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Rowanberry Phenolics: Compositional Analysis and Bioactivities

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Berries contain a large variety of different phenolic compounds such as anthocyanins, flavonols, tannins, and phenolic acids. Due to variation in the nature and content of the phenolic compounds, the antioxidant effect and other bioactivities of berry phenolics are strongly dependent on the berry raw material as the activities differ between the different phenolic constituents. In the present study, wild rowanberries (*Sorbus aucuparia*) and four cultivated sweet rowanberries, Burka, Granatnaja, Titan, and Zoltaja, were characterized for their phenolic composition and screened for antioxidant, antimicrobial, and antiadhesive activities. The HPLC and LC-MS analyses of phenolic composition revealed that the main phenolic constituents were caffeoylquinic acids, varying from 56 to 80% total phenolics. The cultivated species contained less caffeoylquinic acids and more anthocyanins (up to 28.5%). The phenolics derived from wild rowanberries were significantly effective at inhibiting lipid oxidation both in liposomes and in emulsions, especially when assessed by inhibition of the formation of hexanal (86−97% inhibition depending on concentration). The increase in anthocyanin content in the cultivated species did not result in significantly increased antioxidant activity. Both wild and cultivated rowanberry phenolics exhibited a bacteriostatic effect toward *Staphylococcus aureus*. In addition, the phenolic extract from Zoltaja was weakly inhibitory toward *Salmonella* sv. Typhimurium, whereas both Zoltaja- and Granatnaja-derived phenolics retarded *Escherichia coli* growth. The phenolic extracts of wild rowanberries and Burka showed an inhibitory effect on hemagglutination of *E. coli* HB101 (pRR7), which expresses the M hemagglutinin. It can be concluded that cultivation of rowanberries resulted in increased anthocyanin content, but this did not diminish their bioactivity in comparison to wild rowanberries rich in caffeoylquinic acids.

KEYWORDS: Sweet rowanberry; *Sorbus aucuparia*; rowanberry; chlorogenic acid; antioxidant; antimicrobial; antiadhesive

INTRODUCTION

Berries contain a large variety of different phenolic compounds such as anthocyanins, flavonols, and phenolic acids (*1*). Anthocyanins predominate in most berries such as in the genus *Vaccinium*, encompassing blueberries, cranberries, and lingonberries, and in the genus *Ribes*, encompassing currants and gooseberry (*2*). In some berries, such as in cranberries and lingonberries, flavanols and procyanidins are among the main constituents. Cloudberries and raspberries are members of the Rosaceae family, closely related to strawberry in the subfamily Rosoideae and belonging to the geneus *Rubus*, which are well reported to be rich in ellagitannins (*1*−*3*). Another member of the Rosaceae family, *Fragaria* (including strawberry) is also rich in ellagitannins but contains even more anthocyanins. Within berries the most abundant phenolic acids are caffeic acid and its associated derivatives (*3*), such as esters, glycosides, or cell wall-bound forms. Indeed, only a minor fraction of the phenolic acids are in the free form (*4*). In wild rowanberries (*Sorbus aucuparia*), the main phenolic constituents are (neo)chlorogenic acids and flavonols such as quercetin and kaempferol conjugates (*2*, *5*, *6*) Other hydroxycinnamates are also found in rowanberries including hydroxylated cinnamic acids *p*-coumaric acid, ferulic acid, caffeic acid, and associated dicinnamate conjugates (*7*). Rowanberries have been bred for northern conditions and used for jams and jellies.

The antioxidant effect and other bioactivities of berry phenolics are strongly dependent on the berry raw material as the activities differ between the different phenolic constituents (*8*).

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Berry flavonoids and phenolic acids are reported to be important antioxidants due to their ability to retard the oxidation of lipids, thus improving the quality of food (*1*−*3*). Furthermore, plant phenolics can act as chain-breaking antioxidants inhibiting lipid oxidation, thus affecting beneficially food deterioration during storage and processing (*9*). In our previous studies, lipid oxidation was shown to be inhibited by berry phenolics including raspberry ellagitannins, cranberry procyanidins, and black currant anthocyanins in food models consisting of emulsions or liposomes (*10*−*12*). Various berries such as cloudberries, raspberries, and strawberries, rich in ellagitannins, have also been reported to possess antimicrobial properties toward the growth of virulent bacteria, such as *Helicobacter pyroli*, *Campylobacter jejuni*, *Candida albicans*, *Clostridium perfringens*, and *Salmonella* and *Staphylococcus* species (*13*, *14*). The antimicrobial activity of rowanberries has previously been attributed to sorbic acid, which is an organic acid with reported inhibitory effect especially against yeasts and molds, and its action against bacteria is more selective (*15*). In our earlier studies a phenolic extract of wild rowanberry showed a bacteriostatic effect against *C. jejuni* and *Staphylococcus aureus*, but did not inhibit the growth of *Can. albicans* (*13*). There are several reports of berry procyanidins exhibiting antimicrobial effects (*1*−*3*), whereas a number of papers have reported that the procyanidins of American cranberry inhibit the attachment of uropathogenic *Escherichia coli* to uroepithelial and vaginal cells (*16*). Data regarding bioactivities exist for a number of berry species, but the bioactive properties of rowanberries have not been extensively characterized. Therefore, our aim was to characterize the phenolic composition of the wild rowanberry and its five cultivated sweet varieties and to assess their different bioactivities, that is, their antioxidant and antimicrobial activities as well as their effect on bacterial adhesion.

MATERIALS AND METHODS

Materials. All solvents were of HPLC grade and purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). A Milli-Q water purification system was used (Millipore, Bedford, MA). Copper sulfate, Folin−Ciocalteu, and α-tocopherol were from Merck (Darmstadt, Germany). Emultop emulgator was from Degussa Texturants Systems Deutschland GmbH & Co. KG (Hamburg, Germany). Lecithin from soybean was from Cargill Nordic (Espoo, Finland). Gallic acid, catechin, epicatechin, procyanidin B2, caffeic acid, 3-caffeoylquinic acid (chlorogenic acid), ferulic acid, *p*-coumaric acid, sinapic acid, rutin, and cyanidin-3-glucoside were purchased from Extrasynthese (Geney Cedex, France). EDTA-containing human erythrocytes of blood group O were obtained from Ortho-Clinical Diagnostics Inc. (Raritan, NJ). α-Methyl mannoside (α-MM), Luria agar, and phosphate-buffered saline, pH 7.1 (PBS), were used. The microbial growth media MRS (de Man Rogosa Sharpe, Oxoid) and Nutrient (Difco) were used, and peptone saline (Maximal Recovery Diluent, Lab M, Amersham, U.K.) was used in antimicrobial activity analysis.

Berry Samples and Preparation of Phenolic Extracts. The berries used as sources of extracts are listed in **Table 1**. Sweet rowanberries Burka [a cross with chokeberry (*Sorbus aucuparia* × [*Sorbus aria* × *Aronia arbutifolia*])], Granatnaja [a cross with thorn (*Sorbus aucuparia*×*Crataegus sanguine*)], Zoltaja [a cross with pear (*Sorbus aucuparia* × *Pyrus* sp.)], and Titan [a cross with chokeberry, apple, and pear (Burka × *Malus* sp. × *Pyrus* sp.)] were grown and harvested in Koijärvi, Finland. Wild rowanberries (*Sorbus aucuparia*) were grown and harvested in Nurmijärvi, Finland. One sample (1−2 kg) of each cultivar was collected. The isolation of phenolic compounds from rowanberries was carried out by the method outlined by Kähkönen et al. (*2*); briefly, lyophilized berry material (2.0−3.0 g) was weighed into the centrifuge tube as six replicates, 20 mL of 70% aqueous acetone was added, and the sample was homogenized with Ultra-Turrax for 1 min. Samples were centrifuged (1570*g*, 15 min) and the supernatants were collected. The procedure was repeated with another 20 mL of 70% aqueous acetone. Supernatants were combined, evaporated to dryness under vacuum, and dissolved in 25 mL of water. Samples were divided into 5 mL aliquots and were applied onto the C_{18} solid phase extraction columns (Varian Mega Bond Elut, 5 g, 20 mL, loading capacity = 250 mg). Sugars were eluted with 20 mL of 0.01 M HCl, and the phenolic compounds were subsequently eluted with 20 mL of methanol and evaporated by rotary evaporator. Dry phenolic material was reconstituted with 10 mL of water and lyophilized. The moisture content of the samples was measured using an oven-drying method.

Table 1. Berries Studied

berry	wild/cultivated	species
rowanberry	wild	*Sorbus aucuparia*
Burka	cultivated	*Sorbus aucuparia* × [*Sorbus aria* × *Aronia arbutifolia*]
Granatnaja	cultivated	*Sorbus aucuparia* × *Crataegus sanguinea*
Titan	cultivated	Burka × *Malus* sp. × *Pyrus* sp.
Zoltaja	cultivated	*Sorbus aucuparia* × *Pyrus* sp.

HPLC and LC-MS Analyses. The phenolic profiles of berry extracts were determined by HPLC-DAD-FLD and LC-MS methods. The HPLC-DAD-FLD method was used for quantitative and the LC-MS method for qualitative analyses of the phenolic compounds of wild rowanberry and sweet rowanberry cultivars. Phenolic compounds were tentatively identified on the basis of their *m*/*z* properties and their UV spectra and were clustered into five subclasses (anthocyanins, flavonols, hydroxycinnamates, hydroxybenzoates, and flavanols).

The HPLC-DAD-FLD apparatus consisted of a Waters 2690 Alliance (Waters, Milford, MA) separation module, a Waters PDA 996 diode array detector, and a Waters 474 fluorescence detector. Separation was achieved using a Waters Atlantis T3 C_{18}, 5 μm, 4.6 × 150 mm column heated to 40 °C. The mobile phase consisted of a gradient performed with water/0.5% formic acid (solvent A) and acetonitrile/0.5% formic acid (solvent B) at a constant flow rate of 1 mL/min. The gradient (v/v) of B was as follows: 0−1 min, 0%, B; 1−5 min, 0−6.5% B; 5−15 min, 6.5−10% B; 15−25 min, 10−16% B; 25−30 min, 16% B; 30−33 min, 16−24% B; 33−40 min, 24−32% B; 40−43.5 min, 32−64% B; 43−47.5 min, 64% B; 47−50 min, 64−0% B. PDA detection was carried out at 280, 320, 365, and 520 nm. Fluorescence detection had the excitation wavelength at 280 nm and emission wavelength at 324 nm. Analyses were performed in triplicate.

LC-MS analyses were carried out on an LCQ-Deca (Thermo Electron Corp., Waltham, MA) system consisting of a Surveyor autosampler, pump, and diode array detector (DAD) and a ThermoFinnigan mass spectrometer ion trap. The DAD scanned three discrete channels at 280, 320, and 365 nm. The Synergi Hydro C_{18} column with polar end-capping, 2 mm ×150 mm (Phenomonex Ltd.), was run at 40 °C, and the autosampler tray was cooled to 4 °C. Samples were eluted over a gradient from 5% (0.5% formic acid) to 40% acetonitrile (0.5% formic acid) over 75 min at a rate of 400 μL/min. The LCQ-Deca LC-MS was fitted with an electrospray ionization (ESI) interface and analyzed the samples in positive-ion and negative-ion modes. In positive mode the source voltage was set to 5000 V and the cone voltage to 46 V. In negative mode the source voltage was 5000 V and the cone voltage −44.00 V. In both modes the capillary temperature was 275 °C; sheath gas flow, 60.00 L/min; and auxiliary gas flow, 25.00 L/min.

Liposome and Emulsion Oxidation. Liposomes and emulsions were prepared as described in our previous study (*17*). Concentrations of the phenolic extracts (based on the total phenolic content as determined by HPLC) in the liposomes were 2.1, 4.2, and 8.4 μg/mL of a total sample volume and in the emulsions 25, 50, and 100 μg/g of lipid. Oxidation was followed by formation of conjugated diene hydroperoxides and hexanal. Samples (25−100 μL) for conjugated diene hydroperoxide analysis were dissolved in methanol (5 mL) and analyzed spectrophotometrically at 234 nm (Lambda 25 UV−vis spectrometer, Norwalk, CT). Hexanal samples (500 μL) were measured using static headspace gas chromatography (Autosystem XL gas chromatograph equipped with an HS40XL headspace sampler, Perkin-Elmer, Shelton, CT; column NB-54, Nordion).

Antimicrobial Activity. Comparison of the bacterial growth curves in liquid cultures was used for analysis of antimicrobial activities of rowanberry phenolics, as has been described earlier (*13*). Bacterial test strains *E. coli* VTT E-94564^T (ATCC 11775), *Salmonella enterica* sv. Typhimurium VTT E-981151, and *Staphylococcus aureus* VTT E-70045 (ATCC 6538) were grown in or on Nutrient medium (Difco) for 1 day at 37 °C.

Liquid aerobic bacterial cultures were grown with constant agitation of 150 rpm. de Man Rogosa Sharpe (MRS; Oxoid) was used for growth of *Lactobacillus rhamnosus* strain VTT E-96666 (ATCC 53103) in an anaerobic jar ($H_2/CO_2/N_2$; 10:5:85, Anoxomat WS8000, Mart Microbiology, Lichtenvoorde, The Netherlands) at 37 °C for 1−1.5 days.

Bacterial cultures for antimicrobial assays were revived from −70 °C storage cultures on agar plate, and fresh inoculants, for testing antimicrobial activities, were grown in liquid cultures to late logarithmic or stationaric phase. Lyophilized berry extracts were suspended in 10 mL of growth medium to a final concentration of 1 mg/mL, and 0.1% of liquid microbial inoculum was added. Cultures with no berry extract were used as controls. Liquid cultures were incubated as described above for each strain, and microbial growth was followed by plate counts of samples taken five times during the growth period of 24 h. The number of culturable cells in the samples was determined by diluting the sample with peptone saline (Maximal Recovery Diluent, Lab M, Amersham, U.K.), plating in duplicate on the medium and incubating as indicated above for each strain. The effect of each berry extract on the growth of microbial strains in liquid cultures was evaluated by measuring the difference in the plate counts of test and control cultures during the growth period. Each experiment was performed twice.

Inhibition of Bacterial Hemagglutination and Yeast Cell Agglutination by Phenolic Extracts. The recombinant *E. coli* strains used are listed in **Table 2**. These strains, which all express a single type of fimbria, were available from previous work [HB101 (pPIL110-75), HB101 (pPIL291-15), HB101, and LE392 (pBR322)] or kindly provided by Prof. J. Hacker, University of Würzburg, Germany [LE392 (pANN801-13)], Prof. P. Klemm, Technical University of Denmark, Denmark [HB101 (pPKL4)], and Prof. T. K. Korhonen, University of Helsinki, Finland [HB101 [pRR7, HB101 (pBJN406)]. Bacteria were grown for 18 h at 37 °C on Luria agar plates supplemented with appropriate antibiotics, collected, and suspended in phosphate-buffered saline, pH 7.1 (PBS). EDTA-containing human erythrocytes (blood group O) were washed three times in cold PBS and suspended to 4% (v/v) in cold PBS, which results in (2−4) $\times 10^8$ erythrocytes/mL. The cells were kept at 4 °C or on ice. Baker's yeast (*Saccharomyces cerevisiae*) was washed several times in PBS and finally suspended in PBS to a 1% solution (w/v). A yeast cell suspension containing 10 mM α-methyl mannoside was always used in parallel as a control. Hemagglutination (HA) and inhibition of HA (IHA) were performed in round-bottom microtiter wells by mixing 50 μL of bacterial cell suspension and 50 μL of 4% erythrocyte suspension. The plates were incubated for 18 h in 4 °C, and the results were read by eye. The optimal concentration of bacterial cells for inhibition experiments was determined by 2-fold titration of the bacterial suspensions. The bacterial concentration used in inhibition studies was 4 times the minimum agglutinating concentration, which equals $2 \times 10^8-10^9$ bacteria/mL. Yeast cell agglutination (YA) and inhibition of YA (IYA) were performed as for HA and IHA with the use of yeast cells instead of erythrocytes. Freeze-dried phenolic extracts of wild rowanberries and sweet rowanberry cultivars Burka, Granatnaja, Titan, and Zoltaja were dissolved in PBS to obtain a stock solution of 0.3 mg of total phenolics/mL (pH 6.5) and tested for inhibitory effect on bacteria-mediated HA and YA at concentrations between 0.5 and 30 μg of total phenolics/mL. In inhibition analyses bacteria were mixed with the berry sample and incubated for 30 min prior to the addition of erythrocytes or yeast cells.

Table 2. Bacterial Strains Used in the Agglutination Assays

E. coli strain	fimbria or adhesin (serotype)	reference/source
HB101 (pPIL291-15)	P ($F7_1$)	*37*
HB101 (pPIL110-75)	P (F_{11})	*38*
HB101 (pRR7)	M	*39*
HB101 (pBJN406)	Dr	*40*
HB101	no fimbria	ATCC
LE392 (pANN801-13)	S	*41*
LE392 (pPKL4)	type 1	*42*
LE392 (pBR322)	no fimbria	this study

Statistical Analysis. Statistical differences among antioxidant activities were tested by multivariance analysis using SPSS 15.0.1 (SPSS Inc., Chicago, IL). The significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Phenolic Profiles. Chlorogenic acids (i.e., 3- and 5-caffeoylquinic acid) were the most abundant phenolic compounds in the rowanberries (**Table 3**). The cultivar Burka [a cross with chokeberry (*Sorbus aucuparia* × [*Sorbus aria* × *Aronia arbutifolia*])] had 7.31 mg/g dwt (143 mg/100 g fwt) with Zoltaja [a cross with pear (*Sorbus aucuparia* × *Pyrus* sp.)] the lowest at 2.23 mg/g dwt (55 mg/100 g fwt), a 3-fold difference. Conversely, Zoltaja exhibited the greatest level of 3-caffeoylquinic (neochlorogenic) acid (9.22 mg/g dwt, 226 mg/100 g fwt) with wild rowanberry close behind at 8.59 mg/g dwt (213 mg/100 g fwt) and Granatnaja [a cross with hawthorn (*Sorbus aucuparia* × *Crataegus sanguinea*)] the lowest at 3.20 mg/g (59 mg/100 g fwt) (**Table 3**). The values for total caffeoylquinic acids (5- and 3-) saw the wild rowanberry with the hightest content at 13.95 mg/g dwt (346 mg/100 g fwt) and Granatnaja again with the lowest with 6.91 mg/g dwt (128 mg/100 g fwt), an almost 3-fold variation across the samples analyzed. 5-Caffeoylquinic acid has been found in all crossing partners of sweet rowanberries, but neochlorogenic acid has been reported for only black chokeberry (*Aronia melanocarpa*) and wild rowanberries (*5*, *6*, *18*). The levels of caffeolyquinic acids reported here (**Table 3**) are higher than those reported by Mattila et al. (*19*) for the aglycone, caffeic acid, content for wild rowanberries (4.3 mg/g dw) and sweet rowanberries (*Crataegosorbus mitschurinii*) (3.1 mg/g). We also detected hydroxycinnamic acids other than 3- and 5-caffeoylquinic acids at 5−10 times higher levels than Hukkanen et al. (*5*). Interestingly our data contradict the findings of Häkkinen et al. (*20*), who reported that ferulic acid was the most abundant phenolic compound in wild rowanberries and sweet rowanberry cultivar Granatnaja.

The main phenolic group in Titan [a cross with chokeberry, apple, and pear (Burka × *Malus* sp. × *Pyrus* sp.)] was anthocyanins at 6.04 mg/g (115 mg/100 g fwt), probably principally derived from the chokeberry parentage. It is interesting to compare the comparative data of Hukkanen et al. (*5*), who analyzed the phenolic compounds in nine different sweet rowanberry cultivars. They reported the difference between the anthocyanin contents of Zoltaja (10 mg/100 g fwt) and Titan (101.6 mg/100 g fwt) and between Zoltaja (10 mg/100 g fwt) and Burka (156.5 mg/100 g fwt), approximately 10- and 15-fold differences, respectively. The corresponding values here are approximately 12- and 130-fold, respectively, the results being consistent with theirs.

Table 3. Phenolic Composition and Moisture of Selected Rowanberry Cultivars (Milligrams per Gram of Dry Weight, Mean ± SD)

			OH-C[a]					
cultivar	anthocyanins	flavonols	5-caffeoylquinic	3-caffeoylquinic	other	OH-B[b]	flavanols	moisture (%)
Zoltaja	0.36 ± 0.03	0.95 ± 0.17	2.23 ± 0.05	9.22 ± 0.27	0.61 ± 0.02	0.70 ± 0.01	1.16 ± 0.12	75.4 ± 0.4
Titan	6.04 ± 0.2	1.89 ± 0.07	5.58 ± 0.11	4.79 ± 0.09	1.63 ± 0.04	0.21 ± 0.01	1.15 ± 0.49	80.1 ± 0.1
rowanberry	0.12 ± 0.02	1.84 ± 0.04	5.36 ± 0.10	8.59 ± 0.08	1.84 ± 0.07	0.11 ± 0.01	0.97 ± 0.06	75.2 ± 0.5
Granatnaja	2.14 ± 0.01	1.29 ± 0.01	3.71 ± 0.05	3.20 ± 0.01	0.96 ± 0.06	0.16 ± 0.02	0.94 ± 0.04	81.5 ± 0.2
Burka	5.64 ± 0.05	1.18 ± 0.10	7.31 ± 0.21	4.61 ± 0.12	1.34 ± 0.05	0.22 ± 0.03	1.88 ± 0.06	80.5 ± 0.4

[a] OH-C, hydroxycinnamic acids. [b] OH-B, hydroxybenzoic acids.

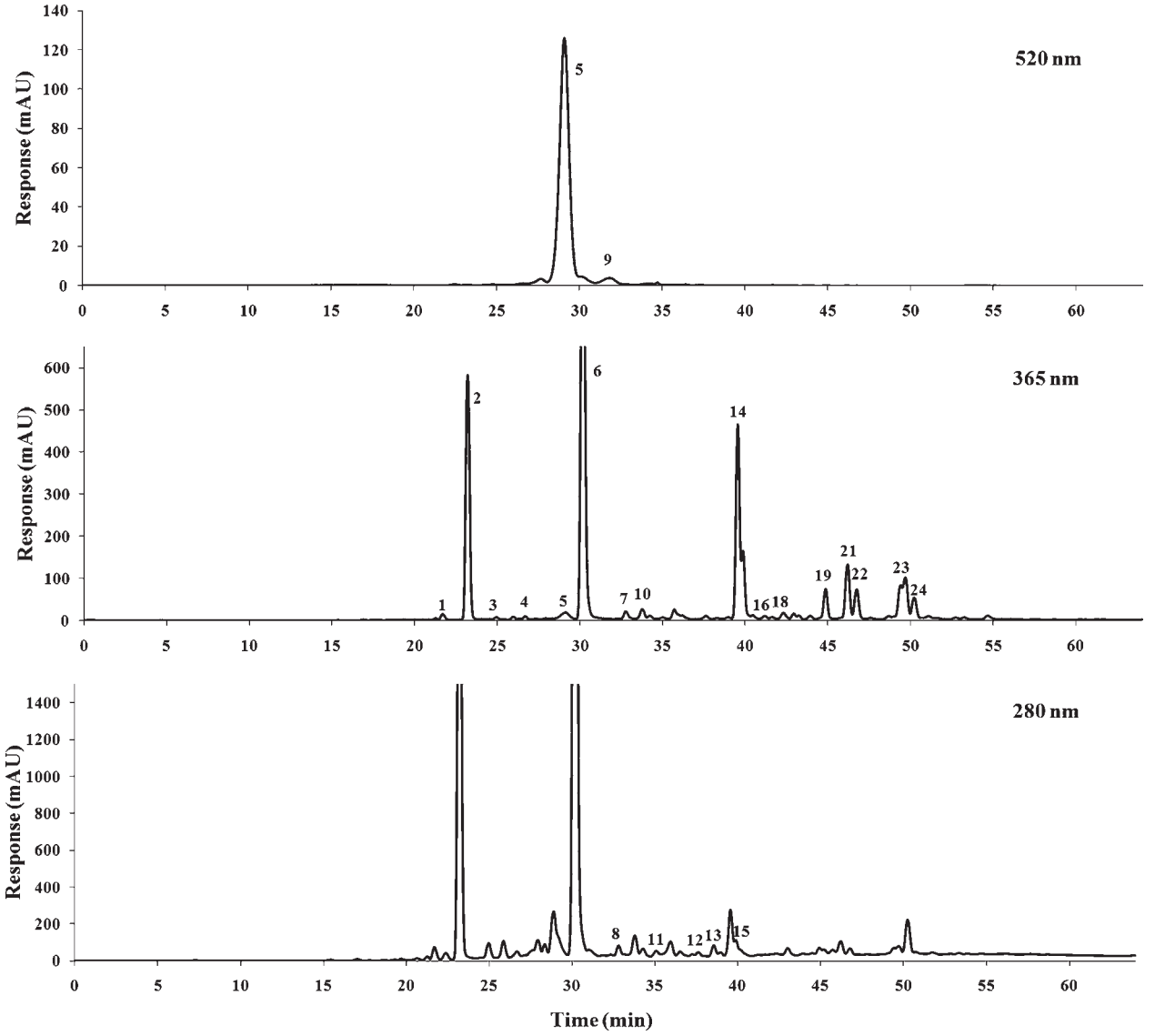

Figure 1. LC chromatograms of wild rowanberry at 280, 365, and 520 nm. See **Table 3** for peak identification.

Both anthocyanins and flavonols, as aglycone and glycosidic forms, have been reported in rowanberries (*18*, *20*, *21*), and in the present study, the range of flavonols in wild and cultivated rowanberries was 0.95−1.89 mg/g dwt (23−36 mg/100 g fwt). The levels of hydroxybenzoic acids were in the range of approximately 0.1−0.2 mg/g (3−4 mg/100 g fwt) except in Zoltaja, which contained 0.7 mg/g (17 mg/100 g fwt) of hydroxybenzoic acids. Sorbic acid and organic acids other than hydroxybenzoic acids were not detected in the rowanberries principally due to their low solubility in water and acetone.

Figure 1 shows the LC chromatogram of wild rowanberry phenolics with the corresponding compounds listed in **Table 4**. The UV spectrum of each compound was recorded to confirm their identities. Caffeoylquinic acids were previously found to be the most abundant phenolic acids in different rowanberry extracts (*5*). Peaks 1, 2, 6, and 10 in **Table 4** were identified as caffeoylquinic acids on the basis of the ions $[M + H]^+$ at *m*/*z* 355 and $[M - H]^-$ at *m*/*z* 353 and their fragmentation to ions *m*/*z* 163 $[M - \text{quinic acid}]^+$ and 191 $[\text{quinic acid} - H]^-$. 3- and 5-Caffeoylquinic acids (peaks 6 and 2) were identified on the basis of previous studies and literature (*6*). Peaks 1 and 10 were identified as 1- and 4-caffeoylquinic acids by comparison to literature data (*22*, *23*). All of the extracts studied contained 3- and 5-caffeoylquinic acids, but other caffeoylquinic acids were not found in Titan. Peak 3 at *m*/*z* 341 in negative mode was identified as caffeoyl glucoside because of the neutral loss of glucose (*m*/*z* 162), yielding an ion at *m*/*z* 179 corresponding to caffeic acid. Peaks 7 and 16 were detected at *m*/*z* 369 $[M + H]^+$ and at *m*/*z* 367 $[M - H]^-$, corresponding to feruloylquinic acid. The MS/MS gave ions at *m*/*z* 163 and *m*/*z* 191, which support this identification. Peaks 3, 7, and 16 were found only in wild rowanberries. Peaks 4 and 24 displayed ions at *m*/*z* 515, which fragmented to ions at *m*/*z* 353, indicating that they were dicaffeoylquinic acids (*24*). Two anthocyanins (peaks 5 and 9) were observed at 520 nm. Peak 5 gave an ion at *m*/*z* 449 in positive mode and at 447 in negative mode, whereas peak 9 gave ions at *m*/*z* 419 and 417, respectively. Both were fragmented to ions at *m*/*z* 287 and 285, which is characteristic of cyanidin. The neutral losses of 162 from 449 and of 132 from 419 are hexose and pentose. On the basis of the standard and literature ion at *m*/*z* 449, peak 5 was identified as cyanidin-3-galactoside and peak 9 (*m*/*z* 419) as a cyanidin-3-arabinoside (*5*, *26*). Peaks 8, 11, 12, 13, 15, and 17 showed ions $[M - H]^-$ at *m*/*z* 289, 577, 865, 1153, 1441, and 1729, which were identified as procyanidin monomers and oligomers on the basis of their fragmentation patterns (*27*). Six different quercetin derivatives and glycosides (peaks 14, 18, 19, 21, 22, and 23) were also

Table 4. Chromatographic, Spectral, and Mass Features of Phenolic Compounds in Wild Rowanberry and Sweet Rowanberries after HPLC by DAD and MS/MS Detection

peak	RT (min)	λ_{max}	$[M+H]^+$ *m/z* (intensity %)	MS/MS fragment (intensity %)	$[M-H]^-$ *m/z* (intensity %)	MS/MS fragment (intensity %)	tentative identity[a]
1	21.7	325	355 (20)	163 (100)	353 (100)	191 (38)	caffeoylquinic acid[a, b, c, e]
2	23.4	325	355 (100)	163 (96)	353 (100)	191 (27)	5-caffeoylquinic acid[a, b, c, d, e]
3	25.1	325			341 (100)	179 (61)	caffeoylglucoside[c]
4	27.2	315			515 (100)	353 (59)	dicaffeoylquinic acid[c, d]
5	28.5	515	449 (87)	287 (100)	447 (100)	285 (28)	cyanidin-3-galactoside[a, b, c, d, e]
6	30.4	325	355 (100)	163 (76)	353 (100)	191 (99)	3-caffeoylquinic acid[a, b, c, d, e]
7	32.4	325	369 (54)	163 (100)	367 (100)	191 (7)	feruloylquinic acid[c]
8	32.9	280			577 (100)	289 (27)	procyanidin dimer[b, c, d, e]
9	32.9	515	419 (100)	287 (84)	417 (100)	285 (31)	cyanidin-3-arabinoside[a, b, c, d, e]
10	33.9	315	355 (47)	163 (100)	353 (100)	191 (99)	caffeoylquinic acid[a, b, c, e]
11	35.2	280			289 (100)	245 (11)	catechin monomer[a, c, e]
12	37.3	280			865 (100)	575 (49)	procyanidin trimer[a, b, c, d, e]
13	38.6	280			1153 (78)	865 (30)	procyanidin tetramer[a, b, c, d]
14	39.6	355	627 (18)	303 (100)	625 (100)	301 (76)	quercetin dihexoside[a, b, c, d, e]
15	40.4	280			1441 (20)	1151 (11)	procyanidin pentamer[a, b, c]
16	41.4	325	369 (100)	163 (80)	367 (100)	191 (4)	feruloylquinic acid[c, e]
17	41.8	280			1729 (27)	1151 (29)	procyanidin hexamer[b]
18	42.4	345			595 (72)	301 (29)	quercetin hexoside pentoside[a, b, c, d, e]
19	45.3	355			609 (100)	301 (30)	quercetin-3-rutinoside[a, b, c, d, e]
20	45.5	325	465 (59)	289 (100)	463 (100)	287 (27)	eriodictyol glucuronide[a, b, d, e]
21	46.3	355	465 (28)	303 (100)	463 (100)	301 (17)	quercetin-3-hexoside[a, b, c, d, e]
22	46.7	355	465 (28)	303 (100)	463 (100)	301 (20)	quercetin-3-hexoside[a, b, c, d, e]
23	49.4	355			549 (12)	505 (93), 301 (40)	quercetin malonylglucoside[a, b, c, d, e]
24	50.3	325			515 (100)	353 (54)	dicaffeoylquinic acid[a, b, c, d, e]

[a] Superscript letters correspond to the cultivar in which the compound was found: [a], Burka; [b], Granatnaja; [c], wild rowanberry; [d], Titan; [e], Zoltaja.

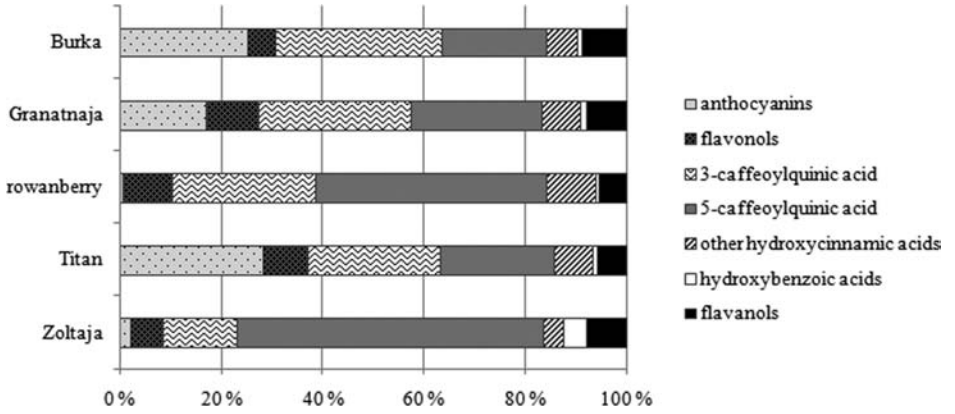

Figure 2. Percentage distribution of groups of phenolic compounds in wild rowanberries and cultivated sweet rowanberries (Burka, Granatnaja, Titan, Zoltaja). The totals were calculated from the values shown in **Table 2**.

detected. They all yielded the MS/MS fragment at *m/z* 301, which corresponds to quercetin. The parent ion of peak 14 was observed at *m/z* 627 $[M+H]^+$, and *m/z* 625 $[M-H]^-$ and was identified as quercetin dihexoside due to the neutral loss of two hexosides. Peak 18 at *m/z* 595 generated the MS/MS ion at *m/z* 301; thus, the neutral loss equates to a hexose and a pentose molecule. The ion at *m/z* 609 $[M-H]^-$ (peak 19) was identified as quercetin-3-rutinoside on the basis of comparative fragmentation (*28*). The MS/MS fragmentation of the parent ion at *m/z* 463 of peaks 21 and 22 corresponds to the neutral loss of hexoside, thus identifying them as quercetin hexosides. The mass difference of 248 between the molecular ion of peak 23 (*m/z* 549) and its aglycone (*m/z* 301) indicates a malonylglucoside moiety (*29*). The molecular ion $[M-H]^-$ of peak 20 was at *m/z* 463, and the MS/MS fragment 287 indicates the loss of glucuronide. The fragment ion at *m/z* 287 corresponds to the flavanone eriodictyol (*30*). Previously, quercetin dihexoside conjugates, quercetin hexose−pentoside conjugates, kaempferol dihexoside conjugates, quercetin glucosides, and quercetin galactosides have been identified in rowanberry juice (*6*).

The phenolic profile of rowanberries revealed that caffeoylquinic acids contributed approximately 80% of the total phenolics in wild rowanberries and sweet rowanberry cultivar Zoltaja, whereas their contribution to other cultivars was lower (56−63%) (**Figure 2**). Anthocyanins contributed a higher amount (17.3−28.4%) of the total phenolics in the sweet rowanberry cultivars Burka, Granatnaja, and Titan compared to wild rowanberries (0.6%) and Zoltaja (2.4%). These results broadly agree with Mattila et al. (*19*) and Hukkanen et al. (*5*), who reported significant (ca. 80%) contribution of hydroxycinnamic acids toward total rowanberry phenolics. The sum of anthocyanins and hydroxycinnamic acids makes up 80−85% of the phenolic content in rowanberries, and the more hydroxycinnamic acids were present, the less anthocyanins were found. The percentual levels of flavanols, hydroxybenzoic acids (OH-B), and flavonols were equal in all

Table 5. Inhibition of Lipid Oxidation As Measured by the Formation of Conjugated Dienes and Hexanal in a Lecithin Liposome Oxidation Model System with 2.1, 4.2, and 8.4 μg/mL Phenolic Compounds[a]

	conjugated diene hydroperoxides			hexanal		
cultivar	2.1 μg/mL[a]	4.2 μg/mL[b]	8.4 μg/mL[c]	2.1 μg/mL[a]	4.2 μg/mL[b]	8.4 μg/mL[b]
Burka	63.0 ± 1.0	74.8 ± 1.3	79.2 ± 0.9	85.9 ± 0.9	96.9 ± 2.1	92.7 ± 1.2
Granatnaja	66.3 ± 0.5	75.4 ± 3.2	77.5 ± 2.4	87.9 ± 0.7	96.6 ± 2.7	96.5 ± 0.9
rowanberry	67.5 ± 2.2	76.9 ± 2.0	77.4 ± 0.5	90.3 ± 1.5	95.7 ± 2.6	96.8 ± 0.1
Titan	63.8 ± 4.2	73.9 ± 2.8	79.8 ± 0.2	86.3 ± 2.3	96.2 ± 2.8	97.2 ± 0.1
Zoltaja	68.4 ± 1.2	73.6 ± 5.9	77.5 ± 0.3	90.4 ± 0.6	97.0 ± 1.0	97.1 ± 0.1

[a] Concentrations based on the sum of phenolic content determined with HPLC (% inhibition, man ± SD). Different letters in the concentration row denote significant difference ($p < 0.05$) in inhibition between the concentrations.

Table 6. Inhibition of Lipid Oxidation As Measured by the Formation of Conjugated Dienes and Hexanal in an Emulsion Oxidation Model System with 25, 50, and 100 μg/g Phenolic Compounds[a]

	conjugated diene hydroperoxides			hexanal		
cultivar	25 μg/g[a]	50 μg/g[b]	100 μg/g[b]	25 μg/g[a]	50 μg/g[a]	100 μg/g[a]
Burka	73.6 ± 13.9	81.2 ± 10.9	88.3 ± 6.9	93.4 ± 5.2	86.5 ± 17.1	91.8 ± 6.1
Granatnaja	79.2 ± 8.5	89.5 ± 2.5	84.7 ± 4.0	92.7 ± 5.2	91.8 ± 6.4	96.7 ± 1.4
rowanberry	79.6 ± 7.0	83.1 ± 7.2	87.4 ± 6.9	93.9 ± 4.4	86.0 ± 11.3	95.0 ± 2.5
Titan	76.4 ± 9.5	88.1 ± 3.3	89.7 ± 3.0	90.3 ± 6,4	89.6 ± 10.2	88.0 ± 8.3
Zoltaja	80.6 ± 8.4	86.7 ± 1.4	83.5 ± 4.7	86.8 ± 11.5	86.3 ± 10.0	92.4 ± 2.9

[a] Concentrations based on the sum of phenolic content determined with HPLC (% inhibition, mean ± SD). Different letters in the concentration row denote significant difference ($p < 0.05$) in the inhibition between the concentrations.

rowanberry extracts. Thus, the differences between different cultivars can be found in the contents of anthocyanins and hydroxycinnamic acids.

Antioxidant Activity. Phenolics in wild rowanberries were highly active in inhibiting lipid oxidation both in liposomes and in emulsions. In the liposome model, the inhibition hexanal formation, a secondary lipid oxidation product, was >90% at the concentrations of 4.2 or 8.4 μg/mL of rowanberry phenolics (**Table 5**). Indeed, the inhibitory effect on lipid oxidation remained high (86−90%) at lower amounts (2.1 μg/mL) of rowanberry phenolics. Also, the wild and cultivated rowanberry phenolics were effective antioxidants toward lipid oxidation in the emulsion model (**Table 6**). Compared to our previous studies in which similar oxidation models at the same concentration levels of berry phenolics were used, the antioxidant activity of rowanberry phenolics was excellent. The inhibition varied between 74 and 80% for conjugated diene hydroperoxides and between 93 and 97% for hexanal in liposomes, whereas the previous results varied between 15 and 70% for conjugated diene hydroperoxides and between 41 and 99% for hexanal (*12*, *31*). In liposomes and emulsions, oxidation was inhibited in a dose-dependent manner with regard to the formation of primary lipid oxidation products, conjugated diene hydroperoxides, although the increase in inhibition accompanying the transition from 50 to 100 μg/g phenolic compound is not statistically significant. It is well accepted that the antioxidant activity of phenolic compounds such as caffeic acid and other hydroxycinnamic acids is related to the number of hydroxyl groups in their molecular structure (*4*). Furthermore, it has previously been shown that the radical scavenging capacities of cultivated sweet rowanberries are high as measured with FRAP and DPPH methods (*5*). However, the antioxidant effect is influenced by the matrix, such as the structure of food (*8*). According to Heinonen et al. (*3*), berries low in anthocyanins and high in hydroxycinnamates were the most potent antioxidants toward liposome oxidation. Among the anthocyanin aglycones, only malvidin appeared to be effective against liposome oxidation at 10, 20, and 40 μM concentrations, whereas cyanidin, delphinidin, and pelargonidin were pro-oxidants (*32*). However, all of these anthocyanins exhibited antioxidant activity in emulsified methyl linoleate (*25*). Pekkarinen et al. (*33*) evaluated the antioxidant activity of different phenolic acids in emulsified system, showing that at concentrations of 50 μM caffeic acid was a prooxidant, whereas at concentrations of 1000 μM it exhibited weak antioxidant behavior. In another study, caffeic acid was found to be effective, whereas its derivative, chlorogenic acid, was a weaker antioxidant (*25*). In the present study, both wild and cultivated rowanberry phenolics rich in chlorogenic acids showed antioxidant activity toward lipid oxidation in liposome and emulsion structures. These findings suggest the antioxidant behavior is dependent on the food matrix as has been suggested earlier by Frankel and Meyer (*34*). The increase in anthocyanin content in the cultivated species did not significantly affect the antioxidant activity.

Table 7. Antimicrobial Activities of Rowanberry Phenolic Extracts (1 mg/mL) on Selected Microbial Strains in Liquid Culture[a]

cultivar	pH	*Staphylococcus aureus* E-70045	*Salmonella* sv. typhimurium E-981151	*Escherichia coli* E-94564^T	*Lactobacillus rhamnosus* E-96666
rowanberry	5.5	+	−	−	−
Burka	6.0	+	−	−	−
Granatnaja	5.5	+	−	+	−
Titan	5.5	++	−	nt	nt
Zoltaja	5.5	++	+	+	−

[a] Inhibition is lidicated from − (no inhibition) to +++++ (strong bacteriocidic effect with decrease of number of cultivable microbial cells below detection limit of 1000 cfu/mL). nt, not tested.

Antimicrobial Effect. Antimicrobial activities of phenolic rowanberry extracts were screened on selected microbial strains in liquid culture. Both wild and cultivated rowanberry phenolics exhibited a weak bacteriosidic effect toward the growth of *Staphylococcus aureus* with Zoltaja phenolics having a weak effect also on *Salmonella* sv. Typhimurium and both Zoltaja and Granatnaja phenolics weakly effective against *E. coli* (**Table 7**). None of the phenolic berry extracts had a bacteriosidic effect on *Lactobacillus rhamnosus*. Puupponen-Pimiä et al. (*14*) studied the bacteriostatic efficacy of extracts from other berries, such as cloudberries, raspberries, sea buckthorn, and black

Table 8. Inhibition of Agglutination Tested at Equal Concentrations of Total Phenolics

	type of *E. coli* adhesin[a]					
cultivar	P	type 1	S	Dr	M	autoagglutionation by berry sample
rowanberry	−	−	−	−	0.94−1.88[b]	3.75−30[c]
Titan	−	−	−	−	−	+
Granatnaja	−	−	−	−	−	+
Burka	−	−	−	−	0.47−0.94[b]	1.88−30[c]
Zoltaja	−	−	−	−	−	3.75−30[c]

[a] −, no inhibition; +, autoagglutination at all tested concentrations. [b] Inhibitory concentration (μg/mL). [c] Autoagglutinating concentration (μg/mL).

currant, and reported that cloudberries and raspberries were the most effective at inhibiting the growth of *Salmonella*, *Escherichia*, and *Staphylococcus* but not *Lactobacillus* or *Listeria*. Similarly, Rauha et al. (*35*) corroborated this efficacy of cloudberries and raspberries and also reported that bilberries and crowberries exhibited effective antimicrobial activity. The antimicrobial activity of the rowanberry phenolics reported here on *Staphylococcus aureus* was clear but not strong, and the effect was bacteriostatic by increasing the lag phase. Zoltaja showed the highest activity, although the differences in activity compared to the other extracts were not significant. Zoltaja exhibited the highest level of 3-caffeoylquinic acid (9.22 mg/g dw), but wild rowan had a level of 8.59 mg/g dw (**Table 3**) but a particularly weak antibacterial profile (**Table 7**). This is perhaps not surprising because Gram-positive lactic acid bacteria and Gram-negative bacteria in general are reported to tolerate berry phenolics, and therefore strong antimicrobial effects are rarely detected (*13*). The source of the elevated activity of Zoltaja remains unexplained.

Effect of Phenolic Extracts on Bacterial Hemagglutination (HA) and Yeast Cell Agglutination (YA). Phenolic extracts of rowanberry and the sweet rowanberries Burka, Granatnaja, Titan, and Zoltaja were analyzed for their inhibitory effect on bacterial HA and YA. The HA of *E. coli* HB101 (pRR7), which expresses the M hemagglutinin, was inhibited by the phenolic extracts of rowanberry and the sweet rowanberry Burka at low concentrations (1−2 μg of total phenolics/mL for rowanberry and 0.5−1 μg of total phenolics/mL for Burka), whereas the extracts of Granatnaja, Titan, and Zoltaja did not inhibit M hemagglutinin-mediated HA (**Table 8**). The extracts did not affect HA of strains HB101 (pPIL110-75), HB101 (pPIL291-15), HB101 (pBJN406), and LE392 (pANN801-13) expressing P, Dr, and S fimbriae or the YA of the type 1 fimbriated strain LE392 (pPKL4) (data not shown). The control strains HB101 and LE392 (pBR322), which do not express fimbriae, were included in all of the analyses. The phenolic extracts agglutinated erythrocytes alone as well as the nonfimbriated control strains when used at higher concentrations (4−30 μg of total phenolics/mL), which hampered evaluation of the inhibitory activity of the extracts at these concentrations. A number of papers have shown that procyanidins of American cranberry inhibit attachment of uropathogenic *E. coli* to uroepithelial and vaginal cells (*16*). In comparison with the P fimbria, which is a potent virulence factor in urinary tract infections caused by *E. coli*, the M hemagglutinin is found very rarely on uropathogenic *E. coli* strains and a homologue of M hemagglutinin named Afa-8 fimbria is highly prevalent in bovine isolates (*36*). Thus, the biological relevance of the inhibitory effect of wild rowanberry and Burka on the M hemagglutinin remains unclear and is not significantly strong enough for speculations with respect to potential therapeutic applications of rowanberries in the inhibition of *E. coli* attachment to the urinary tract.

In summary, wild rowanberries and four cultivated sweet rowanberries, Burka, Granatnaja, Titan, and Zoltaja, were shown to contain high amounts of caffeoylquinic acids varying from 56 to 80%. Most of the cultivated species contained less caffeoylquinic acids and increasing amounts of anthocyanins depending on the crossing species. Both wild and cultivated rowanberries exhibited a potent antioxidant effect both in liposome and in emulsion structures and expressed a weak bacteriostatic effect toward *Staphylococcus aureus*. Cultivation of rowanberries did not diminish their bioactivity in comparison to those of wild rowanberries, which in the long term and following a significant pharmacognostic effort, may be considered as a trait to be aimed for in subsequent rowanberry cultivation.

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