dm^3 potassium acetate, and 170 μL of distilled water were added. Absorbance at 415 nm was determined after 30 min. All samples were made in triplicate, and mean values of flavonoid content were expressed as mg of quercetin equivalents (QE) per g of dw, calculated according to the standard calibration curve.

2.5. Vitamin C content

The method for determination of vitamin C content by Klein and Perry (1982), was adapted for 96-well microplates and used within this study. Briefly, each sample was evaporated *in vacuo* at 40 °C and mixed with *meta*-phosphoric acid (0.1 g/mL) to obtain final concentrations of 60, 90 and 120 mg/mL for all extracts, except for extracts of purée which were prepared in concentrations of 40, 60 and 80 mg/mL. Mixtures were stirred for 45 min at room temperature. The prepared extracts in *meta*-phosphoric acid (30 μL) were mixed with 270 μL of 2,6-dichlorophenolindophenol (72 mg/mL) and absorbance was measured within 5 min at 515 nm. The vitamin C content was determined using the standard calibration curve of vitamin C (ranging from 0 to 320 μg/mL) and results were presented as a mean value of three measurements.

2.6. Antioxidant activity

Scavenger activities of *R. canina* and *R. arvensis* extracts towards 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide, superoxide anion and hydroxyl radical, as well as the reducing power (FRAP assay), and inhibition of lipid peroxidation (LP) was evaluated according to methods previously published by Beara et al. (2014).

2.7. Anti-inflammatory activity

In vitro cyclooxygenase-1 (COX-1) and 12-lipoxygenase (12-LOX) assay was undertaken according to the method previously described by Lesjak et al. (2013). In brief, an aliquot of human platelet concentrate (source of COX-1 and 12-LOX enzymes), viable, but outdated for medical treatment, containing 4×10^8 cells, was suspended in buffer (0.137 mol/dm^3 NaCl, 2.7 mmol/dm^3 KCl, 2.0 mmol/dm^3 KH_2PO_4, 5.0 mmol/dm^3 Na_2HPO_4 and 5.0 mmol/dm^3 glucose, pH 7.2) to obtain the final volume of 2 mL. This mixture was slowly stirred at 37 °C for 5 min. Subsequently, 0.1 mL of extract or standard compounds in DMSO (concentration ranging from 10.0 to 300.0; 0.156 to 5.0 and 0.01 to 0.6 mg/mL for extracts, quercetin and aspirin, respectively), substituted with DMSO in the control and blank probe for control, and 0.1 mL of calcimycin (Calcium Ionophore A23187, 125 μmol/dm^3 in DMSO), substituted with DMSO in blank probe and blank probe for the control, were added and incubated for 2 min at 37 °C, with moderate shaking. Afterwards, 0.3 mL of $CaCl_2$ aqueous solution (16.7 mmol/dm^3), substituted with distilled water in blank probe and blank probe for the control, was added and the mixture was incubated for another 5 min at 37 °C with shaking. Acidification with cold 1% aqueous formic acid (5.8 mL) to pH 3 terminated the reaction. Internal standard prostaglandin B_2 (PGB_2; 50 μL of 6 μg/mL solution in DMSO) was added and extraction of products was done with a mixture of chloroform and methanol (1:1 (v/v), 8.0 mL) with vigorous vortexing for 15 min. After centrifugation at 7012g for 15 min at 4 °C, organic layer was separated, evaporated to dryness, dissolved in methanol (0.5 mL), filtered, and used for further LC–MS/MS analysis. All samples and control were made in triplicate. In order to avoid the need for calibration curves for each compound and to simultaneously compensate for the matrix effects, the internal standard approach was chosen. Analyte-to-internal standard (PGB_2) peak area ratio was used for all calculations. Estimated IC_{50} values were compared to IC_{50} values of standards used as positive controls.

2.8. Cell growth activity

Preparation of samples and standards, experimental conditions for maintaining cell lines, as well as sulforhodamine B (SRB) assay were performed according to previously published procedure (Beara et al., 2014). Each experimental procedure is briefly explained in text below.

2.8.1. Preparation of samples and standards

For the evaluation of the cell growth activity, water stock solutions of extracts and podophyllotoxin (10 mg/mL in DMSO) were diluted in NaCl (9 mg/mL) to obtain range of concentrations 0.1–1.0 $\times 10^3$ and 1.0 $\times 10^{-5}$–1.0 μg/mL, respectively.

2.8.2. Maintaining cell lines

Cell growth activity was evaluated *in vitro* in human cell lines: HeLa (cervix epithelioid carcinoma, ECACC No. 93021013), MCF7 (breast adenocarcinoma, ECACC No. 86012803), HT-29 (colon adenocarcinoma, ECACC No. 91072201), and MRC-5 (human fetal lung, ECACC 84101801). Cell lines were grown in DMEM with 4.5% glucose, supplemented with 10% heat inactivated FCS, 100 IU/mL of penicillin and 100 μg/mL of streptomycin. Cells were cultured in 25 cm^2 flasks at 37 °C, in atmosphere of 5% CO_2 and high humidity, and sub-cultured twice a week. A single cell suspension was obtained using 0.1% trypsin with 0.04% EDTA.

2.8.3. SRB assay

The cell lines were harvested and plated into 96-well microtiter plates at seeding density of 3–5 $\times 10^3$ cells/well, in a volume of 199 or 180 μL, and preincubated in complete medium supplemented with 5% FCS, at 37 °C for 24 h. Serial dilutions of extracts (1 μL) were added in 199 μL of medium, while serial dilutions of podophyllotoxin (20 μL) were added in 180 μL of medium, to

achieve the required final concentrations. All samples were filtered through a 0.22 μm microfilters to obtain sterility. Equal volumes of water and DMSO were added in control wells. Concentration of DMSO in cell culture was ⩽5 μL/mL. After adding the dilutions, microplates were incubated at 37 °C for 48 h. The cell growth was evaluated by the colorimetric SRB assay (Četojević-Simin, Svirčev & Baltić, 2009). Colour development was measured using photometer at 540 nm against 620 nm as a background.

2.9. Statistical analysis

The percentage of inhibition achieved by different concentrations of extracts was calculated by the following equation in the antioxidant assays performed: $I\ (\%) = (A_0 - A)/A_0 \times 100$, where A_0 was the absorbance of the control reaction and A was the absorbance of the examined samples, corrected for the value of blank probe. Percent of inhibition of COX-1 and 12-LOX metabolites formation achieved by different concentrations of extracts was calculated by the following equation: $I\ (\%) = 100 \times (R_0 - R)/R_0$, where R_0 and R were response ratios (metabolite peak area/internal standard peak area) in the control reaction and in the examined samples, respectively. Both R and R_0 were corrected for the value of blank probe. The effect on the cell growth was calculated as: $I\ (\%) = 100 \times A_t/A_c$, where A_t is the absorbance of the test sample and A_c is the absorbance of the control, both obtained after subtracting absorbencies at a reference wave length. Corresponding i nhibition–concentration curves were drawn using Origin software version 8.0, and IC_{50} values (concentration of extract that inhibited DPPH·, ·NO, O_2^{-} and HO·, malondialdehyde, COX-1 and 12-LOX metabolites formation and cell growth by 50%) were determined. For each assay and extract composition determinations, all of the results were expressed as mean ± SD of three or eight (cytotoxic activity) different trials. A comparison of the group means and the significance between the groups were verified by Student's t-test. Statistical significance was set at $p \leqslant 0.05$.

3. Results and discussion

3.1. Phenolic profile

Determination of 44 phenolics and quinic acid in rose hips extracts was performed using LC–MS/MS technique (Orčić et al., 2014), where 14 of 45 examined compounds were present in levels above the limit of quantification (loq) and their content is given in Table 1. In this study, the phenolic profile of *R. arvensis* rose hips, as well as some phenolics in *R. canina* rose hips and fruit preserves, were examined for the first time.

The analysis showed that the qualitative composition of examined phenolics in extracts was similar, but the quantitative differences of phenolics between the species were evident. Gallic and protocatechuic acids were the most dominant phenolic acids, present in ten times higher amount in *R. arvensis* than in *R. canina* extracts. In addition, *p*-cumaric and vanillic acids were indentified only in *R. canina* extracts, while ferulic acid was found only in *R. arvensis* extracts. In *R. arvensis* water extracts and *R. canina* purée, *p*-hydroxybenzoic acid was also present. Previously, presence of caffeic, sinapic and ferulic acids in *R. canina* rose hips was confirmed (Demir et al., 2014), but, in this study, these compounds were not found. The analysis of the selected flavonoids showed that both species, especially *R. arvensis,* contained a notable content of glycosides, such as quercetin-3-*O*-glucoside, hyperoside and kaempferol-3-*O*-glucoside, while quercitrin was the most dominant particularly in water and purée extracts of *R. arvensis*. This is in accordance with the previously published studies regarding phenolic profile of *R. canina* rose hips, where a significant

Table 1
Concentration[a] of detected[b] phenolics in *R. canina* and *R. arvensis* extracts.

Compound	Content of selected phenolics (μg/g of dw)											
	R. canina						*R. arvensis*					
	WF	WD	MF	MD	P	J	WF	WD	MF	MD	P	J
Phenolic acids												
p-Hydroxybenzoic acid	<loq[c]	<loq	<loq	<loq	4.21 ± 0.15 c	<loq	66.7 ± 2.67 a	30 ± 1.48b	<loq	<loq	<loq	<loq
Vanillic acid	<loq	<loq	<loq	<loq	12.8 ± 0.62 a	2.74 ± 0.12 b	<loq	<loq	<loq	<loq	<loq	<loq
Gallic acid	11.3 ± 0.64 g	5.11 ± 0.19 h	1.86 ± 0.09 i	2.32 ± 0.11 i	72.8 ± 0.35 d	22.3 ± 1.14 f	131 ± 5.23 b	170 ± 8.33 a	79.6 ± 4.84 c	80.5 ± 3.05 c	47.9 ± 2.09 e	<loq
Protocatechuic acid	9.79 ± 0.39 f	14.2 ± 0.66 e	8.04 ± 0.32 g	13.7 ± 6.61 e	9.12 ± 0.47 f	1.09 ± 0.05 h	241 ± 12.0 a	161 ± 6.08 b	67.3 ± 2.94 d	65.8 ± 2.60 d	71.4 ± 2.70 c	1.32 ± 0.03 h
p-Coumaric acid	<loq	<loq	1.53 ± 0.07 b	1.48 ± 0.05 b	2.30 ± 0.11 a	<loq	<loq	<loq	<loq	<loq	<loq	<loq
Ferulic acid	<loq	<loq	<loq	<loq	<loq	<loq	9.59 ± 0.46 b	<loq	10.20 ± 0.43 b	25.08 ± 1.14 a	<loq	<loq
Flavonoids												
Amentoflavone	<loq	<loq	<loq	<loq	<loq	<loq	2.09 ± 0.08 a	<loq	2.09 ± 0.10 a	<loq	<loq	2.03 ± 0.03 a
Kaempferol-3-*O*-glucoside	<loq	<loq	1.77 ± 0.06 e	3.04 ± 0.13 d	0.44 ± 0.02 f	1.63 ± 0.06 e	<loq	<loq	92.5 ± 2.49 a	76.1 ± 2.74 b	36.1 ± 1.78 c	<loq
Quercitrin	40.4 ± 1.30 f	27.1 ± 0.10 h	95.2 ± 3.29 e	113 ± 6.78 d	42.1 ± 1.78 f	21.7 ± 0.80 i	25.2 ± 1.01 h	421 ± 9.17 b	134 ± 4.96 c	1.50 ± 0.04 J	464 ± 8.24 a	36.7 ± 0.86 g
Quercetin-3-*O*-glucoside	2.54 ± 0.09 f	<loq	9.40 ± 0.36 d	12.1 ± 0.64 c	0.90 ± 0.05 g	3.61 ± 0.16 e	<loq	3.00 ± 0.11 e	208 ± 7.68 a	209 ± 9.33 a	70.6 ± 3.61 b	<loq
Hyperoside	2.53 ± 0.09 f	<loq	7.73 ± 0.33 d	8.50 ± 0.36 d	1.73 ± 0.06 g	3.86 ± 0.21 e	<loq	<loq	187 ± 8.28 b	193 ± 6.76 a	54.5 ± 2.51 c	<loq
Epicatechin	2.35 ± 0.07 d	1.72 ± 0.06 e	2.92 ± 0.10 d	4.74 ± 0.20 c	14.5 ± 0.52 b	35.1 ± 1.67 a	<loq	<loq	<loq	<loq	<loq	<loq
Catechin	7.83 ± 0.41 d	7.35 ± 0.17 d	4.23 ± 0.15 e	2.37 ± 0.08 f	7.64 ± 0.28 d	4.51 ± 0.19 e	10.7 ± 0.57 c	10.4 ± 0.36 c	16.5 ± 0.71 b	61.5 ± 2.27 a	15.3 ± 0.53 b	<loq
Quinic acid	(1.36 ± 0.02) × 10^3 e	(1.17 ± 0.02) × 10^3 f	(1.52 ± 0.01) × 10^3 d	(1.39 ± 0.02) × 10^3 e	(2.19 ± 0.01) × 10^3 c	(1.19 ± 0.01) × 10^3 f	(3.83 ± 0.02) × 10^3 b	(3.75 ± 0.03) × 10^3 b	(4.48 ± 0.02) × 10^3 a	(4.52 ± 0.02) × 10^3 a	(3.89 ± 0.02) × 10^3 b	(0.27 ± 0.01) × 10^3 g

J – jam extract; MD – methanol extract of air-dried rose hips; MF – methanol extract of fresh rose hips; P – purée extract; WD – water extract of air-dried rose hips; WF – water extract of fresh rose hips.

[a] Values are means ± SD of three measurements. Means within each row with different letters (a–j) differ significantly ($p \leqslant 0.05$).

[b] Compounds analyzed, but peak was below loq; 2,5-dihydroxybenzoic acid, cinnamic acid, syringic acid, 5-*O*-caffeoylquinic acid, caffeic acid, *o*-coumaric acid, 3,4-dimethoxycinnamic acid, sinapic acid (phenolic acids); apigenin, apigenin-7-*O*-glucoside, baicalin, baicalein, apiin, rutin, daidzein, naringenin, vitexin, genistein, isorhamnetin, luteolin, luteolin-7-*O*-glucoside, myricetin, kaempferol, epigallocatechin gallate, chrysoeriol, quercetin (flavonoids); umbelliferone, aesculetin, scopoletin (coumarins); matairesinol, secoisolariciresinol (lignans).

[c] Reference Orčić et al. (2014).

content of quercetin glycosides was also confirmed, while quercetin was absent (Guimarães et al., 2013). Additionally, in our study, epicatechin was found in *R. canina*, while it was not detected previously (Demir et al., 2014; Guimarães et al., 2013). Furthermore, in our study, nine phenolic compounds were quantified in *R. canina* jam, while previously only catechin and quercetin were detected (Türkben, Uylaşer, İncedayi & Çelikkol, 2010). It could be generally noted that all extracts of *R. arvensis*, except the jam, had a higher content of investigated compounds than the *R. canina* extracts. Investigated coumarins and lignans were not detected in examined extracts. The most abundant compound in all extracts was quinic acid, which was expected, since it is an important moiety of many phenolics.

Generally, the results obtained for *R. canina* phenolic profile are in good correlation with earlier studies (Demir et al., 2014; Guimarães et al., 2013), with slight qualitative and quantitative differences. Environmental factors (e.g., light, temperature, soil nutrients) and maturity stages of the fruit, may affect the metabolism and conversions of phenolics, which could be the reason for dissimilarities between these results (Demir et al., 2014). Anyway, Adamczak, Buchwald, Zieliński, and Mielcarek (2012) suggested that the variation of flavonoids in rose hips are of great value for their chemotaxonomy.

The total phenolic content (Table 2) in extracts of *R. canina* ranged from 11.9 to 96.2 mg GAE/g of dw, which was significantly higher than the phenolic content in *R. arvensis* extracts (from 6.63 to 18.7 mg GAE/g of dw), which is opposite to quantitative data of phenolics obtained by LC–MS/MS analysis. This could be due to the weakness of the Folin–Ciocalteu assay, which gives only an estimation of total phenolic content since many of the interfering compounds, including vitamin C, could react with a reagent and elevate the results (Prior, Wu, & Schaich, 2005). Additionally, we only investigated limited number of phenolics by LC–MS/MS. Anyway, the total phenolic content in the *R. canina* rose hips is in agreement with some of the previous studies (Barros et al., 2011; Ercisli, 2007).

Similar amount of flavonoids were found in both species. The highest flavonoid content among *R. canina* extracts was determined in pureé. Interestingly, amongst the *R. arvensis* extracts, the significant amount of flavonoids was found in jam, which could be a consequence of thermal degradation of complex, polymeric polyphenols during cooking (De Paepe et al., 2014).

Table 2
Total phenolic, flavonoid and vitamin C content in *R. canina* and *R. arvensis* extracts.

Extracts	Content[a]		
	Total phenolics (mg GAE/g of dw)	Total flavonoid (mg QE/g of dw)	Vitamin C (mg/g of dw)
R. canina			
WF	74.6 ± 3.08 b	1.22 ± 0.02 f	1.96 ± 0.18 b
WD	61.0 ± 3.37 c	1.14 ± 0.04 fg	2.09 ± 0.20 b
MF	50.9 ± 3.60 c	0.65 ± 0.03 h	1.87 ± 0.14 b
MD	50.3 ± 2.26 c	0.63 ± 0.04 h	1.83 ± 0.17 b
P	96.2 ± 4.35 a	2.94 ± 0.02 b	3.73 ± 0.03 a
J	11.9 ± 0.84 ef	0.61 ± 0.03 h	0.56 ± 0.05 c
R. arvensis			
WF	6.63 ± 0.56 g	2.24 ± 0.05 c	0.13 ± 0.01 f
WD	9.75 ± 0.27 f	1.14 ± 0.02 g	0.23 ± 0.01 e
MF	13.8 ± 0.61 e	4.55 ± 0.10 a	0.32 ± 0.02 d
MD	18.7 ± 0.23 d	1.48 ± 0.01 e	0.42 ± 0.03 c
P	9.54 ± 0.67 f	1.76 ± 0.06 d	0.22 ± 0.02 e
J	7.40 ± 0.65 g	4.26 ± 0.05 a	0.14 ± 0.00 f

J – jam extract; MD – methanol extract of air-dried rose hips; MF – methanol extract of fresh rose hips; P – purée extract; WD – water extract of air-dried rose hips; WF – water extract of fresh rose hips.

[a] Values are means ± SD of three measurements. Means within each column with different letters (a–h) differ significantly ($p \leqslant 0.05$).

To the best of our knowledge, this is the first extensive analysis of phenolic compounds in *R. arvensis* rose hips, and purée and jam of *R. canina*, which is a valuable basis for further research and approval of using them as food with health benefits.

3.2. Vitamin C content

Rose hips are considered to be a valuable natural source of vitamin C. Furthermore, it is regarded that organic acid and flavonoids in rose hips prevent oxidation of vitamin C, which additionally increases its stability and bioavailability in humans (Adamczak et al., 2012). The amount of vitamin C in *R. canina* extracts ranged from 0.56 to 3.73 mg/g of dw (Table 2), which is in good agreement with the previously published results of Barros et al. (2011) and higher than the content determined by Demir et al. (2014). Also, Nojavan et al. (2008) reported that rose hips of *R. canina* contain the highest vitamin C content in fully ripened maturity stage, which was six times higher than that in orange. Despite the differences in experimental conditions, we found the highest content of vitamin C in *R. canina* water extract of dried hips (31.48 mg/100 g of fresh fruit), which was lower than its amount in mild-temperature-dried full ripe rose hips (211 mg/100 g fresh fruit; Nojavan et al., 2008). This is in accordance with previous observations stating that the stability of vitamin C in *R. canina* fruit matrix is higher than in the fruit extract (Nojavan et al., 2008). In our study, no significant difference between vitamin C content in fresh and air-dried *R. canina* rose hips was found, which supports the traditional use of air-dried rose hips as a source of vitamin C. According to the results presented, 540 mL of purée, 360 g of jam, 230 g of fresh or 275 g of air-dried *R. canina* rose hips contained a recommended daily vitamin C intake (90 mg) for adult men (Krinsky et al., 2000).

Vitamin C is present in much smaller amounts in *R. arvensis* rose hips extracts (from 0.13 to 0.42 mg/g of dw), which could be due to environmental influences. Namely, some authors suggested that the level of vitamin C in rose hips could depend on the soil and climate conditions (Adamczak et al., 2012), as well as on the maturity stage (Barros et al., 2011; Nojavan et al., 2008). To the best of our knowledge, there are no previously published data about the vitamin C content in *R. arvensis* hips before our study.

Results of the present study indicate that rose hips of the two investigated *Rosa* species, as well as their fruit preserves, present a valuable natural source of vitamin C and support their traditional use as food rich in vitamin C.

3.3. Antioxidant activity

Epidemiological studies suggested that dietary antioxidants could prevent or slow down the oxidative stress induced by free radicals. Consumption of food rich in phenolics has been associated with a decreased risk of diseases mediated by oxidative stress, such as cardiovascular diseases and certain types of cancer (Knekt et al., 2002). These effects of phenolics from functional food are a consequence of their antioxidant activity, which includes direct free radical scavenging ability and an indirect effect arising from chelation of prooxidant metal ions or activation of the endogenous antioxidant defence system (Shahidi, 2004). In addition, as an electron donor, vitamin C is a powerful water-soluble antioxidant in humans (Padayatty et al., 2003). In order to extensively characterize the antioxidant potency of the widely consumed fruit of the two *Rosa* species, along with their fruit preserves, several *in vitro* assays were performed: DPPH, nitric oxide, hydroxyl and superoxide anion radical scavenger capacity tests, as well as FRAP assay and Fe^{2+}/ascorbate induced LP. The antioxidant effect of extracts and standards were concentration dependent, and the corresponding IC_{50} values are shown in Table 3. Overall, all extracts showed a

Table 3
Antioxidant activity of *R. canina* and *R. arvensis* extracts.

Extracts	Antioxidant activity[a] IC_{50} values					
	DPPH· (μg/mL)	·NO (μg/mL)	O_2^{-} (μg/mL)	HO· (μg/mL)	LP (μg/mL)	FRAP (mg of AAE/g dw)
R. canina						
WF	32.7 ± 1.54 e	126 ± 2.68 d	16.0 ± 0.68 d	(1.48 ± 0.10) × 10^3 j	na[b]	84.5 ± 4.19 bc
WD	35.0 ± 0.86 e	74.4 ± 6.75 b	18.1 ± 1.12 d	600 ± 18.0 f	na	79.0 ± 1.75 c
MF	21.7 ± 2.04 d	136 ± 5.50 d	20.9 ± 1.16 e	475 ± 16.0 e	na	82.2 ± 4.32 bc
MD	36.9 ± 2.74 e	154 ± 0.39 e	13.8 ± 1.11 c	119 ± 58.0 i	na	57.6 ± 3.09 d
P	11.8 ± 0.24 c	109 ± 7.80 c	6.51 ± 0.30 a	na	240 ± 14.8 b	88.2 ± 1.99 b
J	81.1 ± 1.74 f	448 ± 1.50 g	81.5 ± 2.58 i	102 ± 36.0 h	na	13.3 ± 0.94 e
R. arvensis						
WF	313 ± 10.9 J	(2.15 ± 0.02) × 10^3 l	21.8 ± 0.47 ef	631 ± 40.0 f	na	3.81 ± 0.11 J
WD	177 ± 0.67 h	(1.95 ± 0.01) × 10^3 k	24.7 ± 0.88 f	371 ± 5.00 c	(1.96 ± 0.08) × 10^3 d	5.05 ± 0.10 h
MF	103 ± 0.05 g	331 ± 0.90 f	21.5 ± 0.20 ef	760 ± 22.0 g	(2.38 ± 0.10) × 10^3 e	7.67 ± 0.20 g
MD	86.2 ± 3.62 f	622 ± 0.30 i	30.8 ± 1.05 g	431 ± 1.00 d	(1.31 ± 0.10) × 10^3 c	13.2 ± 1.10 e
P	239 ± 9.67 i	526 ± 5.60 h	31.0 ± 1.36 g	569 ± 22.0 f	na	4.74 ± 0.12 i
J	702 ± 50.7 k	(1.51 ± 0.01) × 10^3 j	55.9 ± 1.62 h	na	na	8.33 ± 0.27 f
Standards						
PG	(383 ± 3.62) × 10^{-3} a	6.58 ± 0.43 a	9.68 ± 0.29 b	25.0 ± 0.01 a	na	na
BHT	9.32 ± 0.08 b	na	na	157 ± 5.00 b	14.0 ± 3.00 a	124 ± 11.5 a

J – jam extract; MD – methanol extract of air-dried rose hips; MF – methanol extract of fresh rose hips; P – purée extract; WD – water extract of air-dried rose hips; WF – water extract of fresh rose hips.
[a] Values are means ± SD of three measurements. Means within each column with different letters (a–l) differ significantly ($p \leqslant 0.05$).
[b] na – 50% inhibition not achieved.

considerable antioxidant effect comparable with the well-known synthetic antioxidants butylated hydroxytoluene (BHT) and propyl gallate (PG). Furthermore, our results showed that antioxidant activity of *R. canina* was markedly higher than that of *R. arvensis*. Among *R. canina* extracts, purée exhibited a significant scavenging activity towards DPPH· (IC_{50} = 11.8 μg/mL), which was slightly lower than the activity of standard synthetic antioxidant BHT (IC_{50} = 9.32 μg/mL), while O_2- scavenging activity was better (IC_{50} = 6.51 μg/mL) than the activity of synthetic antioxidant PG (IC_{50} = 9.68 μg/mL). Moreover, purée of *R. canina* also showed a notable reducing power (88.20 mg of ascorbic acid equivalents (AAE)/g of dw), which was slightly weaker than the activity of synthetic antioxidant BHT (124 mg AAE/g of dw). Some previous studies reported a considerable antioxidant activity of the *R. canina* fruits, which was stronger than the activity of some other wild fruits extensively consumed in the diet (blackthorn, strawberry tree, wild blackberry, hawthorn and service tree fruits; Barros et al., 2010; Barros et al., 2011; Egea et al., 2010; Tumbas et al., 2012). In addition, observed *in vitro* antioxidant activity could also contribute to the clinical effect of *R. canina* rose hips preparation in patients with osteoarthritis (Chrubasik et al., 2008).

Antioxidant potential of *R. arvensis* hips extracts has been investigated for the first time in this study. It could be noticed that methanol extracts were more potent in neutralizing DPPH· and ·NO, had a better reducing power, and showed a moderate capacity to inhibit LP than other *R. arvensis* extracts. Nevertheless, jam of both species showed some antioxidant activity compared to other investigated extracts, even though it is considered that thermal treatment in preparation process would greatly degraded compounds that have strong antioxidant activity, such as polyphenolics (De Paepe et al., 2014).

Despite the previously reported strong relationship between the total phenolics content and antioxidant activity (Wenzig et al., 2008), we did not found a clear correlation between the content of the examined compounds and results of antioxidant assays. This indicates a strong additive and synergistic effect of phenolics, flavonoids and vitamin C, as well as other uninvestigated bioactive compounds which could be responsible for a determined antioxidant activity. According to the results presented, it could be concluded that hips of *R. canina* and *R. arvensis*, as well as their fruit preserves, especially purée, present a valuable source of natural antioxidants which could indicate their use as possible agents against prevention of oxidative-stress. Further comprehensive *in vivo* studies are necessary in order to demonstrate rose hips antioxidant effect and support their use as nutraceuticals.

3.4. Anti-inflammatory activity

A number of investigations documented a correlation between increased expression of arachidonic acid metabolites and various diseases prevalence and progression (Hyde & Missailidis, 2009). The important regulatory factors influencing arachidonic acid metabolism, besides substrate availability, are expression levels and activities of the enzymes included in arachidonic acid metabolism. Within the arachidonic acid cascade, COX and LOX enzymes produce potent inflammatory mediators. Inhibition of arachidonic acid cascade has been of great interest not just in treatment of inflammatory conditions, but also in treatment of certain types of cardiovascular diseases and cancer chemoprevention (Hyde & Missailidis, 2009). In order to examine anti-inflammatory activity of *R. arvensis* and *R. canina* hips extracts, *in vitro* test for determination of inhibitory activity towards production of COX-1 and 12-LOX pathway metabolites: 12(*S*)-hydroxy(5*Z*,8*E*,10*E*)-heptadecatrienoic acid (12-HHT), thromboxane B_2 (TXB_2), prostaglandin E_2 (PGE_2) and 12(*S*)-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid (12-HETE) was undertaken.

Only methanol extract of dried hips and purée of *R. canina*, showed potential to inhibit the production of PGE_2 (IC_{50} = 10.3 ± 0.08 mg/mL and IC_{50} = 9.39 ± 0.01 mg/mL) and 12-HETE metabolites (IC_{50} = 8.58 ± 0.18 mg/mL and IC_{50} = 9.35 ± 0.33 mg/mL; Supplementary material). Presented results indicate minor COX-1 and 12-LOX inhibitory activity of examined *R. canina*. Namely, determined activities were significantly lower than the activity of the well-known potent inhibitors of ascorbic acid metabolism aspirin and quercetin, which reached IC_{50} values for inhibition of production PGE_2 metabolite at 5.58 ± 0.53 and 12.75 ± 0.26 μg/mL, respectively. Furthermore, IC_{50} values for 12-LOX inhibition was 7.44 ± 0.65 μg/mL for quercetin, while aspirin was inactive (Lesjak et al., 2013).

Although previous studies have shown that anti-inflammatory activity of the *R. canina* rose hips is the most pronounced in non-polar extracts, probably due to presence of various fatty acid derivatives, in this work the polar extracts (water and methanol), as well as purée and jam, forms in which rose hips are mostly consumed in the diet, were investigated (Chrubasik et al., 2008; Deliorman Orhan et al., 2007; Jäger et al., 2007; Wenzig et al., 2008). The results obtained in this study show that the polar extracts also have some anti-inflammatory activity, indicating that the classes of polar compounds, such as phenolics, could also contribute to observed activity. It is interesting that the purée extract showed generally the best anti-inflammatory potential, which could contribute to its valorisation as a functional food.

Regarding the *R. arvensis* extracts, whose anti-inflammatory activity was not investigated up to this study, only purée inhibited production of PGE_2 metabolite (IC_{50} = 7.46 ± 0.05 mg/mL; Supplementary material), suggesting its COX-1 pathway inhibitory potential. In general, the presented results indicate that purée of both species and methanol extract of dried hips of *R. canina* possess some COX-1 and 12-LOX inhibitory potency. Apparently, rose hips of *R. canina* and *R. arvensis* could present a potential source of natural anti-inflammatory agents. Further in-depth investigation on *Rosa* species effect on arachidonic acid metabolism, as well as production of other inflammation mediators, is required in order to entirely evaluate anti-inflammatory activity of rose hips and their fruit preserves.

3.5. Cytotoxic activity

The cell growth inhibitory activity of twelve rose hips extracts of the two *Rosa* species and their fruit preserves were evaluated *in vitro* by SRB assay using human non-tumor, MRC-5, and three tumor cell lines HeLa, MCF7 and HT-29. Podophyllotoxin, a highly potent cytotoxic agent, was applied as a standard compound.

Only purée of *R. canina* exerted cytotoxic activity regarding HeLa cell lines (IC_{50} = 498 ± 30.5 μg/mL), while podophyllotoxin reached IC_{50} value on the same cell line at concentration of $(4.1 \pm 3.0) \times 10^{-3}$ μg/mL (Supplementary material). All other extracts of *R. canina* did not affect the growth of investigated cell lines. It is important to highlight that extracts did not influenced growth of health MRC-5 cells. Tumbas et al. (2012) investigated *in vitro* antiproliferative activity of *R. canina* rosehip tea fractions in a panel of three human cancer cell lines HeLa, MCF7 and HT-29. The authors found that fraction containing the highest amount of flavonoids exhibited the strongest inhibitory activity toward HeLa cell line growth. These results are in accordance with data obtained in this study, since it was found that single extract whose cytotoxicity was observed, purée of *R. canina*, had the highest content of total polyphenolics and flavonoids. Furthermore, Guimarães et al. (2014) also reported inhibitory activity of *R. canina* rose hip extracts on growth of few cell lines, including HeLa, and absence of cytotoxicity against MCF7. Extracts of *R. arvensis* did not show inhibition potency against growth of the examined cell lines.

These results support future evaluation of *R. canina* rose hips purée as a food with potential health and nutritional benefits.

4. Conclusion

In the present study, phenolic profile, vitamin C content, as well as antioxidant, anti-inflammatory and cytotoxic activity of water and methanol extracts of fresh and air-dried rose hips of the two *Rosa* species, together with their fruit preserves, were investigated. To the best of our knowledge, this is the first report about biopotential and chemical composition of *R. arvensis* rose hips extracts. The LC–MS/MS analysis of the selected plant phenolics revealed that *R. arvensis* rose hips extracts are richer in content of the examined compounds than *R. canina* extracts, with phenolic acids (gallic and protocatechuic acid), quercitrin, quercetin-3-*O*-glucoside and hyperoside being the most dominant in both species. Also, *R. canina* extracts showed a high content of the total phenolics and vitamin C. Evaluation of antioxidant activity demonstrated that *R. arvensis* extracts possess moderate activity in comparison with *R. canina* extracts. Purée of both species and methanol extract of air-dried *R. canina* hips showed some anti-inflammatory activity by means of inhibition of the main arachidonic acid metabolites formed in COX-1 and 12-LOX pathway. Investigation of cytotoxic activity against the applied cell lines (HeLa, MCF7, HT-29 and MRC-5) also highlights purée of *R. canina*, a single extract which exhibited inhibition potency toward HeLa cells. In general, the results obtained in this study support the traditional use of *R. canina* and *R. arvensis* rose hips, purée, jam and tea as food with potential health and nutritional benefits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.07.089.

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