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Antioxidant Activities and Antitumor Screening of Extracts from Cranberry Fruit (*Vaccinium macrocarpon*)

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Polyphenolic compounds in cranberries have been investigated to determine their role in protection against cardiovascular disease and some cancers. Extracts of whole fruit were assayed for radical-scavenging activity and tumor growth inhibition using seven tumor cell lines. Selective inhibition of K562 and HT-29 cells was observed from a methanolic extract in the range of 16−125 μg/mL. Radical-scavenging activity was greatest in an extract composed primarily of flavonol glycosides. Seven flavonol glycosides were isolated and purified from whole fruit for further evaluation; the anthocyanin cyanidin 3-galactoside was also purified for comparison with the flavonoids. Three flavonol monoglycosides were newly identified by ^{13}C NMR as myricetin 3-α-arabinofuranoside, quercetin 3-xyloside, and 3-methoxyquercetin 3-β-galactoside (isorhamnetin); the other four isolated were the previously identified myricetin 3-β-galactoside, quercetin 3-β-galactoside, quercetin 3-α-arabinofuranoside, and quercetin 3-α-rhamnopyranoside. These compounds were evaluated for 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity and ability to inhibit low-density lipoprotein oxidation in vitro. Most of the flavonol glycosides showed antioxidant activity comparable or superior to that of vitamin E; cyanidin 3-galactoside showed activity superior to that of the flavonoids as well as vitamin E or Trolox in both antioxidant assays.

KEYWORDS: Antioxidant; antitumor; cranberry; flavonoid; anthocyanin; phenolic; radical scavenger

INTRODUCTION

Cranberry (*Vaccinium macrocarpon* Ait. Ericaceae) is a native plant of North America; closely related species include lowbush blueberry (*V. angustifolium* Ait.), highbush blueberry (*V. corymbosum* L.), bilberry (*V. myrtillus*), and lingonberry (*V. vitis-idaea*). Cranberry juice and fruits reportedly exhibit various health benefits, including prevention of bacterial adhesion in urinary tract infections (UTI) of *Escherichia coli* and stomach ulcers (*1*, *2*), protection against lipoprotein oxidation (*3*), and in vitro anticancer activity (*4*). Some of these biological effects have been linked to the presence of phenolic compounds in the fruit. Cranberry contained the highest content of total phenolics per serving and by weight among 20 fruits analyzed in a study published by Vinson (*5*) and was ranked sixth in overall antioxidant quality.

Phenolic compounds in cranberries are a diverse group that includes anthocyanins, flavonoids, proanthocyanidins, condensed tannins, and low molecular weight phenolic acids. Both flavonoids and anthocyanins have been identified as strong antioxidants, with the potential to prevent oxidative damage caused by reactive oxygen species (*6*−*9*), and thus protect against cardiovascular disease and some cancers. Previous studies have suggested that crude extracts of cranberry fruits have significant antioxidant activity (*10*−*12*). However, the compounds responsible for this activity have not been fully isolated, characterized, and evaluated for their specific antioxidant activity. Early studies by Francis and collaborators (*13*, *14*) reported the presence of anthocyanins, flavonol glycosides, and other phenolic compounds in cranberry; the flavonol glycosides were further identified through paper chromatography as quercetin 3-galactoside, quercetin 3-arabinoside, quercetin 3-rhamnoside, myricetin 3-arabinoside, and quercetin 3-digalactoside. A screening of flavonoids and phenolic acids in berries by Hakkinen and co-workers (*15*) found that flavonols accounted for 75% of phenolics compared to 25% content of phenolic and hydroxybenzoic acids. Anthocyanins were not quantified in this study; however, Francis found a somewhat higher content by weight of anthocyanins than flavonols. More recent studies on the composition of phenolics in berry extract after hydrolysis and HPLC analysis confirmed the major flavonol aglycons to be myricetin and quercetin (*16*, *17*); however, the sugar moieties were not fully characterized, even

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by HPLC-EIMS (*18*). To provide a better understanding of the antioxidant properties and structures of cranberry flavonoids and anthocyanins, we used assay-guided fractionation to isolate several glycosides of quercetin and myricetin and one of the anthocyanins, cyanidin 3-galactoside, from whole cranberries. Their radical-scavenging activities were evaluated and compared to that of vitamin E by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay. We also evaluated their antioxidant quality using an in vitro assay for lower density lipoprotein (LDL + VLDL) oxidation. This method has been used to measure the dose-dependent ability of food and beverage constituents to prevent oxidation of a biologically relevant target and could be considered to be a predictor of such behavior in vivo (*19*).

The anticancer properties of cranberry and the nature of the compounds that provide protection against tumors are less well-understood. Bomser and co-workers (*4*) found that extracts from berries of *Vaccinium* species were able to inhibit the induction of ornithine decarboxylase, an enzyme involved in tumor proliferation, and induce quinone reductase, an enzyme that can inactivate certain carcinogens. The composition of a cranberry extract which exhibited ODC inhibition was recently reported to include flavonol glycosides and proanthocyanidins (*20*). Guthrie reported that two cranberry extracts inhibited proliferation of MCF-7 and MDA-MB-435 breast cancer cells (*21*); the active species have not yet been reported. In previous studies with Peruvian medicinal plants we have used tumor cell cytotoxicity assays in various cell lines to guide fractionation of plant extracts and identify compounds active against specific tumor cell lines (*22*). Using this approach, we screened cranberry extracts for antitumor activity in H460, ME180, DU145, MCF-7, HT-29, PC3, and K562 cell lines and herein report our initial findings.

MATERIALS AND METHODS

Plant Material. Fresh berries of the cranberry (*V. macrocarpon*), Stevens variety, were harvested in October 1999 from cranberry bogs in Wareham, MA, and kept frozen at −20 °C until use.

Reagents. All reagents were of analytical grade. Dimethyl-d_6 sulfoxide (DMSO), CD_3OD, DPPH, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 4-hydroxycinnamic acid (*p*-coumaric acid), ferulic acid, myricetin, and quercetin dihydrate were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Instrumentation. HPLC analysis and purification were performed on a Waters Millenium HPLC system composed of two Waters 515 pumps with a Waters 996 photodiode array detector. Mass spectra were obtained on a Finnigan TSQ mass spectrometer in EI mode (70 eV). NMR data for ^1H at 300.13 MHz and for ^{13}C at 75.48 MHz were obtained using a Bruker AC-300 NMR spectrometer at room temperature. Chemical shifts were reported relative to tetramethylsilane (TMS) as an internal standard. Spectrophotometric DPPH radical-scavenging assays were performed on a Shimadzu UV-160 UV−visible spectrophotometer.

Initial Screening of Cranberry Fruit Extracts for Antioxidant Activity and Inhibition of Tumor Growth. A bioassay-guided fractionation was used to identify whole fruit extracts with strong antioxidant activity and cytotoxicity toward tumor cells. Extracts were prepared using methods 1 and 2 and tested for ability to scavenge DPPH radicals and to inhibit tumor cell growth. The latter was determined using published procedures (*23*). Cell lines tested included BALB/c3T3, H460, ME180, DU145, MCF-7, HT-29, PC3, and K562. The DPPH radical-scavenging assay is described below.

Method 1. Frozen whole cranberries (100 g) were macerated in a blender containing petroleum ether (300 mL) for 5 min at medium speed. After vacuum filtration to remove the petroleum ether extract (1-PE), the solids were similarly macerated in ethyl acetate (200 mL). After filtration to remove the ethyl acetate extract (1-EA), the remaining solid was similarly macerated in methanol/2% formic acid (1-ME) (200 mL). The methanol extracts were removed by filtration. Extracts were dried in vacuo. Yields from 100 g of fresh weight berries were 1-PE, 0.310 g; 1-EA, 0.465 g; and 1-ME, 2.14 g.

Method 2. A crude acidic methanol extract of whole cranberries (100 g) was prepared by macerating the berries in a blender containing methanol/2% formic acid (300 mL) at medium speed for 5 min. This yielded a crude extract (8.05 g) upon lyophilization, which was then further fractionated using a solvent partitioning protocol. (*24*). The crude extract was first partitioned between water (100 mL) and chloroform (200 mL). The chloroform-soluble material was dried in vacuo and further partitioned between hexane (100 mL) and 90:10 methanol/water (100 mL). The aqueous layer was extracted twice with ethyl acetate (100 mL). Yields from berries (100 g) were as follows: crude extract (2-CR), 8.54 g; chloroform/hexane extract (2-CHX), 0.201 g; chloroform/methanol extract (2-CME), 0.130 g; ethyl acetate extract (2-EA), 0.630 g; and aqueous extract (2-AQ), 4.23 g. The ethyl acetate extract was further characterized by HPLC as described below.

DPPH Assays for Radical-Scavenging Activity. Stock solutions of each test sample including fractions, purified compounds, and standards (vitamin E, quercetin, and myricetin) were prepared in methanol solution for reaction with the DPPH free radical (*25*). Aliquots of stock solution were diluted to various concentrations and mixed with a methanolic solution of DPPH radical (60 μM) in 4 mL cuvettes. Final concentrations of the cranberry extracts and standards ranged from 1 to 0.001 mg/mL depending on the sample (lower concentrations were used for pure compounds). After a reaction period of 60 min at room temperature, absorbances were measured at 515 nm. The percent decrease in absorbance was recorded for each concentration, and percent quenching of DPPH radical was calculated on the basis of the observed decrease in absorbance of the radical. Concentration/% absorbance change curves were used to find the concentrations at which 50% radical scavenging occurred (EC_{50}). These values are reported in micrograms per milliter in **Tables 1** and **4**.

HPLC Analysis of Cranberry Extracts. The ethyl acetate fractions and subsequent column fractions containing flavonol glycosides were analyzed on a Waters 150 × 3.5 mm i.d. Novapak C18 RP column, using a binary solvent system: solvent A, 2% acetic acid; solvent B, methanol in 2% acetic acid. A program of isocratic elution with 100% A from 0 to 10 min, a linear gradient to 100% B from 10 to 45 min, and isocratic elution with 100% B from 45 to 60 min at a flow rate of 0.8 mL/min was used. PDA detection was used to monitor effluent from 250 to 700 nm.

Quantification of Total Flavonol and Anthocyanin Content in Whole Berries. Total flavonol and anthocyanin content of whole cranberries (milligrams of analyte/100 g of fresh weight fruit) was determined spectrophotometrically using the method of Lees and Francis (*13*).

Isolation of Flavonol Glycosides and Cyanidin 3-Galactoside. To evaluate the antioxidant activity of these compounds separately, individual flavonol glycosides were isolated and purified directly from cranberry extract by chromatography in methanol, using a method that we found to yield greater quantities of the flavonol glycosides than purification of the ethyl acetate extract. Cranberry fruits (1.0 kg) were macerated and extracted with 80% aqueous methanol. After concentration in vacuo, the extract was loaded on a 400 × 50 mm Diaion HP-20 column (Supelco) for removal of salts and carbohydrates by washing successively with distilled water and 20% aqueous methanol. Anthocyanins were recovered from the column by eluting with 30% methanol/water containing 5% acetic acid. This fraction was lyophilized and used for the isolation of cyanidin 3-galactoside. Proanthocyanidins were removed with 60% methanol/water. The flavonol glycosides were recovered from the column with 80% methanol/water containing 2% acetic acid. After concentration in vacuo, this extract was loaded on a Toyopearl HW-40C column (Supelco) for further purification, washed with distilled water, and eluted with aqueous methanol in 2% acetic acid, increasing in methanol content by 20% increments. After concentration and lyophilization, each fraction was screened for antioxidant activity using the DPPH radical-scavenging assay. The fraction eluting in 60−80% methanol showed the greatest activity. HPLC analysis matched that of the ethyl acetate extract described above, showing the presence of nine flavonol glycosides (**Table 2**).

Table 1. DPPH Radical-Scavenging Activity and Tumor Growth Inhibition of Initial Fractions Prepared from Whole Cranberry

fraction	EC_{50} for DPPH scavenging (μg/mL)	concn range in which 50% growth inhibition of tumor cells occurs (μg/mL)
1-PE	230	125–500 in all cells[a]
1-EA	40	125–500 in all cells
1-ME	140	~500 in all cells
2-CR	78	>500 in all cells
2-EA	33	>500 in all cells
2-AQ	160	>500 in all cells
2-CME	140	K562 cells: 16–63 HT-29 cells: 31–125 all other cell lines: 63–250

*Cell lines tested: BALB/c3T3, H460, ME180, DU145, MCF-7, HT-29, PC3, and K562.

Purification of Flavonol Glycosides and Cyanidin 3-Galactoside. Individual flavonol glycosides were purified from the flavonoid fraction by semipreparative HPLC on a 300 × 7.8 mm i.d. Nova-Pak HR C18 column (Waters). A binary solvent system of solvent A (20% methanol in 2% acetic acid) and solvent B (100% methanol in 2% acetic acid) was used. An isocratic elution program of 75% A and 25% B at 2.0 mL/min afforded collection of eight flavonol glycosides with the following retention times: **1**, 7.5 min; **2**, 9.1 min; **3**, 10.3 min; **4**, 11.8 min; **5**, 12.6 min; **6**, 13.8 min; **7**, 14.7 min; and **8**, 16.1 min.

Cyanidin 3-galactoside (**10**) was isolated from an aqueous acidic solution of the crude anthocyanin fraction by column chromatography on Toyopearl HW-40C. Elution with methanol/water/acetic acid (10:88:2 v/v) afforded several reddish bands. The second band to elute contained only cyanidin 3-galactoside as confirmed by HPLC analysis using the method described previously. For the purpose of comparison with the flavonol glycosides, its DPPH radical-scavenging activity and ability to inhibit LDL oxidation were evaluated. Results of assays on the purified compounds are given in **Table 4**.

Evaluation of the Antioxidant Activity of Flavonol and Anthocyanin Glycosides. Several of the more plentiful individual myricetin and quercetin glycosides and cyanidin 3-galactoside were assessed for general radical-scavenging activity using the DPPH assay described above. Results are given in **Table 4**. Antioxidant quality as measured by the ability of each antioxidant to inhibit the oxidation of low-density lipoproteins was also determined using a published procedure (*19*). Briefly LDL + VLDL was isolated from plasma and oxidized with cupric ion under physiological conditions of pH and temperature. Oxidation was measured by thiobarbituric acid reactive substances (TBARS). The concentration necessary to inhibit the oxidation 50% versus the control (EC_{50}) was calculated. Vitamin E, quercetin, and myricetin standards were included for comparison. Results are given in **Table 4**.

RESULTS AND DISCUSSION

Fraction 2-CME showed activity against K562 (human chronic myelogenous leukemia) and HT-29 (human colon adenocarcinoma) cell lines with IC_{50} values in the range of 16−125 μg/mL, as compared to the crude extract that only showed 50% growth inhibition at concentrations >500 μg/mL in all cell lines. All other fractions from methods 1 and 2 inhibited tumor cell growth nonselectively and at much higher concentrations (see **Table 1**). Further isolation and identification of the active compounds from 2-CME is underway.

DPPH assays found the strongest radical-scavenging activity (EC_{50} = 30−40 μg/mL) in the ethyl acetate soluble extracts (fractions 1-EA and 2-EA). Radical-scavenging activities of the fractions are given in **Table 1**. HPLC analysis of our extracts with PDA detection showed that the extracts were composed primarily of nine flavonol glycosides, with maximum absorbance wavelengths near 350 nm (**Table 2**). Eight of these were purified by semipreparative HPLC, and seven of the purified flavonoids were present in sufficient quantity for identification by ^{13}C NMR and further evaluation by the DPPH assay for radical-scavenging activity. The most plentiful of these were also evaluated for ability to inhibit LDL + VLDL oxidation.

Table 2. HPLC Analysis of Major Flavonol Glycosides from Cranberry Fruit

compd	retention time (min)	λ_{max} (nm)	relative peak area (%)
1	21.5	357.7, 256.8	5.2
2	22.7	355.3, 261.5	14.7
3	23.4	354.1, 255.7	24.6
4	24.0	355.3, 255.7	9.3
5	24.3	355.3, 255.7	5.0
6	24.8	351.7, 255.7	19.5
7	25.1	349.3, 255.7	13.4
8	26.6	354.1, 255.4	4.1
9	27.2	352.9, 255.4	4.2

Structural Elucidation of Newly Identified Flavonoid Antioxidants from Cranberry. For each sample, the identity of the flavonol moiety was first confirmed by comparison of NMR spectra with published chemical shifts for quercetin and myricetin (*26*). The seven flavonoids that were present in enough quantity for NMR analysis were found to be monosaccharides. Structures are shown in **Figure 1**. Identities of **1**, **3**, and **6**−**8** were assigned by comparison of ^{13}C chemical shifts in DMSO-d_6 or CD_3OD with published data on these glycosides (*27*, *28*). For **2** and **4**, anomeric proton chemical shifts and coupling constants were used to determine the configuration of attached sugars, and comparisons were made with chemical shift data for monosaccharide moieties reported in the literature. Compound **2** was identified on the basis of spectral similarities with **1** and **6**. Whereas **2** has a ^1H spectrum nearly identical to that of **1** in the region of 6.0−8.0 ppm (*29*), with chemical shifts at δ 7.1 (d, 5.82 Hz, H-2′, 6″), 6.40 (d, H-8), 6.19 (d, H-6), its anomeric proton signal appeared at δ 5.53 ppm (J = 2.4 Hz). The ^{13}C NMR spectrum showed signals in the sugar region at δ 103.9, 85.3, 82.0, 76.7, and 60.4 ppm. Hence, we concluded that **2** was the known flavonol glycoside myricetin 3-α-arabinofuranoside, which has been previously isolated from leaves of *Myrsine africana* and other plants (*30*).

^1H NMR signals for **4** in CD_3OD showed a quercetin aglycon [δ 7.60 (1H), 7.57 (1H), 6.85 (1H), 6.39 (1H), 6.20(1H)], whereas the sugar moiety gave a doublet anomeric proton at δ 5.18 (J = 6 Hz), as well as other protons at δ 3.76 (dd), 3.45−3.54 (m), 3.40 (d), 3.34 (s), and 3.10 (dd). The four ^{13}C NMR signals in the sugar region (δ 77.7, 75.4, 71.2, 67.4) suggested that **4** was quercetin 3-xyloside, based on comparison with data on other reported xylosides (*29*). Although this compound has been reported in other plants, for example, *Hypericum pulchrum* (*31*) and *Potentilla anserina* L. (*32*), we did not find complete ^1H and ^{13}C NMR data in the literature. Full ^{13}C NMR data on **4** are given in Table 3. The amount of **5** isolated was insufficient for structure determination.

Cyanidin 3-galactoside **10** was identified on the basis of its UV−visible spectral characteristics (λ_{max} = 515.3 and 279.3 nm) and the known anthocyanin composition of cranberries (*33*), which includes cyanidin 3-galactoside, cyanidin 3-arabinoside, peonidin 3-galactoside, and peonidin 3-arabinoside. Of these, cyanidin and peonidin galactosides are the most abundant. Due to difficulty in separating the other anthocyanins using our preparative HPLC method, only cyanidin 3-galactoside was isolated in a pure enough form to be used in the antioxidant comparison study.

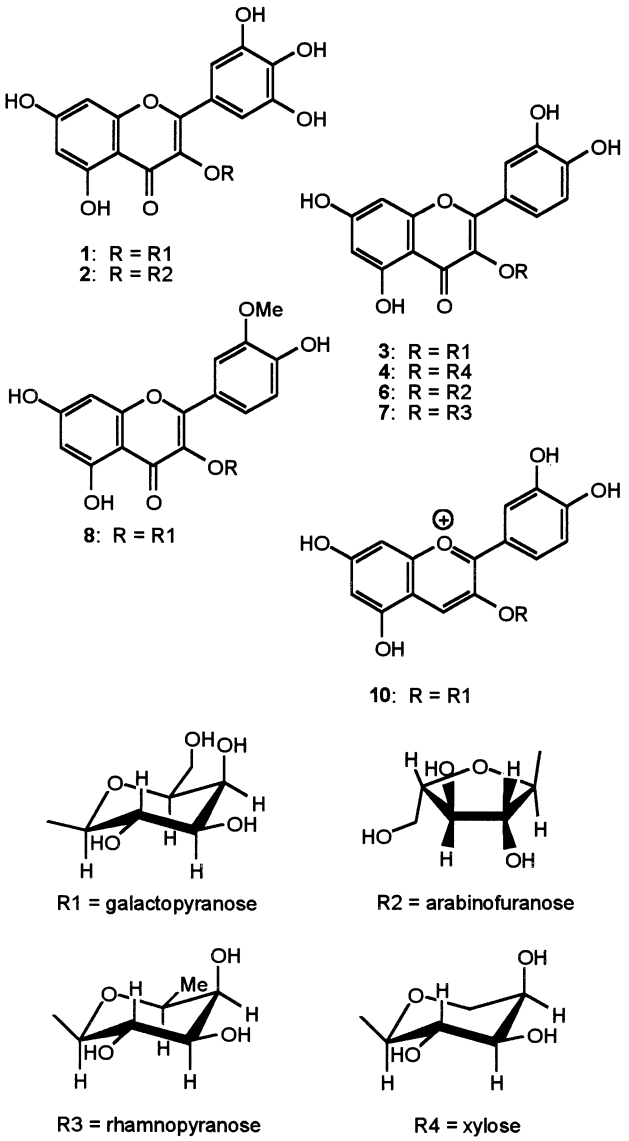

Figure 1. Flavonoids and anthocyanins isolated from cranberry fruit.

Our findings on the identities of flavonol monoglycosides **1**, **3**, **6**, and **7** in cranberry fruit agreed with those of Francis. In addition we have tentatively identified three additional monosaccharide derivatives of myricetin and quercetin in cranberry fruit (**2**, **4**, and **8**). The flavonoids found are identified as myricetin 3-β-galactoside (**1**), myricetin 3-α-arabinofuranoside (**2**), quercetin 3-β-galactoside (**3**), quercetin 3-xyloside (**4**), quercetin 3-α-arabinofuranoside (**6**), quercetin 3-α-rhamnopyranoside (**7**), and 3-methoxyquercetin 3-β-galactoside (**8**). ^{13}C NMR data are summarized in **Table 3**. Francis and Puski (*34*) reported finding myricetin 3-digalactoside on the basis of identification by cellulose chromatography, and Hakkinen and Auriola (*18*) reported finding a dipentose of quercetin on the basis of mass spectrometry. We did not identify any disaccharides in our flavonoid fraction; however, two of the flavonoids eluting in our fraction (compounds **5** and **9** listed in **Table 2**) were not isolated in enough quantity for identification by NMR. It is possible that these may be the previously reported disaccharides.

Radical-Scavenging Activities of Major Flavonol Glycosides in Cranberry Fruit. Initial fractionation of whole cranberry fruit and subsequent DPPH assays indicated that flavonoids play a significant role in the radical-scavenging activity of cranberry fruit, based on our observation that the flavonoid-rich ethyl acetate extract had greater activity than other extracts. The other extracts were not fully characterized in our study. When tested individually, each flavonoid exhibited dose-dependent radical-scavenging activity in the presence of the DPPH radical. EC_{50} values for the isolated flavonol glycosides and several standard antioxidants are given in **Table 4**. The antioxidant activities of each flavonol glycoside varied somewhat but only moderately, ranging from 17.3 to 34.6 μM. Myricetin and quercetin were found to be slightly less active in this assay than most of their glycosides. Myricetin 3-arabinoside (**2**) was the most active flavonol species tested (IC_{50} = 17.3 μM). Previous reports on the DPPH-radical scavenging activity of various flavonoids indicated less activity for the galactoside and rhamnoside of quercetin than quercetin itself (*35*). We also observed this for the quercetin rhamnoside; however, we found the plentiful quercetin galactoside (**3**) more active than free quercetin and nearly as active as **2**. In the lipoprotein oxidation assay (**Table 4**), the flavonol glycosides were able to prevent lipoprotein oxidation at micromolar concentrations. The glycosides exhibit somewhat lower activity

Table 3. ^{13}C NMR Chemical Shifts of Cranberry Flavonol Glycosides[a] (Parts per Million)

carbon	1	2	3	4	6	7	8
C-2	156.2	156.4	156.2	152.0	156.4	157.2	156.0
C-3	133.8	133.3	133.5	130.0	133.3	134.8	134.5
C-4	177.4	177.8	177.4	179.5	177.6	178.2	178.5
C-5	161.2	161.2	161.2	162.0	161.2	161.8	160.2
C-6	98.7	98.7	98.7	100.1	98.9	99.6	101.2
C-7	164.2	164.3	164.4	166.3	164.9	165.3	165.8
C-8	93.4	93.5	93.5	94.9	93.7	94.5	95.4
C-9	156.2	156.4	156.3	158.6	156.8	158.0	158.4
C-10	103.9	103.9	103.8	100.1	103.7	104.5	104.5
C-1′	120.2	119.3	121.6	123.4	121.7	121.5	123.7
C-2′	108.5	108.0	115.2	116.2	115.6	116.1	114.7
C-3′	145.4	145.7	144.8	143.0	145.2	146.0	149.0
C-4′	136.7	136.9	148.5	146.0	148.6	149.0	149.0
C-5′	145.4	145.7	115.6	117.4	115.6	116.2	116.1
C-6′	108.5	107.6	121.1	123.2	120.9	121.4	121.4
C-1″	102.0	103.9	101.8	104.7	107.8	102.3	101.2
C-2″	71.2	76.7	71.2	75.4	76.9	70.9	73.3
C-3″	73.3	82.0	73.2	77.7	82.1	71.2	75.2
C-4″	68.0	85.3	67.9	71.2	85.9	71.7	70.2
C-5″	75.9	60.4	75.8	67.4	60.7	70.6	77.4
C-6″	60.0		60.1			18.0	62.3
OMe							57.1

[a] Solvents: **1**, **2**, **3**, and **7** were dissolved in DMSO-d_6; **4**, **6**, and **8** were dissolved in CD_3OD.

Table 4. DPPH Radical-Scavenging Activities and Inhibition of Lower-Density Lipoprotein Oxidation by Cranberry Flavonoids and Anthocyanins Compared to Standard Antioxidants

	DPPH assay		LDL + VLDL assay
compd	EC_{50} (μg/mL)	EC_{50} (μM)	EC_{50} (μM)
myricetin 3-galactoside	11.0	22.9	5.53
myricetin 3-arabinoside	7.8	17.3	3.54
quercetin 3-galactoside	9.6	20.7	4.32
quercetin 3-rhamnoside	12.0	27.7	9.20
quercetin 3-arabinoside	15.0	34.6	6.13
quercetin 3-xyloside[a]			1.97
cyanidin 3-galactoside	3.5	7.7	1.45
Trolox or vitamin E	7.5	30.0	2.92
myricetin	8.5	26.7	3.35
quercetin dihydrate	9.3	27.5	2.33

[a] The small quantity isolated did not permit DPPH evaluation.

than their aglycons quercetin and myricetin with the exception of quercetin xyloside ($EC_{50} = 1.97\ \mu M$), which was more active than quercetin (2.33 μM), myricetin (3.35 μM), or tocopherol (2.92 μM) standards.

More striking perhaps is the antioxidant activity of cyanidin 3-galactoside **10**. Initial DPPH assays showed less activity in crude anthocyanin-containing fractions, yet the purified cyanidin glycoside was found to be even more effective than the flavonol glycosides and the standards in scavenging free radicals, with an EC_{50} of 7.7 μM compared to 17.3 μM for myricetin 3-arabinoside and 30 μM for Trolox. Its antioxidant quality was the highest of all the compounds tested in the LDL + VLDL oxidation assay, with an EC_{50} of 1.45 μM compared to 1.97 μM for quercetin xyloside and 2.92 μM for tocopherol (vitamin E). Analysis of total anthocyanin and flavonol content of our berry samples showed a slightly higher content of anthocyanins (36.45 $\pm$ 3.12 mg/100 g of berries) than flavonols (27.89 $\pm$ 2.76 mg/100 g of berries). Further isolation and evaluation of anthocyanins is underway.

Other phenolic classes such as catechins and cinnamic acids found in cranberry are capable of significant antioxidant activity, which varies as a function of structure (*36*, *37*). Proanthocyanidins from cranberry have been observed to associate with LDL in vitro and increase the lag time of copper-induced LDL oxidation, presumably due to their ability to bind to LDL and remain associated (*38*). This lipoprotein-bound antioxidant activity has also been demonstrated for catechins, hydroxycinnamic acids, flavonols, flavonol glycosides, and anthocyanins (*39*). We can conclude from our studies that, when compared to a standard dietary antioxidant, vitamin E, both the flavonol glycosides and cyanidin 3-galactoside from whole cranberry are effective in scavenging radicals and preventing LDL oxidation, cyanidin 3-galactoside being superior to the flavonols in this regard. Further studies of the ability of cranberry isolates to scavenge particular reactive oxygen species such as hydroxyl radical are underway. Our data support previously reported observations (*3*, *5*) that cranberry extracts provide some protection against oxidation of lipoproteins and provide additional molecular basis for these observations, highlighting the potential of cranberry and similar flavonoid- and anthocyanin-rich species to protect against cardiovascular disease and other free-radical related conditions.

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