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Antioxidant Activity of Isolated Ellagitannins from Red Raspberries and Cloudberries

Marja Kähkönen,[†] Petri Kylli,[†] Velimatti Ollilainen,[†] Juha-Pekka Salminen,[‡] and Marina Heinonen*[,†]

[†]Department of Food and Environmental Sciences, P.O. Box 27, 00014 University of Helsinki, Finland

[‡]Department of Chemistry, 20014 University of Turku, Finland

ABSTRACT: Ellagitannins from red raspberries (*Rubus idaeus*) and cloudberries (*Rubus chamaemorus*) were isolated by using column chromatography and preparative HPLC. The berry phenolic isolates consisted of 80% (cloudberry) and of 60% (raspberry) of ellagitannins, with raspberries also containing anthocyanins. The main ellagitannins of both raspberries and cloudberries were identified by ESI-MS to consist of the dimeric sanguiin H-6 and the trimeric lambertianin C. Monomeric ellagitannins such as casuarictin in raspberries and pedunculagin in cloudberries were also found. The antioxidant activity of the berry phenolic isolate, ellagitannin isolate (mixture), ellagitannin main fraction (dimer and trimer), and ellagic acid was studied in bulk and emulsified methyl linoleate, in human low-density lipoprotein in vitro, and the radical scavenging activity was studied in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. Cloudberry and red raspberry ellagitannins were highly effective as radical scavengers. Berry ellagitannins also showed significant antioxidant activity toward oxidation of both human LDL and methyl linoleate emulsions. However, only weak or moderate antioxidant activity was exhibited by ellagitannins toward oxidation of bulk oil. Thus, ellagitannins contribute significantly to the antioxidant capacity of cloudberries and red raspberries in lipoprotein and lipid emulsion environments, the latter being more relevant for food applications.

KEYWORDS: *ellagitannins, dimers and trimers, cloudberries, red raspberries, chromatographic isolation, antioxidant activity, methyl linoleate, emulsion, human LDL, DPPH*

INTRODUCTION

Ellagitannins together with gallotannins form the group of hydrolyzable tannins. These tannins are less widely distributed in the plant kingdom than proanthocyanidins, also known as condensed tannins, and many other phenolic compounds.[1−3] Ellagitannins are present especially in berries belonging to the genus *Rubus* (raspberry, blackberry, cloudberry, arctic bramble) and the genus *Fragaria* (strawberries), in other berries such as sea buckthorn and rose hip, and in pomegranate, nuts, and oak-aged wines.[2−6] Berries are the major sources of ellagitannins in Western diets.[3,7] The average dietary intake of ellagitannins in the Finnish population has been estimated to be 12 mg/day, of which 99% was obtained from berries and berry dishes.[8]

Ellagitannins have renewed the concept of tannins with over 500 compounds isolated and structurally determined since 1975.[9] The ellagitannin monomers tend to form dimers, trimers and even higher oligomers via phenolic oxidative coupling reactions. In raspberries (*Rubus idaeus* L.) the major ellagitannins (Figure 1) have been identified as the dimeric sanguiin H-6 and the trimeric lambertianin C.[9−12] Also other ellagitannins are present in raspberries, such as monomeric casuarictin, potentillin, pedunculagin, sanguiin H-10, dimeric nobotanin A,[9,13] and tetrameric lambertianin D.[14]The ellagitannin composition of cloudberries (*Rubus chamaemorus* L.) is less studied apart from McDougall et al.[15] reporting lambertianin C as the predominating ellagitannin.

Ellagitannins are of interest due their antioxidant properties and other bioactive properties. Regarding radical scavenging activity and antioxidant activity toward both lipid and protein oxidation, berry ellagitannins have been reported to exert promising activities compared to phenolic and other antioxidants.[12,16−22] Sanguiin H-6 has been reported to account for 30−45%[10,19,22] and lambertianin C for 14%[19,22] of the radical scavenging activity of red raspberries with vitamin C and anthocyanins also contributing to the effect. In our previous studies with food model systems, we have reported high antioxidant activity of ellagitannin-rich phenolic extracts of both red raspberry and cloudberry in bulk methyl linoleate.[12,16,17] Ellagitannins in raspberries were also found to be potent toward oxidation of liposomes incorporated with lactalbumin.[18]

The aim of this research was to evaluate the antioxidant activity toward lipid oxidation in different environments (bulk and emulsified methyl linoleate and human LDL) and the radical scavenging activity (DPPH) of monomeric, dimeric, and trimeric ellagitannins isolated from both red raspberries and cloudberries.

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). Ellagic acid and Amberlite XAD-7 nonionic polymeric adsorbent were purchased from Sigma Chemical Co. (St. Louis, MO). α-Tocopherol, copper sulfate, Na_2HPO_4, and formic acid were purchased from Merck (Darmstadt, Germany). Methyl linoleate, MeLo, was from Nu Check Prep (Elysina, MN, USA), and Emultop (partially hydrolyzed soybean lecithin) was from Lucas Meyer Gmbh (Hamburg, Germany). Ellagic acid and human LDL were obtained from Sigma.

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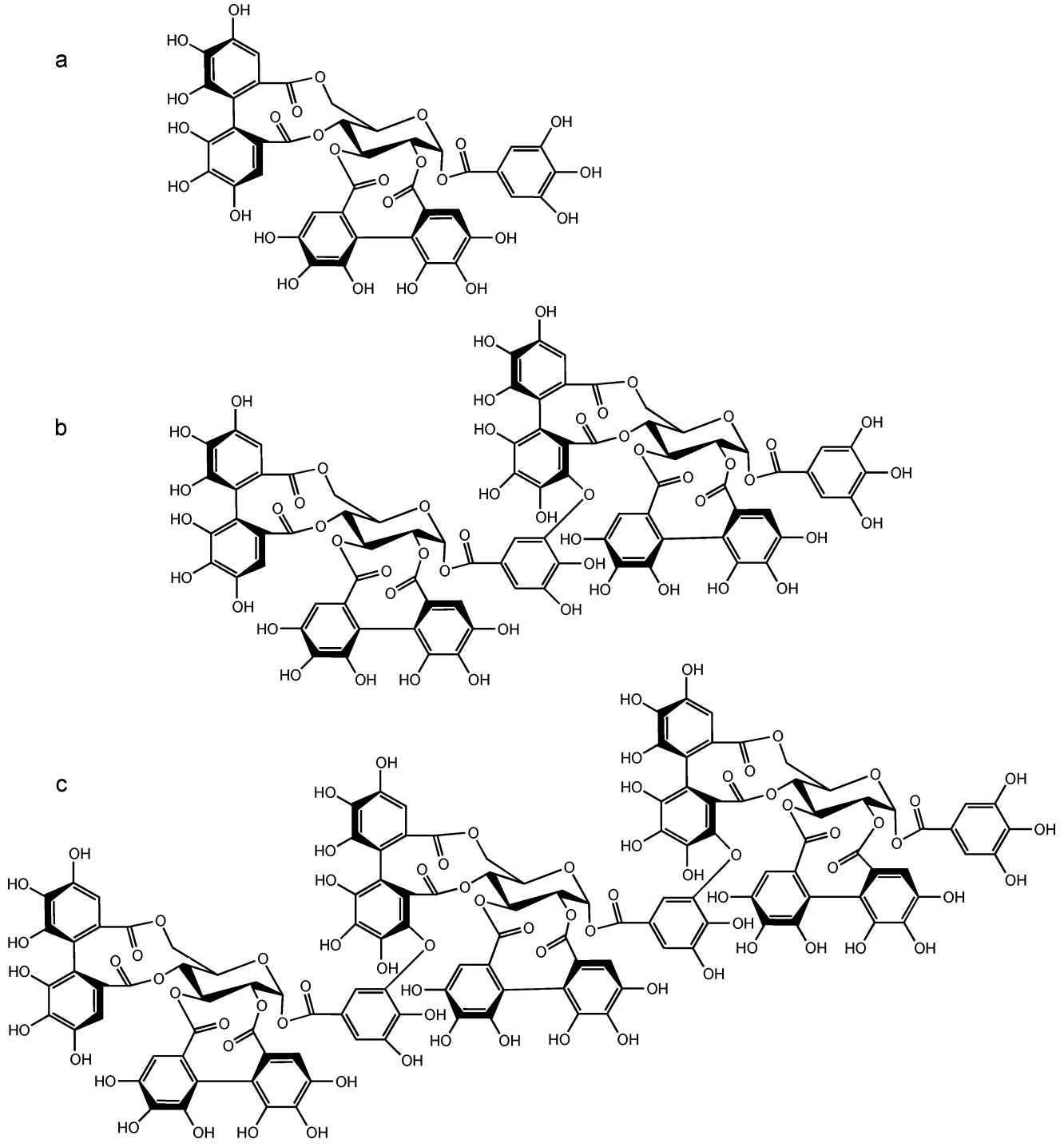

Figure 1. Structures of the main ellagitannin monomer (casuarictin) (a), dimer of casuarictin (sanguiin H6) (b), and trimer of casuarictin (lambertianin C) (c) found in raspberries (*Rubus idaeus* L.).

Extraction and Isolation of Berry Phenolics. Red raspberries (*Rubus idaeus L.*), cultivar Ottawa, and cloudberries (*Rubus chamaemorus*) were purchased as fresh berries from a local market place. The berries were packed immediately into vacuum sealed bags and stored in a freezer at −20 °C. The phenolic berry isolates (ET extract) were produced by using 70% aqueous acetone as the solvent acccording to a method outlined in ref 17. Berries (3 g) were weighted to a centrifuge tube, 25 mL of solvent was added, and sample was homogenized for 1 min (Ultra-Turrax T25 mixer, Janke & Kunkel, Germany). Tubes were centrifuged (3000 rpm, 15 min) and the clear supernatant was collected. The procedure was repeated twice with another 25 mL of solvent, and the supernatants were combined and taken to dryness with rotary evaporator. The solid residues were dissolved in water for further purification. The berry phenolics were used in antioxidant testing as freeze-dried powders. Settings for the freeze-drying procedure for both berry material and extract were as follows: prefreezing, −20 °C for 10 min; primary drying, −20 °C/0.2 mbar for 2 h, −10 °C/0.2 mbar for 5 h, 0 °C/0.5 mbar for 10 h, 5 °C/0.5 mbar for 10 h, 10 °C/0.5 mbar for 4 h, 15 °C/0.5 mbar for 4 h, 20 °C/0.5 mbar for 1 h; secondary drying, 25 °C for 1 h; total time, 37 h (Heto FD8, Jouan Nordic A/S, Allerød, Denmark).

Phenolic berry extracts (ET extract) were further purified using Amberlite XAD-7 column chromatography. The sample was introduced into the column (diameter 40 mm, length 300 mm), and

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free sugars and organic and phenolic acids were washed out with 6% CH_3CN (CH_3CN:TFA:H_2O 6:0.5:93.5 V/V/V). Elution was continued with CH_3CN (CH_3CN:TFA 99.5:0.5) to yield a fraction containing flavonols, anthocyanins, and ellagitannins. To separate ellagitannins from the other phenolics, this fraction was introduced to a column of similar size packed with Sephadex LH-20. The sample was introduced into the column, and flavonols and anthocyanins were eluted with 50% MeOH. In this step, no ellagitannins were eluted. Ellagitannins were eluted with aqueous acetone (70:30 V/V). Acetone was evaporated in a rotavapor (Büchi Rotavapor R) at 35 °C, and the aqueous residue was lyophilized to produce ellagitannin mixture (ET isolate) for antioxidant testing.

The main ellagitannins (ET dimer and trimer) were isolated from the ellagitannin mixture by using semipreparative HPLC. The HPLC system (Waters, Milford, MA, USA) consisted of a WISP 712 autosampler, three 501 pumps and a pump control module, a column oven with a temperature control module, a PDA996 diode array detector, and a Millennium 2020C/S software data module. A Develosil ODS-HG-5 column (250 × 20 mm; 5 μm; Phenomenex, USA) with a guard column (50 × 20 mm) was used for separation. The mobile phase consisted of 1% formic acid (solvent A) and 100% CH_3CN (solvent B). The elution conditions were as follows: isocratic elution 0% B, 0−5 min; linear gradient from 0% B to 15% B, 5−100 min; to 30% B, 140 min; to 100% B, 150−170 min. Flow rate was 5.0 mL/min, and column oven temperature was 35 °C. Detection wavelength was set to 280 nm. Sample was introduced to the column using an HPLC pump. Dimeric and trimeric ellagitannins eluting at 120−130 min were collected, and the organic solvent was evaporated, after which the remaining fraction was lyophilized.

HPLC Analysis, HPLC−ESI-MS and UPLC−ESI-MS Identification. Ellagitannin analysis was performed using the same equipment as in preparative HPLC. Analytical separation of ellagitannins was carried out according to Kähkönen et al.[17] on a Nova Pak C18 column (150 mm × 4.6 mm, 5 μm; Waters, USA) equipped with a C18 guard column. The temperature of the column oven was set at 40 °C. The elution conditions were as follows: isocratic elution 100% AB, 0−5 min; linear gradient from 100% A to 96% A/4% B, 5−15 min; to 92% A/8% B, 15−25 min; stepwise to 8% B/92% C, 25−25.01 min; linear gradient to 20% B/80% C, 25.01−45 min; 40% B/60% C, 45−55 min; to 80% B/20% C, 55−65 min; isocratic elution 80%B/20% C, 65−70 min; linear gradient to 100% A, 70−75 min; post-time 15 min before next injection; flow rate 0.5 mL/min. Detection wavelength was 280, and the spectra from 200 to 600 nm were recorded for all peaks. The purity of the ellagitannin fractions in different stages of the isolation was determined by HPLC at wavelength 280 nm as the percentage of total ellagitannin peak areas from all peak areas (Table 1).

Table 1. Characterization of Red Raspberry (*Rubus idaeus* L.) and Cloudberry (*Rubus chamaemorus* L.) Phenolic and Ellagitannin Isolates

sample	composition and purity
	Red Raspberry
phenolic extract	60% ellagitannins, 8% anthocyanins
ellagitannin isolate (mixture)	≥94% pure by HPLC
ellagitannin dimers and trimers	>95% pure by HPLC
	Cloudberry
phenolic extract	80% ellagitannins, 7% phenolic acids, 3% flavonols
ellagitannin isolate (mixture)	≥95% pure by HPLC
ellagitannin dimers and trimers	>95% pure by HPLC

HPLC−ESI-MS analyses on ellagitannins were performed using a Perkin-Elmer Sciex API 365 triple quadrupole mass spectrometer (Sciex, Toronto, Canada) equipped with an ion-spray interface. The mass spectrometer was operated in the negative mode, and mass spectra were obtained by acquiring data between 100 and 2000 atomic mass units. Additional analyses on berry phenolics were performed with ACQUITY UPLC system (Waters, USA) coupled with Bruker Esquire-LC MS fitted with an ESI (electrospray ionization) interface and analyzing the samples in negative-ion mode according to method described by Kylli et al.[23] The column (Waters HSS T3 C18, 2.1 mm × 150 mm × 1.8 μm) was heated to 40 °C, and the autosampler tray was cooled to 4 °C. Samples were eluted over a gradient from 0% (0.5% formic acid) to 40% acetonitrile (0.5% formic acid) over 28 min at a rate of 0.5 mL/min. The flow was split in two before the PDA and MS detectors.

Radical Scavenging Activity and Antioxidant Activity. *Radical Scavenging Activity.* The ability of the berry phenolics and ellagitannin isolates to act as free radical scavengers against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was tested spectrophotometrically by measuring the disappearance of the absorbance at 517 nm after adding the antioxidant solution.[24] The samples were tested at concentrations of 250 and 500 μg/mL (berry phenolic extract and ET mixture) and 2, 5, and 10 μM (ET dimer and trimer, and ellagic acid). The absorption was monitored at 15 s intervals for 5 min. The results are expressed as the percentage of radicals scavenged after 4 min of reaction time.

Antioxidant Activity in Bulk and Emulsified Methyl Linoleate (MeLo). The assays were conducted as reported previously.[4,24] In bulk methyl linoleate, the berry phenolic and ellagitannin mixtures were tested at concentrations of 100 and 500 μg/g and the ellagitannin dimers and trimers and ellagic acid at concentrations of 50, 100, and 250 μM. Oxidation of MeLo was carried out in the dark at 40 °C, and the level of oxidation was followed by measuring the formation of MeLo hydroperoxides by HPLC at 234 nm. The antioxidant activity was expressed as percentual (%) inhibition of formation of MeLo hydroperoxides after 72 h of oxidation. The oxidation experiment in 10% oil-in-water emulsion was carried out using 0.400 g of MeLo and 3.6 mL of 1% (w/V) Emultop, a partially hydrolyzed soybean lysolecithin, distilled in Milli-Q water. The method has been described in detail in our previous paper.[24] The emulgator contained some α- and γ-tocopherols (62 and 40 μg/g, respectively). The test concentrations used were 100 and 500 μg/g for ellagitannin mixtures, and 50 and 250 μM for the ellagitannin dimer and trimer fraction and ellagic acid. The antioxidants, MeLo, and the emulsifier solution were combined in glass vials, and emulsions were prepared by sonicating the mixture for 3 min in an ice bath with a U 50 Control Ikasonic sonicator (Janke & Kunkel GmbH & Co. KG, Staufen, Germany). Oxidation was carried out in the dark at 40 °C, and the level of oxidation was followed by measuring the formation of conjugated diene hydroperoxides spectrophotometrically (Perkin-Elmer lambda 15 UV−vis spectrophotometer, Norwalk, CT) at 234 nm. The antioxidant activity was expressed as percentual (%) inhibition of formation of MeLo hydroperoxides after 72 h of oxidation.

Antioxidant Activity in Human LDL ex Vivo. The ability of berry phenolics including ellagitannins to inhibit the oxidation of human LDL ex vivo was studied using the assay described previously.[20,25] Human LDL (Sigma) was diluted to a protein concentration of 0.2 mg/mL with 0.01 M phosphate buffer solution (pH 7.4) containing 0.15 M NaCl. The samples were incubated at 37 °C with 10 μM copper sulfate solution in sealed headspace vials. The berry phenolic extracts were tested at concentrations of 1.4, 2.8, and 4.2 μg/mL and the ellagitannin dimers and trimers at 1.4 and 4.2 μg/mL. After 2 h of incubation, the extent of oxidation was determined by measuring the formation of hexanal using static headspace gas chromatography (PerkinElmer Autosystem XL gas chromatography equipped with PerkinElmer HS 40XL Headspace Sampler, Shelton, CT).

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

Identification of Ellagitannins and Other Phenolics. The phenolic profile of red raspberries consisted of 60% ellagitannins and 8% anthocyanins, with the remaining

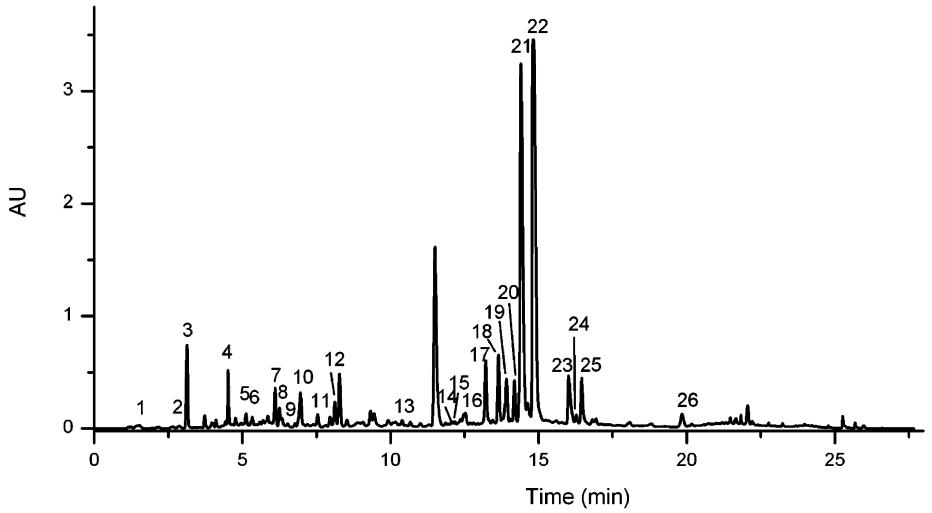

Figure 2. UPLC chromatogram with UV detection at 280 nm of ellagitannin-rich extract of cloudberries. Peak numbers correspond to Table 2.

phenolics mainly belonging to the groups of flavonols and phenolic acids (Table 1). These results are in accordance with previous findings.[4,10,18] Ellagitannins were isolated from red raspberries both as a mixture of monomers, dimers, and trimers and as ellagitannin main fraction consisting of dimers and trimers. The purities of the red raspberry ellagitannin mixture and ellagitannin monomer fractions were ≥93% and ≥94%, respectively. The molar mass of the monomeric ellagitannins was 936 g/mol corresponding to either casuarictin or potentillin. In red raspberries the main ellagitannins found in the mixture correspond to the dimeric sanguiin H-6, $[M - H]^-$ at m/z 1869, and trimeric lambertianin C, $[M - 2H]^{2-}$ at m/z 1401. These findings correspond to the earlier reports.[10−12,18,19,21,26] In addition, these two ETs were identified by coelution with authentic standards.[27]

In cloudberries, the ellagitannins (80%) predominated followed by phenolic acids (7%) and flavonols (3%) (Table 1). This phenolic compound profile is in accordance with previous reports.[12,15] According to more recent findings cloudberry extract is reported to consist of ellagitannins (77.1%), proanthocyanidins (5.9%), hydroxybenzoic acids (3.3%), hydroxycinnamic acids (8.9%), ellagic acids (2.1%), flavonols (2.6%), and anthocyanins (0.4%).[28] The purity of both the cloudberry ellagitannin isolate and the isolate consisting of ellagitannin dimers and trimers was ≥95%. The identification of cloudberry ellagitannins was based on the fragmentation data reported in the literature.[10,11,14,15,27−35]

Peaks 1 and 3 showed the $[M - H]^-$ at m/z 969 and were fragmented to 925 (loss of CO_2) and 481 (HHDP-glu). The fragmentation pattern was similar to Zhang et al.[28] reported for phyllanthusiin G. Peaks 2, 6, 8, 9, and 13 had the $[M - H]^-$ at m/z 951, which fragmented to 907 (loss of CO_2), 783 (loss of galloyl group), and 469 (loss of HHDP-glu). Based on the fragmentation patterns, peaks were identified as trisgalloyl HHDP glucose.[31] Peaks 4 and 7 were observed at m/z 1567, which was $[2M - H]^-$ (ref 27) of an ion m/z 783. The ion at m/z 1567 was fragmented to 783 ($[M - H]^-$ and 481 (loss of HHDP). Peak 11 appeared at m/z 783 and was fragmented the same way as peaks 4 and 7. They were identified as pedunculagin/casuariin isomers.[27,31,32] Peak 5 at m/z 1433 fragmented to 951 (loss of HHDP-glu). Further fragmentation followed the fragmentation pattern of the peaks 2, 6, 8, 9, and 13. Therefore, the molecule has trisgalloyl HHDP glucose structure + HHDP-glucose. Peak 10 at m/z 1121 yielded after a loss of galloyl group again the fragment ion at m/z 951. The ion at m/z 951 fragmented to ions at m/z 907, 783, and 469, indicating that it has trisgalloyl HHDP glucose + galloyl group. Peaks 14 and 15 had the $[M - H]^-$ at m/z 935 and were further fragmented to 633 (loss of HHDP). On the basis of the fragmentation and previously published data,[33,34] the peaks were identified as potentillin/casuarictin. Peaks 16, 18, and 19 exhibited at the same m/z 1567 as peaks 4 and 7 but fragmented differently. The fragment ions were at m/z 1265 (loss of HHDP), 1103 (loss of HHDP-glu), and 933 (loss of HHDP-glu-galloyl). Peaks were identified as sanguiin H-10.[15,35] Peaks 17 and 22 had the $[M - H]^-$ at m/z 1869 and gave fragment ions at m/z 1567 (loss of HHDP), 1265 (loss of two HHDP groups), 1103 (loss of HHDP-HHDP-glu), and 933 (loss of HHDP-HHDP-glu-galloyl), which are characteristics for sanguiin H-6. Peak 21 at m/z 1401 was a doubly charged ion of the molecule having molecular weight of 2804 (lambertianin C). The fragment ions at m/z 1869, 1567, 1265, 1103, and 933 confirmed the identity.[10,14,15,32] Peak 23 was identified as ellagic acid with an authentic standard. Both the standard and the peak 23 were fragmented similarly giving ion at m/z 257 and 229.[11] Peaks 24 and 26 gave the $[M - H]^-$ at m/z 1103, which fragmented to 1059 (loss of CO_2), 933 (loss of galloyl), and 633 (HHDP-glu-galloyl). Tanaka et al.[14] and Kool et al.[35] reported similar fragmentation for sanguiin H-2. Peak 25 had the $[M - H]^-$ at m/z 935 and the fragment ions at 933 and 301 like peaks 14 and 15. Peaks 12 and 20 gave the fragment of 301 showing that they were ellagitannins. Other characteristic losses such as loss of HHDP were seen, but the identity remained unknown.

The dimeric and trimeric ellagitannins found in red raspberries, i.e. dimeric sanguiin H-6, $[M - H]^-$ at m/z 1869, and trimeric lambertianin C, $[M - 2H]^{2-}$ at m/z 1401, were also the predominant ellagitannins in cloudberries (Figure 2, Table 2). Other ellagitannins tentatively identified were pedunculagin, sanguiin H-6 and H-10 isomers, potentillin, and casuarictin. All peaks listed gave the fraction ion at m/z 301 in negative mode representing ellagic acid, thus they were identified as ellagitannins. Kool et al.[35] reported that in another *Rubus* berry, boysenberry, sanguiin H-6 and sanguiin H-10 were the most abundant ellagitannins with sanguiin H-2 found as a minor fraction. McDougall et al.[15] found that lambertianin C

Table 2. Chromatographic and Mass Features of Ellagitannins in Cloudberry after UPLC by MS/MS Detection

peak	RT (min)	tentative identity	m/z $[M - H]^-$	MS^2 fragment ions m/z	ref
1	1.5	phyllanthusiin G	969	925 ($[M - H]^- - CO_2$, 481 ($[HHDP\text{-}glu]^-$), 301	30
2	2.8	trisgalloyl HHDP glucose isomer	951	907 ($[M - H]^- - CO_2$), 783 (bis-HHDP-glu), 469 ($[M - H]^-$ − HHDP-glu), 301	31
3	3.6	phyllanthusiin G	969	925 ($[M - H]^- - CO_2$, 481 ($[HHDP\text{-}glu]^-$), 301	30
4	4.5	pedunculagin/casuariin	1567 ($[2M - H]^-$)	783 ($[M - H]^-$), 481 ($[M - H]^-$ − HHDP), 301	27, 31, 32
5	5.1	trisgalloyl HHDP glucose + HHDP glucose	1433	951 ($[M - H]^-$ − HHDP-glu), 907 ($[M - H]^-$ − HHDP-glu − CO_2), 469 ($[M - H]^-$ − HHDP-glu − CO_2 − HHDP-glu), 301	
6	5.3	trisgalloyl HHDP glucose isomer	951	907 ($[M - H]^- - CO_2$), 783 (bis-HHDP-glu), 469 ($[M - H]^-$ − HHDP-glu), 301	31
7	6.1	pedunculagin/casuariin	1567 ($[2M - H]^-$)	783 ($[M - H]^-$), 481 ($[M - H]^-$ − HHDP), 301	27, 30−32
8	6.3	trisgalloyl HHDP glucose isomer	951	907 ($[M - H]^- - CO_2$), 783 (bis-HHDP-glu), 469 ($[M - H]^-$ − HHDP-glu), 301	31
9	6.5	trisgalloyl HHDP glucose isomer	951	907 ($[M - H]^- - CO_2$), 783 (bis-HHDP-glu), 469 ($[M - H]^-$ − HHDP-glu), 301	31
10	7	trisgalloyl HHDP glucose + galloyl	1121	1077 ($[M - H]^- - CO_2$), 951 ($[M - H]^-$ − galloyl), 783 (bis-HHDP-glu), 301	
11	7.3	pedunculagin/casuariin	783	481 ($[M - H]^-$ − HHDP), 301	27, 31, 32
12	8.1	ellagitannin	1567	1235, 933, 783, 301	
13	10.4	trisgalloyl HHDP glucose isomer	951	907 ($[M - H]^- - CO_2$), 783 (bis-HHDP-glu), 469 ($[M - H]^-$ − HHDP-glu), 301	31
14	11.8	casuarictin/potentillin	935	633 ($[M - H]^-$ − HHDP), 301	33, 34
15	12.2	casuarictin/potentillin	935	633 ($[M - H]^-$ − HHDP), 301	33, 34
16	12.5	sanguiin H-10	1567	1265 ($[M - H]^-$ − HHDP), 1103 ($[M - H]^-$ − HHDP-glu), 933 ($[M - H]^-$ − HHDP-glu-galloyl), 301	15, 35
17	13.2	sanguiin H-6	1869	1567 ($[M - H]^-$ − HHDP), 1265 ($[M - H]^-$ − HHDP-HHDP), 1103 ($[M - H]^-$ − HHDP-HHDP-glu), 933 ($[M - H]^-$ − HHDP-HHDP- glu-galloyl), 631 ($[M - H]^-$ − HHDP-HHDP-glu-galloyl-HHDP), 301	14, 27, 32
18	13.7	sanguiin H-10	1567	1265 ($[M - H]^-$ − HHDP), 1103 ($[M - H]^-$ − HHDP-glu), 933 ($[M - H]^-$ − HHDP-glu-galloyl), 301	15, 35
19	13.9	sanguiin H-10	1567	1265 ($[M - H]^-$ − HHDP), 1103 ($[M - H]^-$ − HHDP-glu), 933 ($[M - H]^-$ − HHDP-glu-galloyl), 301	15, 35
20	14.1	ellagitannin	1251	1235, 1099, 933, 633, 301	
21	14.4	lambertianin C	1401 $[M - 2H]^{2-}$	1869 ($[M - H]^-$ − galloyl-bis-HHDP-glu), 1567 ($[M - H]^-$ − galloyl-bis-HHDP-glu-HHDP), 1265 ($[M - H]^-$ − galloyl-bis-HHDP-glu-HHDP-HHDP), 1103 ($[M - H]^-$ − galloyl-bis-HHDP-glu-HHDP-HHDP-glu), 933 ($[M - H]^-$ − galloyl-bis-HHDP-glu-HHDP-HHDP-glu-galloyl), 631 ($[M - H]^-$ − galloyl-bis-HHDP-glu-HHDP-HHDP-glu-galloyl-HHDP), 301	10, 14, 15, 32
22	14.8	sanguiin H-6	1869	1567 ($[M - H]^-$ − HHDP), 1265 ($[M - H]^-$ − HHDP-HHDP), 1103 ($[M - H]^-$ − HHDP-HHDP-glu), 933 ($[M - H]^-$ − HHDP-HHDP-glu-galloyl), 631 ($[M - H]^-$ − HHDP-HHDP-glu-galloyl-HHDP), 301	14, 27, 32
23	16.1	ellagic acid	301	257, 229	11
24	16.3	sanguiin H-2	1103	1059 ($[M - H]^- - CO_2$), 933 ($[M - H]^-$ − galloyl, 633 (HHDP-glu-galloyl)	14, 35
25	16.4	galloyl-bis-HHDP glucose isomer	935	633 ($[M - H]^-$ − HHDP), 301	
26	19.9	sanguiin H-2	1103	1059 ($[M - H]^- - CO_2$), 933 ($[M - H]^-$ − galloyl, 633 (HHDP-glu-galloyl)	14, 35

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Table 3. Radical Scavenging (DPPH) and Antioxidant Activity of Red Raspberry and Cloudberry Phenolic Extracts and Isolated Ellagitannin Mixtures (ET-Isolate)

	DPPH test[a] (mg/mL)		MeLo[b] (μg/g)		emulsion[c] (μg/g)		LDL[d] (μg/mL)		
sample	0.25	0.5	100	500	100	500	1.4	2.8	4.2
raspberry extract	23 ± 0.2	35 ± 0.4	45 ± 1.8	97 ± 0.0			−15 ± 1.1	96 ± 0.6	97 ± 1.2
raspberry ET-isolate	49 ± 0.4	79 ± 0.6	16 ± 1.8	67 ± 3.2	94 ± 0.7	97 ± 0.7	95 ± 0.3		97 ± 0.0
cloudberrry extract	25 ± 0.3	36 ± 1.5	60 ± 3.5	94 ± 0.0			−7 ± 0.9	93 ± 2.3	96 ± 1.0
cloudberry ET-isolate	54 ± 0.5	85 ± 0.4	14 ± 0.0	56 ± 1.4	95 ± 0.7	96 ± 0.7	63 ± 0.6		94 ± 1.7

Expressed as

[a]Radicals scavenged (%) after 4 min reaction time. [b]Inhibition (%) of methyl linoleate (MeLo) hydroperoxide formation after 72 h of oxidation. [c]Inhibition (%) of conjugate diene hydroperoxide formation after 72 h of oxidation. [d]Inhibition (%) of hexanal formation after 2 h of oxidation.

Table 4. Radical Scavenging (DPPH) and Antioxidant Activity of Ellagitannin Main Fraction (ET Dimer and Trimer) Isolated from Red Raspberries and Cloudberries

	DPPH-test[a] (μM)			MeLo[b] (μM)			emulsion[c] (μM)	
sample	2	5	10	50	100	250	50	250
raspberry ET dimers and trimers	20 ± 0.4	40 ± 0.1	79 ± 0.3	24 ± 4.9	37 ± 0.0	37 ± 3.2	90 ± 0.7	96 ± 0.4
cloudberry ET-dimers and trimers	21 ± 0.1	47 ± 0.2	74 ± 1.7	21 ± 4.9	13 ± 3.2	59 ± 3.2	91 ± 0.0	95 ± 0.0
ellagic acid	13 ± 0.1	29 ± 0.4	49 ± 0.3	56 ± 1.4	21 ± 1.1	14 ± 1.4	83 ± 0.4	89 ± 0.0

[a]Expressed as radicals scavenged (%) after 4 min reaction time. [b]Expressed as inhibition (%) of methyl linoleate (MeLo) hydroperoxide formation after 72 h of oxidation. [c]Expressed as inhibition (%) of conjugate diene hydroperoxide formation after 72 h of oxidation.

predominated in cloudberries and sanguiin H-6 in raspberries with other abundant phenolic compounds in cloudberries being sanguiin H-6, sanguiin H-10, and ellagic acid.

Radical Scavenging and Antioxidant Effect. The antioxidant activity of the red raspberry and cloudberry phenolic extracts, ellagitannin mixture (ET-isolate), ellagitannin dimeric and trimeric fraction, and ellagic acid was studied in bulk and emulsified methyl linoleate, in human low-density lipoprotein (LDL) in vitro, and the radical scavenging activity in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. Cloudberry and red raspberry ellagitannins were highly effective as radical scavengers. While the radical scavenging activity of the berry phenolics containing 60% ellagitannins of the total phenolic content (red raspberries) or 80% (cloudberries) remained weak, the isolated ellagitannins were effective as DPPH radical scavengers especially at higher concentrations of 0.5 mg/mL (mixture of ellagitannin monomers, dimers, and trimers with also ellagic acid present) and 10 μM (ET dimers and trimers) (Tables 3 and 4). The radical scavenging activity of the complex ellagitannins was higher than that of the metabolic product, ellagic acid. Significant radical scavenging activity of isolated berry ellagitannins has also been reported previously using other assays such as the oxygen radical absorbance capacity ORAC assay with muscadine grapes,[36] the reactive oxygen species assays with pomegranate punicalagins,[29] and electron spin resonance spectroscopy with red raspberries.[10] Sanguiin H-6 and lambertianin C were found to be responsible for 58% of the antioxidant capacity measured of red raspberries based on FRAP assay.[22] The strong correlation between antiradical activity measurements and ellagitannins can be attributed to the high number of hydroxyl groups in the ellagitannin structures. Gil et al.[37] showed that a high radical scavenging activity using the DPPH method was observed for punicalagin containing 16 phenolic hydroxyls per molecule.

Radical scavenging activity measured in aqueous or organic solutions does not predict antioxidant activity in complex lipid environments such as food and other biological materials. In the present paper, it is reported for the first time that red raspberry and cloudberry ellagitannins show significant antioxidant activity toward lipid oxidation in complex environments such as human LDL and methyl linoleate emulsions (Tables 3 and 4). The isolated ellagitannin fractions inhibited LDL oxidation already at lower concentration of 1.4 μg/mL (95 and 63% inhibition for red raspberry and cloudberry, respectively) corresponding to a range below 10 μM (Table 3). The berry ellagitannin isolates containing dimeric sanguiin H-6 and trimeric lambertianin C among other ellagitannins (Table 2) were as potent antioxidants as the more complex phenolic extracts of red raspberries and cloudberries at higher concentrations of 4.2 μg/mL (94−97% inhibition). The in vitro LDL assay is however not very selective in comparing activities of berries rich in different phenolic antioxidants since most berries rich in ellagitannins, anthocyanins, or flavonols exhibit excellent antioxidant properties when measuring the copper induced prolongation of oxidation lag time of LDL.[12,20,25] As ellagitannins are not readily absorbed in vivo with a metabolite ellagic acid found in plasma in concentrations up to 18 μM but rather may convey their biological activity through microbial metabolites,[38] the role of these phenolic compounds regarding human health most likely lies beyond antioxidant properties.

Berry ellagitannins at concentrations of 100 μg/g and higher resulted in significant protection toward oxidation of food emulsions (94 and 95% inhibition for red raspberry and cloudberry, respectively, Table 3). The current result adds to our earlier findings for red raspberry phenolics and isolated ellagitannins having been found effective toward oxidation of liposomes, with or without dairy proteins incorporated.[18,25] For the first time it is shown that dimeric and trimeric ellagitannins contribute significantly to the oxidative protection of food emulsion (Table 4). On the other hand, only weak or moderate antioxidant activity was exhibited by isolated berry ellagitannins (mixture or isolated dimers and trimers) toward oxidation of bulk oil while the phenolic extracts of both red raspberries and cloudberries exhibited excellent antioxidant activities (97 and 94% inhibition, respectively) at higher concentrations of 500 μg/g (Tables 3 and 4). The latter is in accordance with

previous findings[4,16] indicating that other phenolics rather than ellagitannins in cloudberries and red raspberries provide the protection toward oxidation in bulk oil. In conclusion, ellagitannins from berries of the *Rubus* family including dimeric sanguiin H-6, sanguiin H-10, and trimeric lambertianin C function both as radical scavengers and as antioxidants toward lipid oxidation in food emulsions. This capacity may well be exploited in developing foods with high oxidative stability.

AUTHOR INFORMATION

Corresponding Author

*Tel: +358 9 191 58224. Fax: +358 9 191 58475. E-mail: marina.heinonen@helsinki.fi.

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