Published in final edited form as:

J Agric Food Chem. 2011 April 13; 59(7): 3020–3026. doi:10.1021/jf200367j.

Edible Neotropical Blueberries: Antioxidant and Compositional Fingerprint Analysis

KEYVAN DASTMALCHI[†], GEMA FLORES[†], VANYA PETROVA[†], PAOLA PEDRAZA-PEÑALOSA[§], and EDWARD J. KENNELLY^{†,*}

[†]Department of Biological Sciences, Lehman College, and The Graduate Center, The City University of New York, 250 Bedford Park Boulevard West, Bronx, New York

[§]Institute of Systematic Botany, New York Botanical Garden, Bronx, 200th street & Kazimiroff, New York

Abstract

Edible blueberry species are well recognized for their potential health benefits. Ericaceae fruits including the North American highbush blueberry (*Vaccinium corymbosum* L.) and five less common edible blueberry relatives from the New World tropics, *Anthopterus wardii* Ball, *Cavendishia grandifolia* Hoerld, *Macleania coccoloboides* A. C. Sm., *Sphyrospermum buxifolium* Poepp. & Endl., and *Sphyrospermum cordifolium* Benth, were investigated for their antioxidant properties and phenolic profiles. The Neotropical berries *C. grandifolia* and *A. wardii* exhibited significantly higher DPPH[•] and ABTS^{•+} scavenging and iron chelation activities than *V. corymbosum*. Total phenolic content and HPLC-PDA compositional fingerprint analyses were also carried out. Significant correlations were observed among total phenolic contents, DPPH[•] and ABTS^{•+} scavenging, and iron chelation activities. Using HPLC-PDA, the phenolic constituents in the berries were identified as chlorogenic acid, *p*-coumaric acid, hyperoside, quercetin-3-*O*-glucoside, isoorientin, isovitexin, orientin and vitexin. Principal components analysis reduced the dimensions of antioxidant and total phenolic data to two components, which accounted for 95% of total variation among the six fruits. Each fruit species formed its own cluster, and therefore the antioxidant profile of each species was shown to be distinct.

Keywords

antioxidants; Ericaceae; Neotropical blueberries; HPLC-PDA; free radical scavenging; iron chelation; PCA

INTRODUCTION

Reactive oxygen species (ROS) are products of normal cellular metabolism and are involved in cellular signalling mechanisms (1). However, if there is excessive formation of ROS, the body's antioxidant defence mechanisms can be overwhelmed, and result in oxidative stress, which plays an important role in the pathogenesis of many diseases such as cardiovascular disorders, diabetes, central nervous system diseases, and chronic obstructive pulmonary disease (COPD) (2, 3). In the case of COPD, studies have shown that the ROS present in the cigarette smoke induce inflammation, resulting in further generation of ROS (4).

*CORRESPONDING AUTHOR: Tel.: +1 718 960 1105, Fax.: +1 718 960 8236, Edward.kennelly@lehman.cuny.edu (E.J. Kennelly).

SUPPLEMENTAL INFORMATION

A table containing information about linear regression parameters, LOD and LOQ and a figure representing the loading plot are provided.

There is overwhelming epidemiological evidence that correlates consumption of fruits and vegetables with lower incidence of certain chronic diseases (5), and this may explain the numerous articles in the past decade on natural antioxidants. There is strong evidence to suggest the beneficial effects of fruits and vegetables can be attributed, at least in part, to phenolic antioxidants (6).

Antioxidants are particularly abundant in Ericaceae berries (7–9), which have been championed as “super fruits” in the popular literature and commercial advertisements. The most economically valuable edible Ericaceae fruits include three temperate species, highbush blueberries (*Vaccinium corymbosum* L.), cranberries (*V. macrocarpon* Aiton), and lingonberries (*V. vitis-idaea* L.). *Vaccinium* L. species are well known for their nutritional and medicinal uses, and the earliest evidence dates back to the Bronze Age (10). Much antioxidant research on Ericaceae has focused on the genus *Vaccinium*, which is only one of the 32 berry producing genera within the tribe (11).

More than 600 different species of berry-producing Ericaceae are native to the New World tropics (Neotropics) (11). There are very few published accounts of uses of Neotropical blueberries and their potential in horticulture and in medicine has yet to be realized. Although understudied, ethnobotanical observations confirm culinary, cultural, and medicinal uses of certain Neotropical blueberries (11). Phytochemical investigations of Neotropical blueberries are practically absent and only the leaf flavonoids and simple phenols of 116 species of *Cavendishia* Lindl. have been studied, and the flavonols quercetin, kaempferol, and myricetin were detected using paper chromatography, a low-resolution method (12). In the present study, the antioxidant activity and composition of the fruits of five species of Neotropical blueberries were investigated for the first time. The species here investigated, *Anthopterus wardii* Ball, *Cavendishia grandifolia* Hoerld, *Macleania coccoloboides* A. C. Sm., *Sphyrnospermum buxifolium* Poepp. & Endl., and *Sphyrnospermum cordifolium* Benth, cover three distinct lineages. The antioxidant properties and chemical profiles of these species were compared to the common edible highbush blueberry, *V. corymbosum*.

MATERIALS AND METHODS

Reagents

1,1-Diphenyl-2-picrylhydrazyl, disodium salt of ethylenediaminetetraacetic acid, Folin-Ciocalteu reagent, gallic acid, potassium peroxosulfate, trichloroacetic acid, Trizma base and Trolox, and chlorogenic acid were purchased from Sigma Chemical-Aldrich (St. Louis, MO, USA). 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonate) diammonium salt, was from TCI-Ace (Tokyo, Japan). Vitexin, isovitexin, orientin, isoorientin, hyperoside, quercetin-3-O-glucoside, *p*-coumaric acid, and chlorogenic acid were from Chromadex (Irvine, CA, USA). HPLC-grade MeCN, MeOH and formic acid were purchased from J.T. Baker (Philipsburg, NJ, USA) and GR grade MeOH and EtOH from VWR Inc. (Bridgeport, PA, USA). Ultrapure water was prepared using a Millipore Milli-RO 12 plus system, Millipore Corp. (Bedford, MA, USA).

Plant material

Fruits of *A. wardii*, *C. grandifolia*, *S. buxifolium*, and *S. cordifolium* were collected at The New York Botanical Garden Nolen Greenhouses and Enid A. Haupt Conservatory. *Macleania coccoloboides* fruits were from Atlanta Botanical Garden. Fruits of *V. corymbosum* cultivar Brigitta, which is here used as a positive control, were purchased at a local supermarket.

Preparation of fruit extracts

The freeze-dried fruits were homogenized using a blender and 70% (v/v) MeOH as the extractant. The ratio of material to the extractant was 1:20 (w/v). Extracts were filtered and the marc extracted two more times. Extracts were combined and concentrated *in vacuo* (45 °C), freeze-dried and stored at 4 °C.

Total phenol content

Total phenol content was assessed by Folin-Ciocalteu method (13, 14). Three aliquots were analyzed in triplicate ($n = 6$). To 100 μL of sample or gallic acid, 1 mL of Folin-Ciocalteu reagent was added, mixed, and incubated for 5 min at room temperature prior to addition of 1 mL of 10% Na_2CO_3 solution. This mixture was then kept for 90 min at room temperature, and the absorbance was determined at 765 nm. Total phenol content was estimated as gallic acid equivalents (GAE, mg gallic acid/g dry fruit material).

1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH $^{\bullet}$) scavenging

The DPPH $^{\bullet}$ scavenging activity was assessed according to the method described by Smith *et al.* (15) with minor modifications. To 50 μL aliquot of the sample 150 μL of DPPH (400 μM) were added and the absorbance at 515 nm was recorded after 30 min of incubation at 37 °C using a Molecular Devices Versa $_{\text{max}}$ microplate reader (Sunnyvale, CA, USA). The percentage inhibition values for different concentrations were calculated using equation 1 (Eq. 1). A plot of percentage inhibition versus concentration was made for the reference standard, Trolox. On the basis of this plot the Trolox equivalent antioxidant capacity (TEAC, 3mol Trolox/g dry fruit material) values for different samples were calculated.

$$\text{Percentage Inhibition} = \left[\frac{(\text{Absorbance}_{\text{control}}) - (\text{Absorbance}_{\text{sample}})}{(\text{Absorbance}_{\text{control}})} \right] \times 100 \quad \text{Eq. 1}$$

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulphonate) free radical (ABTS $^{\bullet+}$) scavenging

The determination of ABTS $^{\bullet+}$ scavenging was carried out based on the method of Re *et al.* (16). The ABTS $^{\bullet+}$ was generated by reacting an ABTS (7 mM) aqueous solution with $\text{K}_2\text{S}_2\text{O}_8$ (2.45 mM) in the dark for 12–16 h, at ambient temperature, and adjusting the $\text{Abs}_{734 \text{ nm}}$ to 0.700 (± 0.020) with ethanol. To 2 μL aliquot of the sample 198 μL ABTS $^{\bullet+}$ was added and the absorbance at 734 nm was recorded after initial mixing and subsequently at 5 min intervals (for 40 min in total) using a Molecular Devices Versa $_{\text{max}}$ microplate reader (Sunnyvale, CA, USA). The results were expressed as the TEAC (3mol Trolox/g dry fruit material) values at different time intervals (5).

Iron chelation

The iron chelation activity was assessed using the method of Carter (17) with minor modification. To 20 μL of the sample, 10 μL of iron (II) chloride tetrahydrate (2 mM) and 90 μL of methanol were added. The reaction mixture was incubated for 5 min, thereafter 40 μL of ferrozine (5mM) were added. After 10 min the absorbance was measured at 562 nm, using a Molecular Devices Versa $_{\text{max}}$ microplate reader (Sunnyvale, CA, USA). The percentage chelation was calculated using Eq. 2. A plot of percentage chelation vs. concentration was made for the reference standard, disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA).

$$\text{Percentage Chelation} = \left[\frac{(\text{Absorbance control}) - (\text{Absorbance sample})}{(\text{Absorbance control})} \right] \times 100 \quad \text{Eq.2}$$

The results were expressed as the Na₂EDTA equivalent (3mol Na₂EDTA/g dry fruit material) values.

Chromatographic fingerprint analysis

HPLC-PDA analyses of the extracts were performed using Waters (Milford, MA, USA) Alliance 2695 system equipped with 2695 separation module unit and 2996 PDA detector using a 250 × 4.6 mm, 4μ Phenomenex Synergi Hydro-RP 80A column (Torrance, CA, USA). The mobile phase consisted of solvents (A) 0.1% aqueous formic acid solution and (B) 0.1% formic acid solution in MeCN. Step-wise gradient elution was performed using 85% A for 5 min and 85–80% A in 35 min. The composition was then changed to initial condition in 5 min, and maintained for 10 min. The flow rate and the injection volume were 1 mL/min and 10 μL, respectively. The results were monitored using a wavelength range of 210–800 nm.

Stock solutions of the standard compounds were prepared in 70 % (v/v) methanol to final concentration of 1 mg/mL. Each stock solution was further diluted to obtain six concentrations of the standard. Calibration curves of the standards were established on six data points, and each standard dilution was injected in triplicate. The fruit extracts were also reconstituted in 70% (v/v) methanol and injected in triplicate at a concentration of 20 mg/mL. Peak areas for the extracts and standards were integrated from HPLC-PDA chromatograms using Waters Empower2 software. Limits of detection (LODs) and limits of quantitation (LOQs) of the standards were determined on the basis of their signal to noise (S/N) ratios of 3 : 1 and 10 : 1, respectively.

Statistical analysis

Data are presented as mean values ± 95% confidence interval. Analysis of variance was performed using ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $P < 0.05$. Pearson's correlation coefficient was calculated at a level of $P < 0.05$. Principal component analysis of the antioxidant data (n = 6) of the fruits were carried out. The JMP version 8 software was used for statistical analyses.

RESULTS AND DISCUSSION

Of the five Neotropical blueberries examined, two species, *C. grandifolia* and *A. wardii*, showed stronger antioxidant properties than the highbush blueberry *V. corymbosum*.

Extract yield and total phenolic content

The highest extraction yield of the five tested Neotropical blueberries was obtained from *A. wardii* and the lowest yield was from *C. grandifolia* (Table 1). The total phenolic contents of the dried fruits (Table 1), using the Folin-Ciocalteu method, decreased in the following order: *C. grandifolia* > *A. wardii* and *V. corymbosum* (not significantly different, $P > 0.05$) > *S. buxifolium* > *M. coccoloboides* > *S. cordifolium*. *Cavendishia grandifolia* had a significantly higher total phenolic content than *V. corymbosum*. There was no significant correlation observed between the extraction yield and the total phenolic contents ($r = -0.18$), indicating that phenolic compounds are not the only major class of constituent extracted from these fruits.

DPPH[•] scavenging

The order of DPPH[•] scavenging activity for the fruits is: *Cavendishia grandifolia* > *A. wardii* > *S. buxifolium* > *V. corymbosum* > *M. coccoloboides* and *S. cordifolium* (not significantly different, $P > 0.05$) (Figure 1A). Thus three of the Neotropical blueberries, *C. grandifolia*, *A. wardii*, and *S. buxifolium*, demonstrated significantly higher scavenging activities than the positive control (*V. corymbosum*). However, the other Neotropical species (*M. coccoloboides* and *S. cordifolium*) showed mild to moderate DPPH[•] scavenging activities.

There is a significant correlation between total phenolics content of the fruit extracts and their DPPH[•] scavenging activities ($r = 0.89$). This indicates that the phenolic compounds are the major constituents in Neotropical blueberries contributing to the DPPH[•] scavenging activity. In a study of wild blueberries (*V. myrtillus*) and four highbush blueberry (*V. corymbosum*) cultivars, a significant correlation between total phenolics content and DPPH[•] scavenging activity was also observed (18).

Free radicals play a significant role in the progression of oxidative stress and thus scavenging these species is an important mechanism of antioxidant action (3). Therefore fruits of *C. grandifolia*, *A. wardii*, and *S. buxifolium* may prevent free radical mediated oxidative damage *in vivo*.

Iron chelation

Three of the Neotropical blueberries, *C. grandifolia*, *A. wardii*, and *M. coccoloboides*, demonstrate significantly higher chelation activities than highbush blueberry (*V. corymbosum*) (Figure 1B). The order of iron chelation activity for the fruits is as follows: *Cavendishia grandifolia* and *A. wardii* (not significantly different, $P > 0.05$) > *M. coccoloboides* > *V. corymbosum* (Figure 1B).

Like many other transition metals, iron in its ferrous form, is responsible for the formation of ROS *in vivo* which can lead to oxidative stress (19). Therefore, some plant polyphenols by forming redox stable complexes with transition metal ions have prevented formation of ROS which is termed as the secondary antioxidant effect (20). This mechanism of antioxidant action is of value in the treatment of diseases mediated by oxidative stress such as Alzheimer's disease and COPD (2, 21). For example, clioquinol is an iron chelator that has been tested clinically for the treatment of Alzheimer's disease (2).

A significant correlation was observed between iron chelation and total phenolic contents ($r = 0.57$). The ABTS^{•+} ($r = 0.57$) and DPPH[•] ($r = 0.59$) scavenging activities also showed significant correlation with the chelation activity. This shows that antioxidant constituents present in the Neotropical blueberries exert their effect via different mechanisms, which can be of value in prevention of oxidative stress.

ABTS^{•+} scavenging

The order of ABTS^{•+} scavenging activity at 0 min was: *C. grandifolia* > *A. wardii* and *V. corymbosum* (not significantly different, $P > 0.05$) > *M. coccoloboides*, *S. buxifolium* and *S. cordifolium* (not significantly different, $P > 0.05$) (Figure 2). The order of scavenging activity changed at 5, 10, and 15 min; however, throughout this period *C. grandifolia* followed by *A. wardii* had the highest scavenging activities. From 15 min onwards the order of activity remained the same and was as follows *C. grandifolia* > *A. wardii* > *S. buxifolium* and *V. corymbosum* (not significantly different, $P > 0.05$) > *S. cordifolium* > *M. coccoloboides* (not significantly different, $P > 0.05$) (Figure 2).

The ABTS^{•+} scavenging activity of *C. grandifolia*, *M. coccoloboides*, and *S. buxifolium* increased up to 40 min, after which it did not change significantly. There was a rise in the activity of *A. wardii*, *S. cordifolium*, and *V. corymbosum* up to 30 min, and thereafter no significant change was observed (Figure 2). Due to the changes in ABTS^{•+} scavenging activities of the fruits over time the order of activity did not remain constant. Therefore, to take into account these variations the overall ABTS^{•+} scavenging capacities of the fruit extract were calculated in terms of area under the curve (AUC) values. The overall scavenging activities (AUC) is: *C. grandifolia* (8637.49 ± 392.15) > *A. wardii* (2624.45 ± 102.12) > *S. buxifolium* (1717.52 ± 97.72) and *V. corymbosum* (1852.50 ± 114.25) (not significantly different, $P > 0.05$) > *M. coccoloboides* (1231.47 ± 37.32) and *S. cordifolium* (1291.00 ± 143.19) (not significantly different, $P > 0.05$) (Figure 2). Therefore, *C. grandifolia* and *A. wardii* have significantly higher scavenging activities than the positive control, *V. corymbosum*. The activity of *S. buxifolium* was also not significantly different from the control. However, the other Neotropical blueberries (*M. coccoloboides* and *S. cordifolium*) showed mild to moderate ABTS^{•+} scavenging activities.

Although the ABTS^{•+} and DPPH[•] scavenging assays relies on the same principal, the ability of the former assay to evaluate the activity of both lipophilic and hydrophilic compounds, overcomes solubility problems associated with the latter assay (20). The wavelength at which the absorbance reading is taken (734 nm) is high enough to prevent absorbance interference from most phytochemical substances, including blueberry anthocyanins. The assay is not influenced by variations in the pH (22). Absence of steric hinderence, unlike that of DPPH[•], enables evaluation of the activities of large compounds (23).

As the free radical scavenging activity was monitored over time, the slow acting antioxidants had enough time to exert their effects. Due to their contribution to the scavenging activity the order of activity among the fruits changed during the assay. The total phenolics content of the fruits and their ABTS^{•+} scavenging activities showed significant correlation ($r=0.88$). Other studies have also reported similar correlation for *V. corymbosum* and *V. macrocarpon* at different stages of their maturation (24, 25). This indicates that the phenolic compounds are the major constituents in the fruits contributing to the ABTS^{•+} scavenging activity. The DPPH[•] scavenging ($r=0.95$) and iron chelation ($r=0.57$) activities also showed significant correlation with the ABTS^{•+} scavenging activity.

Qualitative and quantitative HPLC compositional analysis

The data from qualitative-quantitative HPLC analysis of the fruit extracts are presented in Table 1. The extracts contained flavonoids and cinnamic acid derivatives, some of which were identified and quantitated. All the fruits contained chlorogenic acid (Figure 3) and their content of chlorogenic acid decreased in the following order. *Cavendishia grandifolia* > *V. corymbosum* > *S. buxifolium* > *S. cordifolium* > *A. wardii* > *M. coccoloboides*. *Cavendishia grandifolia* contained the highest amount of chlorogenic acid, 11 times greater than *V. corymbosum* (Table 1), and others have also reported presence of large quantities of chlorogenic acid in *V. corymbosum* and other *Vaccinium* species (26). Chlorogenic acid is the most abundant chemical constituent in *Cavendishia grandifolia* (Table 1, Figure 4A).

There was significant correlation observed between the contents of chlorogenic acid, total phenolic contents ($r=0.83$), DPPH[•] ($r=0.88$), and ABTS^{•+} ($r=0.98$) scavenging activities. Since chlorogenic acid is one of the major phenolic constituents present in all five Neotropical berries studied, much of the free radical scavenging properties can be attributed to this caffeic acid derivative. However, there is no significant correlation between chlorogenic acid content and iron chelation ($r=0.49$). This may be due to larger contribution of other phytoconstituents to the chelating activity of the fruits. Chlorogenic acid is a potent antioxidant compound with a wide range of antioxidant properties (27). This

well studied compound has also been reported to have a wide range of biological and pharmacological activities (28).

All the fruits with the exception of *A. wardii* contained hyperoside (Figure 3) and their content of hyperoside decreased in the following order (Table 1). *Vaccinium corymbosum* > *C. grandifolia* > *S. buxifolium* > *M. coccoloboides* > *S. cordifolium*. The presence of quercetin-3-*O*-glucoside (Figure 3) was detected in *C. grandifolia*, *V. corymbosum*, and *S. buxifolium*. *Cavendishia grandifolia* had the highest and *S. buxifolium* had the lowest amount of quercetin-3-*O*-glucoside. Other researchers have also reported the presence of these quercetin glycosides and other quercetin derivatives in *V. corymbosum* and other *Vaccinium* spp. (26, 29). In a study done by by our group (unpublished results) orientin, isoorientin, vitexin and isovitexin were identified in *A. wardii*, (Figure 3) and in the current study these constituents were quantitated (Table 1). The most abundant constituents present in *A. wardii* were vitexin and isovitexin (Table 1, Figure 4B). Vitexin, orientin, and isoorientin have been reported to be present in acai berries (30). Chlorogenic acid and orientin were the least abundant constituents identified in *A. wardii* (Table 1, pFigure 4B). Trace quantities of -coumaric acid (Figure 3) was found in *S. buxifolium*.

The remaining phenolic constituents present in the fruits, were classified and quantitated as hydroxycinnamic acid derivatives and flavonoids. The content of non-identified flavonoids in the fruits decreased in the following order. *Cavendishia grandifolia* > *V. corymbosum* > *S. buxifolium* > *A. wardii* > *S. cordifolium* > *M. coccoloboides* (Table 1). *Cavendishia grandifolia* had the highest and *M. coccoloboides* had the lowest content of non-identified flavonoids (Table 1). The content of non-identified hydroxycinnamic acid derivatives in the fruits decreased in the following order. *Cavendishia grandifolia* > *S. buxifolium* > *S. cordifolium* > *M. coccoloboides* > *V. corymbosum* > *A. wardii* (Table 1). *Cavendishia grandifolia* had the highest and *A. wardii* had the lowest content of non-identified hydroxycinnamic acid derivative (Table 1). The total content of non-identified phenolic constituents in the fruits decreased in the following order. *Cavendishia grandifolia* > *S. buxifolium* > *V. corymbosum* > *S. cordifolium* > *A. wardii* > *M. coccoloboides*. *Cavendishia grandifolia* had the highest and *M. coccoloboides* had the lowest content of total non-identified phenolic compounds (Table 1).

Principal Component Analysis

The two principal components (PCs), account for 95% of total variation among the fruits (PC1 = 81.21% and PC2 = 14.26%). ABTS^{•+} and DPPH[•] scavenging activities and total phenolic contents, were features with high positive loading on PC1 and negative loading on PC2. However, iron chelation showed high positive loading on PC1 and PC2. Based on the rotation factors ABTS^{•+} (0.93), DPPH[•] (0.93), and total phenolic contents (0.90) were responsible for most of the variations among the fruits. Iron chelation (0.31) contributed to the variation to a lesser degree.

The species here studied represent different lineages (different genera and different clades) within the blueberry tribe Vaccinieae according to recent phylogenetic analyses (31, 32). Montane Neotropical groups are, geologically speaking, young and had undergone rapid adaptive radiation. In the score plot (Figure 5) six clusters can be seen, one for each species. Moreover, different genera are distributed in different areas of the plot (Figure 5), which can be divided in four major groups. Only *Sphyrnospermum* and *Vaccinium* were found in the same quadrant; although these genera were once thought to be closely related, molecular studies have shown that is not the case (32). However, since the growth and storage conditions for the commercial *V. corymbosum* is different from the greenhouse-grown Neotropical blueberries under study, the influence of environmental factors should also be considered.

The proximity of the clusters representing species belonging to the genus *Sphyrospermum* should be noted (Figure 5). *Sphyrospermum buxifolium* and *S. cordifolium* are considered sister species, if not equal (33). Both species had similar antioxidant profiles; however, their ABTS^{•+} and DPPH[•] scavenging activities were significantly different which contributed to formation of different clusters for each species.

In conclusion, *C. grandifolia* and *A. wardii* showed significantly higher antioxidant activities in all the assays when compared to the highbush blueberry. With highbush blueberry being referred to in the popular literature as a “superfruit”, the two Neotropical blueberries have the potential to be even more highly promising edible fruits, based on our findings. We are currently conducting additional chemical and biological studies on *C. grandifolia* and *A. wardii*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the staff of the Nolen Greenhouses at The New York Botanical Garden, and especially the Manager Marc Hachadourian, for his assistance in providing fruit samples. Staff members of the Atlanta Botanical Garden are thanked for providing *M. coccoloboides* fruits. We express our gratitude to Dr. James Luteyn for his assistance with developing this project. This work was supported by NIH-NHLBI, grant 5SC1HL096016. We would like to acknowledge statistical assistance from Mr. Uchenna Unachukwa, and technical assistance from Dr. Chunhui Ma, Mr. Adam Kavaliar, Ms. Amy Keller, and Mr. James Lyles.

LITERATURE CITED

1. Singh M, Arseneault M, Sanderson T, Murthy V, Ramassamy C. Challenges for research on polyphenols from foods in Alzheimer's disease: Bioavailability, metabolism, and cellular and molecular mechanisms. *J Agric Food Chem*. 2008; 56:4855–4873. [PubMed: 18557624]
2. Dastmalchi K, Damien DHJ, Vuorela H, Hiltunen R. Plants as potential sources for drug development against Alzheimer's disease. *Int J Biomed Pharm Sci*. 2007; 1:83–104.
3. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007; 39:44–84. [PubMed: 16978905]
4. Foronjy R, D' Armiento J. The effect of cigarette smoke-derived oxidants on the inflammatory response of the lungs. *Clin Appl Immunol Rev*. 2006; 6:53–72.
5. Dastmalchi K, Dorman DHJ, Oinonen PP, Darwis Y, Laakso I, Hiltunen R. Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. *LWT--Food Sci Technol*. 2007; 41:391–400.
6. Kroon P, Williamson G. Polyphenols: dietary components with established benefits for human health. *J Sci Food Agric*. 2005; 85:1239–1240.
7. Kähkönen MP, Hopia AI, Heinonen M. Berry phenolics and their antioxidant activity. *J Agric Food Chem*. 2001; 49:4076–4082. [PubMed: 11513713]
8. Connor AM, Luby JJ, Hancock JF, Berkheimer S, Hanson EJ. Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. *J Agric Food Chem*. 2002; 50:893–898. [PubMed: 11829664]
9. Määttä-Riihinen KR, Kähkönen MP, Törrönen AR, Heinonen IM. Catechins and procyanidins in berries of *Vaccinium* species and their antioxidant activity. *J Agric Food Chem*. 2005; 53:8485–8491. [PubMed: 16248542]
10. Trehane, J. Blueberries, Cranberries and Other Vacciniums. Timber Press; Portland, Or: 2004.
11. Luteyn, JL.; Pedraza-Peñalosa, P. [Accessed: February 9, 2011] Neotropical blueberries: The plant family Ericaceae. <<http://www.nybg.org/bsci/res/lut2>>

12. Luteyn J, Harborne J, Williams C. A Survey of the flavonoids and simple phenols in leaves of *Cavendishia* (Ericaceae). *Brittonia*. 1980; 32:1–16.
13. Singleton VL, Rossi JA Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965; 16:144–158.
14. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem*. 1999; 47:3954–3962. [PubMed: 10552749]
15. Smith RC, Reeves JC, Dage RC, Sanderson T. Antioxidant properties of 2-imidazolones and 2-imidazolthiones. *Biochem Pharmacol*. 1987; 36:1457–1460. [PubMed: 3579984]
16. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med*. 1999; 26:1231–1237. [PubMed: 10381194]
17. Carter P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Anal Biochem*. 1971; 40:450–458. [PubMed: 5551554]
18. Giovanelli G, Buratti S. Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. *Food Chem*. 2009; 112:903–908.
19. Chvátalová K, Slaninová I, Brezinová L, Slanina J. Influence of dietary phenolic acids on redox status of iron: Ferrous iron autoxidation and ferric iron reduction. *Food Chem*. 2008; 106:650–660.
20. Dastmalchi, K. Doctoral Dissertation. University of Helsinki; Helsinki: 2008. *Dracocephalum moldavica*, L. and *Melissa officinalis*, L., Chemistry and bioactivities relevant in Alzheimer's disease therapy.
21. Ghio AJ, Hilborn ED, Stonehuerner JG, Dailey LA, Carter JD, Richards JH, Crissman KM, Foronjy RF, Uyeminami DL, Pinkerton KE. Particulate matter in cigarette smoke alters iron homeostasis to produce a biological effect. *Am J Respir Crit Care Med*. 2008; 178:1130–1138. [PubMed: 18723436]
22. Magalhães LM, Segundo MA, Reis S, Lima JLFC. Methodological aspects about in vitro evaluation of antioxidant properties. *Anal Chim Acta*. 2008; 613:1–19. [PubMed: 18374697]
23. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem*. 2005; 53:4290–4302. [PubMed: 15884874]
24. Castrejón ADR, Eichholz I, Rohn S, Kroh LW, Huyskens-Keil S. Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. *Food Chem*. 2008; 109:564–572.
25. Çelik H, Özgen M, Serçe S, Kaya C. Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit. *Sci Hort*. 2008; 117:345–348.
26. Vasco C, Riihinen K, Ruales J, Kamal-Eldin A. Chemical composition and phenolic compound profile of Morti3o (*Vaccinium floribundum* Kunth). *J Agric Food Chem*. 2009; 57:8274–8281. [PubMed: 19719139]
27. Shahidi F, Chandrasekara A. Hydroxycinnamates and their in vitro and in vivo antioxidant activities. *Phytochem Rev*. 2010; 9:147–170.
28. Higdon JV, Feri B. Coffee and health: A review of recent human research. *Crit Rev Food Sci Nutr*. 2006; 46:101–123. [PubMed: 16507475]
29. Määttä-Riihinen KR, Kamal-Eldin A, Mattila PH, González-Paramás AM, Törrönen AR. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *J Agric Food Chem*. 2004; 52:4477–4486. [PubMed: 15237955]
30. Kang J, Li Z, Wub T, Jensen GC, GSA, Wua X. Anti-oxidant capacities of flavonoid compounds isolated from acai pulp (*Euterpe oleracea* Mart.). *Food Chem*. 2010; 122:610–617.
31. Kron KA, Powell EA, Luteyn JL. Phylogenetic relationships within the blueberry tribe (Vaccinieae, Ericaceae) based on sequence data from MATK and nuclear ribosomal ITS regions, with comments on the placement of *Satyria*. *Am J Bot*. 2002; 89:327–336. [PubMed: 21669741]
32. Pedraza-Peñalosa P. Insensitive blueberries: a total-evidence analysis of *Disterigma s.l* (Ericaceae) exploring transformation costs. *Cladistics*. 2010; 26:388–407.
33. Luteyn JL, Pedraza-Peñalosa P. A synopsis of the neotropical genus *Sphyrrosperrum* (Ericaceae: Vaccinieae), including five new species. *Brittonia*. In Press.

Figure 1.

(A) DPPH scavenging and (B) iron chelation activities of Neotropical blueberries. Values are expressed as means $\pm$ 95% confidence intervals ($n = 6$). Bars with different letters (a–e) are significantly ($P > 0.05$) different. Analysis of variance was performed by ANOVA procedures, with significant differences between means determined by Tukey's pairwise comparisons. ND, not detected.

Figure 2. ABTS^{•+} scavenging activity of Neotropical blueberries. Values are expressed as means $\pm$ 95% confidence intervals ($n = 6$). Trolox Equivalent Antioxidant Capacity (TEAC) which is expressed as (3mol Trolox/g dry fruit material).

Figure 3. Structural formulas of **1**, chlorogenic acid; **2**, hyperoside; **3**, quercetin-3-*O*-glucoside; **4**, isoorientin; **5**, orientin; **6**, vitexin; **7**, isovitexin; and **8**, *p*-coumaric acid

Figure 4. HPLC-PDA chromatograms of (A) *C. grandifolia* and (B) *A. wardii* at 360 nm. **1**, chlorogenic acid; **2**, hyperoside; **3**, quercetin-3-*O*-glucoside; **4**, isorientin; **5**, orientin; **6**, vitexin; and **7**, isovitexin.

Figure 5. Score plot PC1 and PC2 for classification of the fruits. The fruit samples are **1**, *A. wardii*; **2**, *C. grandifolia*; **3**, *M. coccoloboides*; **4** *S. buxifolium*; **5** *S. cordifolium*; and **6** *V. corymbosum*

Table 1
Extract Yield, Total Phenols and Identified Chemical Constituents in Neotropical Blueberries

Fruits	<i>A. wardii</i>	<i>C. grandifolia</i>	<i>M. coccoboloides</i>	<i>S. buxifolium</i>	<i>S. cordifolium</i>	<i>V. corymbosum</i>
Extract yield (mg/g dry fruit)	818.2	487.6	637.2	594.3	531.3	522.0
Total phenolic contents ^a (mg gallic acid/g dry fruit)	11.59 ± 0.42 A	20.25 ± 0.26 B	3.81 ± 0.17 C	6.97 ± 0.37 D	3.05 ± 0.11 E	12.23 ± 0.35 F
Identified components (μg/g dry fruit) ^b						
Chlorogenic acid	58.42 ± 0.49 A	16572.86 ± 275.64 B	27.49 ± 0.62 C	168.83 ± 9.44 D	66.86 ± 2.71 E	1456.62 ± 19.50 F
<i>p</i> -Coumaric acid	ND ^c	ND	ND	Tr. ^d	ND	ND
Isovitexin	892.59 ± 13.06	ND	ND	ND	ND	ND
Vitexin	576.18 ± 12.99	ND	ND	ND	ND	ND
Isoorientin	135.44 ± 2.00	ND	ND	ND	ND	ND
Orientin	60.49 ± 1.68	ND	ND	ND	ND	ND
Hyperoside	ND	406.83 ± 13.40 A	128.30 ± 0.72 B	174.61 ± 0.49 C	126.39 ± 0.18 D	754.14 ± 9.39 E
Quercetin-3- <i>O</i> -glucoside	ND	282.56 ± 1.97 A	ND	61.00 ± 0.37 B	ND	153.69 ± 3.94 C
Non-identified components (μg/g dry fruit) ^b						
Flavonoids ^e	210.21 ± 1.66 A	1866.35 ± 1.16 B	31.57 ± 0.88 C	223.32 ± 1.90 D	108.37 ± 1.16 E	557.87 ± 13.18 F
Hydroxycinnamic acid derivatives ^f	20.32 ± 0.11 A	1530.20 ± 8.36 B	140.35 ± 0.87 C	1139.83 ± 31.77 D	150.43 ± 1.21 E	54.47 ± 1.77 F
Total	230.52 ± 1.53 A	3396.55 ± 7.31 B	171.92 ± 1.27 C	1363.15 ± 33.50 D	258.80 ± 2.37 E	612.34 ± 12.17 F

^aValues are expressed as means ± 95% confidence intervals ($n = 6$).

^bValues are expressed as means ± 95% confidence intervals ($n = 3$).

^cND, not detected below LOD.

^dTr., Trace below below LOQ.

^eQuantitated using vitexin as a standard.

^fQuantitated using chlorogenic acid as a standard. Values with different upper case letters (A–F) within each row are significantly ($P > 0.05$) different. Analysis of variance was performed by ANOVA procedures, with significant differences between means determined by Tukey's pairwise comparisons.