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Antioxidant and Metabolite Profiling of North American and Neotropical Blueberries Using LC-TOF-MS and Multivariate Analyses

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Supporting Information

ABSTRACT: There are many neotropical blueberries, and recent studies have shown that some have even stronger antioxidant activity than the well-known edible North American blueberries. Antioxidant marker compounds were predicted by applying multivariate statistics to data from LC-TOF-MS analysis and antioxidant assays of 3 North American blueberry species (*Vaccinium corymbosum*, *Vaccinium angustifolium*, and a defined mixture of *Vaccinium virgatum* with *V. corymbosum*) and 12 neotropical blueberry species (*Anthopterus wardii*, *Cavendishia grandifolia*, *Cavendishia isernii*, *Ceratostema silvicola*, *Disterigma rimbachii*, *Macleania coccoloboides*, *Macleania cordifolia*, *Macleania rupestris*, *Satyria boliviana*, *Sphyrospermum buxifolium*, *Sphyrospermum cordifolium*, and *Sphyrospermum ellipticum*). Fourteen antioxidant markers were detected, and 12 of these, including 7 anthocyanins, 3 flavonols, 1 hydroxycinnamic acid, and 1 iridoid glycoside, were identified. This application of multivariate analysis to bioactivity and mass data can be used for identification of pharmacologically active natural products and may help to determine which neotropical blueberry species will be prioritized for agricultural development. Also, the compositional differences between North American and neotropical blueberries were determined by chemometric analysis, and 44 marker compounds including 16 anthocyanins, 15 flavonoids, 7 hydroxycinnamic acid derivatives, 5 triterpene glycosides, and 1 iridoid glycoside were identified.

KEYWORDS: *Ericaceae, blueberry, multivariate analysis, chemometrics, iron chelation, antioxidant markers*

INTRODUCTION

Metabolite fingerprinting has been used as an effective tool in comprehensive metabolite profiling for diverse applications such as disease diagnosis, toxicology, and drug discovery.[1] In the field of plant sciences, metabolite profiling was initially used to assess the relationship between global metabolite pool and specific environmental conditions or the impact of herbicide treatment.[2] Recently, metabolite profiling has been increasingly used in the area of phytomedicine to standardize botanical products or herbal medicines for their quality and safety.[2] However, correlating marker ions and biological activity using metabolite profiling has not been widely employed, especially in studies of edible or medicinal plants.

A great deal of phytochemical and bioactivity research on Ericaceae has focused on temperate species of *Vaccinium* L.,[3−6] which is only 1 of the 32 berry-producing genera within the tribe Vaccinieae of the plant family Ericaceae.[7,8] More than 600 different species of berry-producing Ericaceae are native to the New World tropics (neotropics).[7,8] Berries of several genera of neotropical blueberries are consumed raw or in different types of preparations, including those of several species in the genera here studied (Pedraza-Peñalosa, personal correspondence). Perhaps the best example of a neotropical blueberry as a food source is some *Vaccinium* plant berries that are traditionally harvested for human consumption in several regions of South America. The growing interest in them has spurred a variety of enterprises experimenting with the development and commercialization of food products or the establishment of plantations; in many regions, the trade of these species has already moved from local markets to specialized supermarkets. Despite the potential, it must be also noted that the determination of the taxonomic identity of some species remains difficult, an important factor among dozens of potentially edible species with relatives with uninvestigated reports of toxicity (Pedraza-Peñalosa, personal correspondence). Although the exploration of the uses of neotropical blueberries is an open and promising field, it should be undertaken with caution as the edibility and nutritional value of most of the species, including those in this study, remain poorly undocumented or at early stages. There are very few published accounts on the potential health benefits of neotropical relatives of blueberries.[9] In a previous study we

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have reported that *Anthopterus wardii* and *Cavendishia grandifolia* have stronger antioxidant activities than the North American highbush blueberry, *Vaccinium corymbosum*.[10] The berries from the neotropical species *A. wardii* contain antioxidant constituents of potential therapeutic relevance in chronic obstructive pulmonary disease.[9] Therefore, it is important to analyze the phytochemical constituents present in neotropical species of Ericaceae and also to investigate differences in chemical composition with the well-studied temperate *Vaccinium* species.

The objective of this study was to use chemometric and fragmentation analyses of LC-MS-TOF data for the identification of markers specific to neotropical and temperate blueberry species. Differences in metabolite profiles between different genera and species of neotropical blueberries were also examined. A second objective was to predict marker compounds contributing to the antioxidant activities of the blueberry samples. This is achieved by correlating the marker ion data from chemometric analysis with the results from antioxidant studies using multivariate statistical analysis. The antioxidant properties investigated were 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH$^{\bullet}$) scavenging and iron chelation activities, which are of relevance to the potential health benefits of blueberries, such as the prevention of cancer and chronic obstructive pulmonary disease (COPD).[9,11] Therefore, antioxidant marker constituents detected as a result of this study may have potential health benefits. This information along with the knowledge obtained from the metabolite profiling may help to determine which neotropical species can be prioritized for domestication and agricultural development.

MATERIALS AND METHODS

Reagents. HPLC-MS grade acetonitrile, water (J. T. Baker, Phillipsburg, NJ, USA), and formic acid (Sigma-Aldrich, St. Louis, MO, USA) were used for HPLC-TOF-MS analysis. Guaranteed reagent grade methanol (EMD, Gibbstown, NJ, USA) and deionized water were used for the extraction of the fruit materials. Delphinidin-3-*O*-glucoside (**2**), cyanidin-3-*O*-galactoside (**4**), cyanidin-3-*O*-glucoside (**5**), cyanidin-3-*O*-arabinoside (**6**), petunidin-3-*O*-glucoside (**8**), peonidin-3-*O*-galactoside (**10**), and malvidin-3-*O*-glucoside (**14**) were purchased from WuXi AppTec Inc. (Tianjin, China). Luteolin-8-*C*-glucoside (orientin) (**20**), luteolin-6-*C*-glucoside (isoorientin) (**22,**), apigenin-8-*C*-glucoside (vitexin) (**23**), apigenin-6-*C*-glucoside (isovitexin) (**24**), and chlorogenic acid (**32**) were from Chromadex (Irvine, CA, USA).

Fruit Material. Fruits of *Anthopterus wardii* Ball, *Cavendishia grandifolia* Hoerld, *Cavendishia isernii* Sleumer, *Ceratostema silvicola* A. C. Sm., *Disterigma rimbachii* (A. C. Sm.) Luteyn, *Macleania coccoloboides* A. C. Sm., *Macleania cordifolia* Benth., *Macleania rupestris* (Kunth) A. C. Sm., *Satyria boliviana* Luteyn, *Sphyrospermum buxifolium* Poepp. & Endl., *Sphyrospermum cordifolium* Benth., and *Sphyrospermum ellipticum* Sleumer were collected when fully ripened at The New York Botanical Garden (Bronx, NY, USA), and fruit collection and identification were supervised by Dr. Paola Pedraza-Peñalosa. Fruits of *Vaccinium corymbosum* L. cv. Brigitta were purchased at a local supermarket. Frozen fruits of wild blueberries *Vaccinium angustifolium* Aiton were purchased from Jasper Wyman and Son (Milbridge, ME, USA). The blueberry powder used was a mixture of *Vaccinium virgatum* Aiton/*V. corymbosum* obtained from the U.S. Highbush Blueberry Council (Folsom, CA, USA).[12]

Sample Preparation. The freeze-dried fruits were homogenized using a blender with 70% (v/v) MeOH for 5 min. The ratio of material to solvent was 1:20 (w/v). Extracts were filtered, and the residue was extracted two more times for 5 min each. Extracts were combined and concentrated in vacuo (45 °C), frozen at −20 °C, and subsequently freeze-dried using a Büchi lyophilizer at a vacuum of 45 Torr and a temperature of −60 °C and stored at −20 °C.

Liquid Chromatography. Separation was achieved by HPLC using a Waters 2695 separations module (Milford, MA, USA), equipped with a 2998 photodiode array detector (PDA). The separations were carried out on a 100 × 2.0 mm i.d., 3.0 μm Gemini C18 column (Phenomenex, Torrance, CA, USA). All analyses were performed at 50 °C with a flow rate of 0.2 mL/min. The sample volume injected was 5 μL. Each sample was injected twice. The mobile phase was composed of 1.0% aqueous formic acid (A) and acetonitrile (B) using a stepwise gradient elution of 2% B for 5 min, 2−12% B at 5−20 min, 12% B for 26 min, 12−16% B at 46−65 min, 16−25% B at 65−66 min, 25−72% B at 66−98 min, 72−75% B at 98−99 min, 75−90% B at 99−113 min, 90−100% B at 113−133 min, and this proportion of solvent kept for 10 min. The UV−vis spectra were recorded from 190 to 700 nm.

Mass Spectrometry. High-resolution electrospray ionization mass spectrometry was performed using an LCT premier XE TOF mass spectrometer (Waters, Milford, MA, USA) equipped with an ESI interface and controlled by MassLynx v.4.1 software. Mass spectra were acquired in both the positive and negative modes over the range m/z 100−1000. The capillary voltages were set at 3000 V (positive mode) and 2800 V (negative mode), respectively, and the cone voltage was 20 V. Nitrogen gas was used both for the nebulizer and in desolvation. The desolvation and cone gas flow rates were 300 and 20 L/h, respectively. The desolvation temperature was 400 °C, and the source temperature was 120 °C. For the dynamic range enhancement (DRE) lockmass, a solution of leucine enkephalin (Sigma-Aldrich) was infused by a secondary reference probe at 200 pg/mL in acetonitrile/water (1:1) containing 0.1% formic acid with the help of a model 515 LC pump (Waters). The reference mass was scanned once every five scans for each positive and negative data collection. Both positive and negative ESI data were collected using a scan time of 0.2 s, with an interscan time of 0.01 s, and a polarity switch time of 0.3 s.

DPPH$^{\bullet}$ Scavenging. The DPPH$^{\bullet}$ scavenging activity was assessed according to the method described by Smith et al.[13] with minor modifications. To a 50 μL aliquot of the sample was added 150 μL of DPPH (400 μM), and the absorbance at 515 nm was recorded after 30 min of incubation at 37 °C using a Molecular Devices Versa$_{max}$ microplate reader (Sunnyvale, CA, USA). The percentage inhibition values for different concentrations were calculated using eq 1. A plot of percentage inhibition versus concentration was made for the common reference standard, Trolox. On the basis of this plot the Trolox equivalent antioxidant capacity (TEAC, μmol Trolox/g dried fruit) values for different samples were calculated.

$$\%\ \text{inhibition} = \left[\frac{(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}\right] \times 100 \quad (1)$$

Iron Chelation. The iron chelation activity was assessed using the method of Carter[14] with minor modification. To 20 μL of the sample were added 10 μL of iron(II) chloride tetrahydrate (2 mM) and 90 μL of methanol. The reaction mixture was incubated for 5 min, and thereafter 40 μL of ferrozine (5 mM) was added. After 10 min, the absorbance was measured at 562 nm, using the same Molecular Devices Versa$_{max}$ microplate reader. The percentage chelation was calculated using eq 2. A plot of percentage chelation versus concentration was made for the common reference standard, disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA).

$$\%\ \text{chelation} = \left[\frac{(\text{Abs}_{\text{control}}) - (\text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}\right] \times 100 \quad (2)$$

The results were expressed as the Na_2EDTA equivalent (μmol Na_2EDTA/g dried fruit) values.

Chemometric Data Analysis. The HPLC-TOF-MS data of the 15 samples and their DPPH$^{\bullet}$ scavenging and iron chelation activities were analyzed by principal component analysis (PCA) and multivariate analyses using Markerlynx v.4.1 and JMP v.8. The aim was to identify discriminate variables and discover the correlations between

the marker ions and the different activities. Peak detection and alignment and the filtering of raw HPLC-TOF-MS data were carried out using MarkerLynx v4.1. The parameters used included a retention time range of 4−110 min, a mass range of 100−1000, and a mass tolerance of 0.05. Isotopic peaks were excluded for analysis; noise elimination level was set at 6.00, the intensity threshold (counts) of collection parameters was set at 100; retention time tolerance was set at 0.6 min. The retention time and m/z data pair for each peak was determined by the software. After all of the marker ions were obtained, they were arranged by their significance values in descending order. The first 250 marker ions along with the DPPH$^{\bullet}$ scavenging and iron chelation activity data were selected for further multivariate analyses using JMP v.8 software. The raw relative contents of all the marker ions were determined by the MarkerLynx software and were exported and transferred to the JMP v.8 software using Microsoft Excel.

RESULTS AND DISCUSSION

Metabolic Fingerprinting and Principal Component Analyses. The PCA based on the positive mode data displayed a clear differentiation of the 15 samples (species of *Anthopterus*, *Cavendishia*, *Ceratostema*, *Disterigma*, *Macleania*, *Satyria*, *Sphyrospermum*, and *Vaccinium*) into three clusters, A1, A2, and A3, which belonged to North American *Vaccinium* species, neotropical *Cavendishia* species, and the remainder of the neotropical species, respectively (Figure 1A). The presence of *M. rupestris*, *M. cordifolia*, *A. wardii*, *C. silvicola*, *S. boliviana*, and *D. rimbachii* in the same cluster, A3, showed that they contained similar chemical constituents. However, even though *C. grandifolia* and *C. isernii* are of neotropical origin, they formed a separate cluster, A2, well differentiated from A3, and somewhat closer to A1, which represents *Vaccinium* species (Figure 1A). This result can be explained in terms of high contents of anthocyanins in the two *Cavendishia* species. Anthocyanins can be ionized to positive charged ions under the ESI ionization mode, like the marker ions at m/z 493.1313, 463.1206, and 493.1318 shown in the loading plot (Figure 1B). The three ions were present in large quantities in both *Vaccinium* and *Cavendishia* genera; however, the remainder of the neotropical species had low amounts of them. These ions contributed to the clusters formed in Figure 1A.

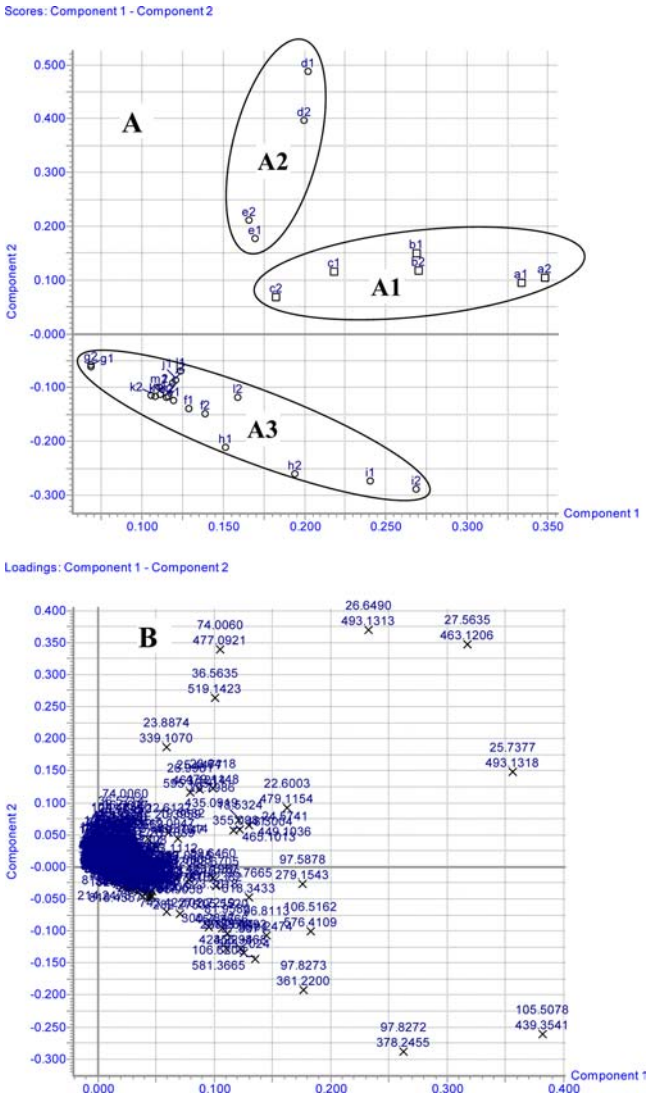

Figure 1. (A) Scores and (B) loading plots of blueberry samples by PCA processing based on the MS data obtained in positive mode.

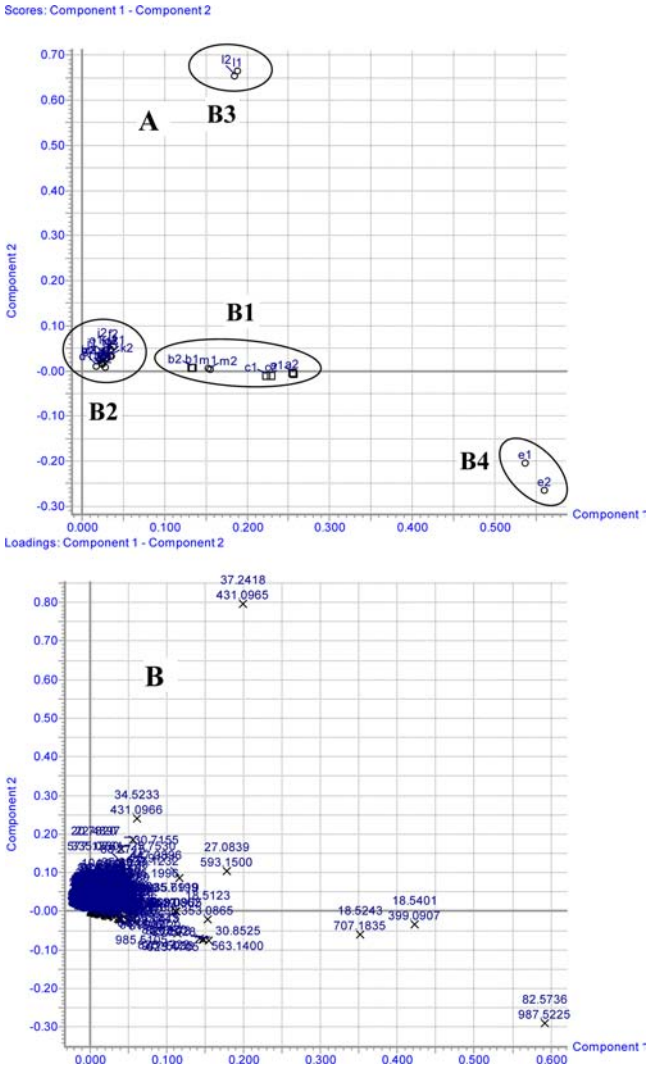

Figure 2. (A) Scores and (B) loading plots of blueberry samples by PCA processing based on the MS data obtained in negative mode.

The PCA based on the negative mode data displayed four clusters, B1, B2, B3, and B4 (Figure 2A). Cluster B1 contained the North American *Vaccinium* and the neotropical *C. silvicola*. B3 and B4 were monospecific containing *A. wardii* and *C. isernii*, respectively; the remaining neotropical species clustered in B2. Comparing the corresponding locations in scores and

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Table 1. Anthocyanins: Tentative Structure Identification of Marker Ions Obtained by PCA

no.	RT (min)	UV−vis	$[M]^+$, $[M + H]^+$ or $[M - H]^-$ (MF,[a] ppm)	adduct and fragment ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	tentative identification	plant sources[b,c]
1	18.1	521, 277	465.1013 $[M]^+$ ($C_{21}H_{21}O_{12}$, −4.3) 463.0900 $[M - 2H]^-$ ($C_{21}H_{19}O_{12}$, −5.0)	303.0504 [M − galactosyl group]$^+$ ($C_{15}H_{11}O_7$, −0.3) 499.0632 $[M - H + Cl]^-$ ($C_{21}H_{20}O_{12}Cl$, −2.2); 509.0923 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{14}$, −1.6)	delphinidin-3-*O*-galactoside	a−c, d, e, i, l
2	19.7	521, 278	465.1046 $[M]^+$ ($C_{21}H_{21}O_{12}$, 2.8) 463.0865 $[M - 2H]^-$ ($C_{21}H_{19}O_{12}$, −2.6)	303.0492 [M − glucosyl group]$^+$ ($C_{15}H_{11}O_7$, −4.3) 499.0620 $[M - H + Cl]^-$ ($C_{21}H_{20}O_{12}Cl$, −4.6); 509.0913 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{14}$, −3.5)	delphinidin-3-*O*-glucoside	b−e, a, f, i, j
3	20.8	520, 276	435.0914 $[M]^+$ ($C_{20}H_{19}O_{11}$, −3.0) 433.0779 $[M - 2H]^-$ ($C_{20}H_{17}O_{11}$, 1.8)	303.0493 [M − arabinosyl group]$^+$ ($C_{15}H_{11}O_7$, −4.0) 469.0555 $[M - H + Cl]^-$ ($C_{20}H_{18}O_{11}Cl$, 3.6); 479.0822 $[M - 2H + HCOOH]^-$ ($C_{21}H_{19}O_{13}$, −0.8)	delphinidin-3-*O*-arabinoside	a−e, i, k, l, n
4	20.4	516, 279	449.1063 $[M]^+$ ($C_{21}H_{21}O_{11}$, −4.7)	287.0561 [M − galactosyl group]$^+$ ($C_{15}H_{11}O_6$, 1.7) 483.0703 $[M - H + Cl]^-$ ($C_{21}H_{20}O_{11}Cl$, 1.9); 493.0977 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{13}$, −1.0)	cyanidin-3-*O*-galactoside	a−c, e, d, i, l, m
5	21.9	516, 279	449.1066 $[M]^+$ ($C_{21}H_{21}O_{11}$, −4.0)	287.0545 [M − glucosyl group]$^+$ ($C_{15}H_{11}O_6$, −3.8) 483.0673 $[M - H + Cl]^-$ ($C_{21}H_{20}O_{11}Cl$, −4.3); 493.0968 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{13}$, −2.8)	cyanidin-3-*O*-glucoside	b-e, a, i, k, m-o
6	22.6	520, 279	419.0971 $[M]^+$ ($C_{20}H_{19}O_{10}$, −1.7)	287.0543 [M − arabinosyl group]$^+$ ($C_{15}H_{11}O_6$, −4.5) 453.0601 $[M - H + Cl]^-$ ($C_{20}H_{18}O_{10}Cl$, 2.9); 463.0891 $[M - 2H + HCOOH]^-$ ($C_{21}H_{19}O_{12}$, 3.0)	cyanidin-3-*O*-arabinoside	a-e, i, m
7	22.6	521, 277	479.1168 $[M]^+$ ($C_{22}H_{23}O_{12}$, −4.6) 477.1042 $[M - 2H]^-$ ($C_{22}H_{21}O_{12}$, 1.9)	317.0645 [M-galactosyl group]$^+$ ($C_{16}H_{13}O_7$, −5.0) 513.0807 $[M - H + Cl]^-$ ($C_{22}H_{22}O_{12}Cl$, 1.4); 523.1069 $[M - 2H + HCOOH]^-$ ($C_{23}H_{23}O_{14}$, −3.6)	petunidin-3-*O*-galactoside	a−c, e, j, d, i, l
8	23.6	523, 277	479.1195 $[M]^+$ ($C_{22}H_{23}O_{12}$, −1.0) 477.1037 $[M - 2H]^-$ ($C_{22}H_{21}O_{12}$, 0.8)	317.0661 [M − glucosyl group]$^+$ ($C_{16}H_{13}O_7$, 0.0) 513.0782 $[M - H + Cl]^-$ ($C_{22}H_{22}O_{12}Cl$, −3.5); 523.1077 $[M - 2H + HCOOH]^-$ ($C_{23}H_{23}O_{14}$, −2.1)	petunidin-3-*O*-glucoside	b−e, a, g, h, i, j, k, n, o
9	24.5	523, 277	449.1078 $[M]^+$ ($C_{21}H_{21}O_{11}$, −1.3) 447.0944 $[M - 2H]^-$ ($C_{21}H_{19}O_{11}$, 3.8)	317.0647 [M − arabinosyl group]$^+$ ($C_{16}H_{13}O_7$, −4.4) 483.0701 $[M - H + Cl]^-$ ($C_{21}H_{20}O_{11}Cl$, 1.4); 493.0977 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{13}$, −1.0)	petunidin-3-*O*-arabinoside	a−e, I
10	24.2	520, 278	463.1247 $[M]^+$ ($C_{22}H_{23}O_{11}$, 1.5)	301.0706 [M − galactosyl group]$^+$ ($C_{16}H_{13}O_6$, −2.0) 497.0826 $[M - H + Cl]^-$ ($C_{22}H_{22}O_{11}Cl$, −5.0); 507.1116 $[M - 2H + HCOOH]^-$ ($C_{23}H_{23}O_{13}$, −4.5)	peonidin-3-*O*-galactoside	a−c, e, d, j, l
11	25.5	520, 279	463.1251 $[M]^+$ ($C_{22}H_{23}O_{11}$, 2.4)	301.0710 [M − glucosyl group]$^+$ ($C_{16}H_{13}O_6$, −0.7) 497.0849 $[M - H + Cl]^-$ ($C_{22}H_{22}O_{11}Cl$, −0.4); 507.1116 $[M - 2H + HCOOH]^-$ ($C_{23}H_{23}O_{13}$, −4.5)	peonidin-3-*O*-glucoside	b−e, a, f, g, i, j, o
12	26.2	520, 278	433.1131 $[M]^+$ ($C_{21}H_{21}O_{10}$, −0.9)	301.0723 [M − arabinosyl group]$^+$ ($C_{16}H_{13}O_6$, 3.7) 467.0752 $[M - H + Cl]^-$ ($C_{21}H_{20}O_{10}Cl$, 1.5); 477.1032 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{12}$, −0.2)	peonidin-3-*O*-arabinoside	a−e, i, j, m
13	25.7	525, 276	493.1341 $[M]^+$ ($C_{23}H_{25}O_{12}$, −1.0) 491.1190 $[M - 2H]^-$ ($C_{23}H_{23}O_{12}$, 0.0)	331.0803 [M − galactosyl group]$^+$ ($C_{17}H_{15}O_7$, −4.5) 527.0953 $[M - H + Cl]^-$ ($C_{23}H_{24}O_{12}Cl$, −0.6); 537.1244 $[M - 2H + HCOOH]^-$ ($C_{24}H_{25}O_{14}$, 0.0)	malvidin-3-*O*-galactoside	a−c, e, j, l, d, f, h, i
14	26.6	527, 277	493.1337 $[M]^+$ ($C_{23}H_{25}O_{12}$, −1.8)	331.0823 [M − glucosyl group]$^+$ ($C_{17}H_{15}O_7$, 1.5)	malvidin-3-*O*-glucoside	b−e, a, f−k, n, o

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Table 1. continued

no.	RT (min)	UV−vis	$[M]^+$, $[M + H]^+$ or $[M - H]^-$ (MF,[a] ppm)	adduct and fragment ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	tentative identification	plant sources[b,c]
			491.1182 $[M - 2H]^-$ ($C_{23}H_{23}O_{12}$, −1.6)	527.0958 $[M - H + Cl]^-$ ($C_{23}H_{24}O_{12}Cl$, 0.4); 537.1236 $[M - 2H + HCOOH]^-$ ($C_{24}H_{25}O_{14}$, −1.5)		
15	27.5	526, 277	463.1218 $[M]^+$ ($C_{22}H_{23}O_{11}$, −4.8)	331.0816 [M − arabinosyl group]$^+$ ($C_{17}H_{15}O_7$, −0.6)	malvidin-3-*O*-arabinoside	a−e, j, f, h, i, l
			461.1082 $[M - 2H]^-$ ($C_{22}H_{21}O_{11}$, −0.4)	497.0857 $[M - H + Cl]^-$ ($C_{22}H_{22}O_{11}Cl$, 1.2); 507.1141 $[M - 2H + HCOOH]^-$ ($C_{23}H_{23}O_{13}$, 0.4)		
16	28.6	526, 279	507.1117 $[M]^+$ ($C_{23}H_{23}O_{13}$, −4.3)	463.1210 [M − acetyl group]$^+$ ($C_{22}H_{23}O_{11}$, −6.5); 303.0493 [M − acetyl group − hexosyl group]$^+$ ($C_{15}H_{11}O_7$, −4.0);	delphinidin-3-acetylglucoside	c, b
			505.0965 $[M - 2H]^-$ ($C_{23}H_{21}O_{13}$, −3.4)	541.0735 $[M - H + Cl]^-$ ($C_{23}H_{22}O_{13}Cl$, −2.6); 551.1010 $[M - 2H + HCOOH]^-$ ($C_{24}H_{23}O_{15}$, −4.9)		

[a]MF, molecular formula. [b]a, *Vaccinium corymbosum*; b, *Vaccinium virgatum* and *corymbosum*; c, *Vaccinium angustifolium*; d, *Cavendishia grandifolia*; e, *Cavendishia isernii*; f, *Sphyrospermum buxifolium*; g, *Sphyrospermum cordifolium*; h, *Sphyrospermum ellipticum*; i, *Macleania coccoloboides*; j, *Macleania rupestris*; k, *Macleania cordifolia*; l, *Anthopterus wardii*; m, *Ceratostema silvicola*; n, *Satyria boliviana*; o, *Disterigma rimbachii*. [c]The underscored marker compounds are "high content" as defined under Results and Discussion.

loading plots (Figure 2), we could find that the marker ions at m/z 431.0965 and 987.5225 corresponding to compounds **24** and **39**, respectively (Tables 2 and 3) were responsible for the separation of *A. wardii* and *C. isernii* from other neotropical species samples. Two other marker ions at m/z 707.1835 and 399.0907 corresponding to compound **32** contributed to clustering of the three *Vaccinium* samples with *C. silvicola* in cluster B1. Compound **32** (chlorogenic acid) could be detected more easily when it was negatively charged under ESI ionization technique. Altogether, 44 molecular marker ions were found to be useful in distinguishing these 15 samples and were selected for further identification.

Mass Fragmentation Analysis of Standards and Marker Compounds. Compounds **2**, **4**, **5**, **6**, **8**, **10**, **14**, **20**, **22**−**24**, and **32** were identified by comparison with the standards of their retention times and mass spectra profiles (Tables 1−3). All other marker compounds were identified by fragmentation cleavage analysis and comparison with previously published data.[3,5]

Analysis of Anthocyanins and Flavonoids. The tentative identifications of anthocyanins and flavonoids were mostly based on their UV−vis absorbance, mass fragmentation analyses, and retention times. Using unique UV−vis absorbance characteristics, compounds **1**−**16** were classified as anthocyanins and compounds **17**−**30** as flavonoids (Tables 1 and 2). Furthermore, the different UV−vis absorbance profiles were helpful in the determination of the aglycone moiety of the flavonoids. For instance, the determinations of myricetin, apigenin, laricitrin, quercetin, kaempherol, rhamnetin (isorhamnetin), and syringetin were mostly based on the comparison of their UV−vis spectra with previously published values (Table 2).

Besides UV−vis absorbance data, MS data were instrumental in the tentative identification of constituents. The aglycone moeity of 16 anthocyanins, including delphinidin, cyanidin, petunidin, peonidin, and malvidin, was determined by its positive fragment ions at m/z 303, 287, 317, 301, and 331, respectively. According to the mass spectra data, these anthocyanins were all monoglycosides. Usually, they are galactoside, glucoside, and arabinoside, most typically attached at the 3-position of the anthocyanins. Similarly, the positive fragment ions at m/z 319, 271, 333, 303, 287, and 317 helped to confirm the identities of myricetin, apigenin, laricitrin, quercetin, kaempferol, and rhamnetin (isorhamnetin) aglycones of flavonoids, which were already determined by the UV−vis data.

In the case of anthocyanins, retention times were vital to determine the types of glycosides. All 16 monoglycosides were eluted on the C-18 column in the order of increasing retention time: galactoside, glucoside, and arabinoside.[15−18] Additionally, acylated derivatives eluted after nonacylated anthocyanins.[19] The acyl groups are most commonly attached at the 6-position of the sugar group. The sugar moieties of the anthocyanins were determined by this elution series rule, and this determination has been reported in the literature previously.[16,17,20,21] For flavonoid monoglycosides, a similar rule exists for reporting their elution sequence: galactoside, glucoside, xyloside, arabinoside, and rhamnoside.[21−24] However, there is an exception to the rule: arabinoside has been reported to elute before xyloside.[17] Because there were exceptions to the elution rule, and also some of the flavonoids were identified as diglycosides, we reported most of the sugars in flavonoid glycosides as hexosides or pentosides instead of giving them precise names (Table 2). Only when the flavonoid was confirmed by comparison with authentic standards (e.g., **20** and **22**−**24**) was the name of the sugar reported.

Analysis of Hydroxycinnamic Acid Derivatives. Caffeic acid and coumaric acid derivatives proved to be useful to distinguish the temperate *Vaccinium* from its neotropical counterparts. The characteristic UV absorbance maxima at around 320−325 and 310−315 nm indicated the presence of caffeoyl and coumaroyl groups in the structures. The same molecular ions at m/z 355.1024 $[M + H]^+$/353.0867 $[M - H]^-$ of compound **31** and at m/z 355.1016 $[M + H]^+$/353.0865 $[M - H]^-$ of compound **32** showed they are isomers. The fragment ions at m/z 191.0554 [M − H − caffeoyl group]$^-$ and 179.0347 $[M - H - \text{quinic acid} + H_2O]^-$ indicated that **31** was an isomer of chlorogenic acid (Table 3). Compound **35** differed from **31** and **32**, in the presence of a coumaroyl group instead of caffeoyl, which could be determined by the molecular and fragment ions at m/z 339.1074 $[M + H]^+$ and 165.0546 $[M + H - \text{quinic acid} + H_2O]^+$ of **35**. The UV absorbance maxima of compounds **33** and **34** also indicated the presence of a coumaroyl group in the structures (Table 3).

Besides a coumaroyl group, the fragment ion at m/z 209.0289 $[M - H - \text{coumaric acid} + H_2O]^-$ of **33** and 209.0296 $[M - H - \text{coumaric acid} + H_2O]^-$ of **34** indicate there might be a sugar moiety, glucaric or galactaric group, in

Table 2. Flavonoids: Tentative Structure Identification of Marker Ions Obtained by PCA

no.	RT (min)	UV−vis	$[M]^+$, $[M + H]^+$, or $[M - H]^-$ (M, ppm)	adduct and fragment ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	tentative identification	plant sources
17	27.1	348, 266	613.1387 $[M + H]^+$ ($C_{26}H_{29}O_{17}$, −2.9) 611.1219 $[M - H]^-$ ($C_{26}H_{27}O_{17}$, −4.4)	481.0992 $[M + H - \text{pentosyl group}]^+$ ($C_{21}H_{21}O_{13}$, 2.1); 319.0437 $[M + H - \text{pentosyl group} - \text{hexosyl group}]^+$ ($C_{15}H_{11}O_8$, −5.3)	myricetin-pentosylhexoside	f, g, k, n, h, i
18	29.3	354, 267	481.0969 $[M + H]^+$ ($C_{21}H_{21}O_{13}$, −2.7) 479.0816 $[M - H]^-$ ($C_{21}H_{19}O_{13}$, −2.1)	319.0450 $[M + H - \text{hexosyl group}]^+$ ($C_{15}H_{11}O_8$, −1.3)	myricetin-hexoside	a−c, e−g, k, n, h, m
19	29.5	332, 273	565.1543 $[M + H]^+$ ($C_{26}H_{29}O_{14}$, −2.5) 563.1411 $[M - H]^-$ ($C_{26}H_{27}O_{14}$, 1.8)	433.1129 $[M + H - \text{pentosyl group}]^+$ ($C_{21}H_{21}O_{10}$, −1.4); 271.0585 $[M + H - \text{pentosyl group} - \text{hexosyl group}]^+$ ($C_{15}H_{11}O_5$, −7.7)	apigenin-pentosylhexoside	e, d, l
20	30.6	341, 272	449.1063 $[M + H]^+$ ($C_{21}H_{21}O_{11}$, −4.0) 447.0913 $[M - H]^-$ ($C_{21}H_{19}O_{11}$, −3.4)		orientin	e, l, f, m
21	30.8	336, 271	565.1549 $[M + H]^+$ ($C_{26}H_{29}O_{14}$, −1.4) 563.1389 $[M - H]^-$ ($C_{26}H_{27}O_{14}$, −2.1)	433.1139 $[M + H - \text{pentosyl group}]^+$ ($C_{21}H_{21}O_{10}$, 0.9)	apigenin-pentosylhexoside	e, d, l
22	31.4	341, 270, 251	449.1067 $[M + H]^+$ ($C_{21}H_{21}O_{11}$, −3.8) 447.0912 $[M - H]^-$ ($C_{21}H_{19}O_{11}$, −3.4)		isoorientin	j
23	34.5	335, 271, 236	433.1124 $[M + H]^+$ ($C_{21}H_{21}O_{10}$, −2.5) 431.0975 $[M - H]^-$ ($C_{21}H_{19}O_{10}$, −0.7)		vitexin	e, j, l, a
24	37.1		433.1141 $[M + H]^+$ ($C_{21}H_{21}O_{10}$, 1.4) 431.0972 $[M - H]^-$ ($C_{21}H_{19}O_{10}$, −1.4)		isovitexin	e, j, l
25	39.3	352, 264	495.1126 $[M + H]^+$ ($C_{22}H_{23}O_{13}$, −2.6) 493.0967 $[M - H]^-$ ($C_{22}H_{21}O_{13}$, −3.0)	333.0595 $[M + H - \text{hexosyl group}]^+$ ($C_{16}H_{13}O_8$, −4.5)	laricitrin-3-*O*-hexoside	a−c, e, f, k, n, d, i, l, m
26	40.2	353, 255	435.0934 $[M + H]^+$ ($C_{20}H_{19}O_{11}$, 1.6) 433.0770 $[M - H]^-$ ($C_{20}H_{17}O_{11}$, −0.2)	303.0505 $[M + H - \text{pentosyl group}]^+$ ($C_{15}H_{11}O_7$, −0.0) 469.0559 $[M + Cl]^-$ ($C_{20}H_{18}O_{11}Cl$, 4.5); 479.0823 $[M - H + HCOOH]^-$ ($C_{21}H_{19}O_{13}$, −0.6); 867.1624 $[2M - H]^-$ ($C_{40}H_{35}O_{22}$, 0.5); 301.0360 $[M - H - \text{pentosyl group}]^+$ ($C_{15}H_9O_7$, 4.0)	quercetin 3-*O*-pentoside	d, m, f, g, i, k, l, n, o
27	44.7	350, 255	435.0923 $[M + H]^+$ ($C_{20}H_{19}O_{11}$, −0.9) 433.0772 $[M - H]^-$ ($C_{20}H_{17}O_{11}$, 0.2)	303.0501 $[M + H - \text{pentosyl group}]^+$ ($C_{15}H_{11}O_7$, −1.3) 469.0539 $[M + Cl]^-$ ($C_{20}H_{18}O_{11}Cl$, 0.2); 479.0805 $[M - H + HCOOH]^-$ ($C_{21}H_{19}O_{13}$, −4.4); 867.1626 $[2M - H]^-$ ($C_{40}H_{35}O_{22}$, 0.7); 301.0360 $[M - H - \text{pentosyl group}]^+$ ($C_{15}H_9O_7$, 4.0)	quercetin 3-*O*-pentoside	d, f, g, i, k, l, n, o
28	48.4	345, 265	595.1638 $[M + H]^+$ ($C_{27}H_{31}O_{15}$, −4.2) 593.1477 $[M - H]^-$ $C_{27}H_{29}O_{15}$, −4.9)	617.1469 $[M + Na]^+$ ($C_{27}H_{30}O_{15}Na$, −2.1); 287.0546 $[M + H - \text{rhamnosyl group} - \text{hexosyl group}]^+$ ($C_{15}H_{11}O_6$, −3.5) 639.1525 $[M - H + HCOOH]^-$ ($C_{28}H_{31}O_{17}$, −5.6); 447.0903 $[M - H - \text{rhamnosyl group}]^-$ ($C_{21}H_{19}O_{11}$, −5.4)	kaempferol-3-*O*-rutinoside	a, c, m, b, h
29	55.0	354, 266, 254	625.1760 $[M + H]^+$ ($C_{28}H_{33}O_{16}$, −1.4)	647.1577 $[M + Na]^+$ ($C_{28}H_{32}O_{16}Na$, −1.7); 479.1185 $[M + H - \text{rhamnosyl group}]^+$ ($C_{22}H_{23}O_{12}$, −1.0); 317.0656 $[M + H - \text{rhamnosyl group} - \text{hexosyl group}]^+$ ($C_{16}H_{13}O_7$, −1.6) 659.1394 $[M + Cl]^-$ ($C_{28}H_{32}O_{16}Cl$, 2.3); 669.1630 $[M - H + HCOOH]^-$ ($C_{29}H_{33}O_{18}$, −5.5); 477.1048 $[M - H - \text{rhamnosyl group}]^-$ ($C_{22}H_{21}O_{12}$, 3.1)	isorhamnetin 3-*O*-rutinoside or rhamnetin 3-*O*-rutinoside	b, c, m, a

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Table 2. continued

no.	RT (min)	UV−vis	$[M]^+$, $[M + H]^+$, or $[M - H]^-$ (M, ppm)	adduct and fragment ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	tentative identification	plant sources
30	57.5	357, 266, 253	509.1282 $[M + H]^+$ ($C_{23}H_{25}O_{13}$, −2.6) 507.1121 $[M - H]^-$ ($C_{23}H_{23}O_{13}$, −3.5)	347.0753 $[M + H -$ hexosyl group$]^+$ ($C_{17}H_{15}O_8$, −4.0)	syringetin-3-*O*-hexoside	a−c, f, g, j, k, n, d, e, i, l, m

the structures. Therefore, compounds **33** and **34** were tentatively identified as coumaroylglucaric acid or coumaroylgalactaric acid, which were previously reported in citrus species.[25,26] Compounds **36** and **37** were tentatively identified as vaccinoside or andromedoside on the basis of their molecular ion at *m/z* 535 $[M - H]^-$ and the fragment ions at *m/z* 357 $[M + H - H_2O -$ hexosyl group$]^+$, 193 $[M - H - H_2O -$ hexosyl group − coumaric acid$]^-$, and 165 $[M + H -$ monotropein + $H_2O]^+$ (Table 3). These two compounds have been reported in certain *Vaccinium* species including *V. myrtillus* (bilberry) and *V. vitis-idaea* (lingonberry).[5,27]

Analysis of Triterpene Glycosides. On the basis of their exact molecular weights compounds **38**−**42** were identified as triterpene glycosides. These compounds had the same aglycone according to their similar mass spectra profiles. The aglycone also contained the carbonyl group, which can be determined by the fragment ion at *m/z* 409 [aglycone + H − 2 × H_2O − CO]$^+$ (Table 3). The aglycone of these five compounds could be tentatively identified as hydroxyursolic acid or hydroxyoleanolic acid on the basis of their occurrences in the blueberry species such as *V. marcocarpon*.[3,27,28] Among them, compounds **38** and **39** and compounds **41** and **42** were two pairs of isomers. These compounds were specific to *Cavendishia* species (Table 3). All relevant data used for marker compound identification are listed in Tables 1−3.

Comparison of Chemical Constituent between Temperate and Tropical Species. The principal component analyses showed that differences in the relative contents of some marker ions could be useful to differentiate neotropical from North American samples. The relative contents of all marker compounds in samples were determined by their peak intensities in the ion extracted chromatograms. A measure of the relative contents of compounds **1** and **2** in different blueberry samples can be obtained by looking at their peak intensities. If the peak intensity of a compound in a sample is higher than $^1/_{10}$ of the highest peak intensity in that sample, then the compound is considered to be present in "high content". If the intensity is lower than $^1/_{10}$ of the highest peak intensity, the compound is said to be present in "low content". The differences in the contents of marker compounds were helpful in distinguishing these species and may also influence their bioactivities.

The studied North American blueberries contained very high contents of anthocyanins, compared to the neotropical species. Anthocyanin **16** was detected only in *V. corymbosum* and *V. virgatum*. Anthocyanin **1** was present in large quantities in the studied North American blueberries, and its content in some of the neotropical relatives was quite low (Table 1). Additionally, most of the anthocyanins were present in both *Vaccinium* and *Cavendishia* genera, some of which, such as compounds **2** and **5**, are known for their antioxidant properties and bioacitivities relevant in the treatment of COPD.[29] This finding might explain the strong DPPH$^\bullet$ scavenging activities of these species. The difference in the profile of anthocyanins can help in distinguishing extracts of different blueberry species. In addition to being present in *Vaccinium* and *Cavendishia* genera, the anthocyanins **7**, **13**, and **15** could also be detected in high amount in berries of neotropical species *M. rupestris* and *A. wardii*. Therefore, these three marker compounds can be useful in differentiating *M. rupestris* from the other two *Macleania* species in the current study.

Flavonoids (**17**, **19**−**24**, **26**, **27**), hydroxycinnamic acids (**31**, **33**−**37**), triterpene glycosides (**38**−**42**), and one iridoid glycoside (**43**) were present in large amounts in some of the neotropical species; however, they could not be detected in the North American species. These are the most significant differences in metabolite profiles between neotropical and North American species. Compounds **27**, **35**, and **38**−**42** could be detected in high amounts only in *Cavendishia* species. Compounds **38**−**42** were all triterpene glycosides, which suggested the triterpene glycosides to be marker compounds for *Cavendishia* species; especially compounds **38**, **41**, and **42** could be found only in *C. isernii*. Similarly, detection of compound **22** in *M. rupestris* only may suggest that this compound could be used as a marker for this species. However, if the findings related to these marker compounds are validated in further studies, they can be used in future identification of the extracts and products containing neotropical and temperate Ericaceae fruits. This information may help to determine which neotropical species will be prioritized for domestication and agricultural development. Another important aspect of this finding is the beneficial effects of flavonoids and hydroxycinnamic acid derivatives. Epidemiological studies have pointed to the beneficial effects of dietary intake of flavonoids in the prevention of diseases such as asthma and COPD.[30] Hydroxycinnamic acid derivatives have also been reported for a wide range of biological and pharmacological activities. Therefore, the presence or absence of these compounds may have a significant effect on the potential health benefits of the blueberry species under study.

DPPH$^\bullet$ Scavenging. Free radicals play a significant role in the progression of oxidative stress, and thus scavenging these species is an important mechanism of antioxidant action.[31] As oxidative stress contributes to the pathogenesis of many diseases such as cardiovascular diseases, neurodegeneration, and COPD, its prevention would be of great health benefit.[32,33] However, there are limitations associated with the assay such as steric hindrance, the influence of pH, and the polarity of the test samples. Therefore, it is possible that some of the antioxidants present in the berry extracts will not be measured precisely by this assay only.

In a previous study carried out by our group, berries of six Ericaceae species, *A. wardii*, *C. grandifolia*, *M. coccoloboides*, *S. buxifolium*, *S. cordifolium*, and *V. corymbosum*, were screened for DPPH$^\bullet$ scavenging activity.[10] In the current study we assessed berries from nine additional Ericaceae species for their DPPH$^\bullet$ scavenging properties. The scavenging data from the previous study were used for comparing the activities of different fruit

Table 3. Other Compounds: Tentative Structure Identification of Marker Ions Obtained by PCA

no.	RT (min)	UV−vis	$[M]^+$, $[M + H]^+$, or $[M - H]^-$ (MF, ppm)	adduct and fragment ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	tentative identification	plant sources
31	8.9	324, 245, 234	355.1024 $[M + H]^+$ ($C_{16}H_{19}O_9$, −1.4)		caffeoylquinic acid (chlorogenic acid isomer)	d, f, k, n, b, e, g, h, i, l, m, o
			353.0867 $[M - H]^-$ ($C_{16}H_{17}O_9$, −1.7)	707.1842 $[2M - H]^-$ ($C_{32}H_{35}O_{18}$, 2.7); 191.0554 $[M - H -$ caffeoyl group$]^-$ ($C_7H_{11}O_6$, −1.0); 179.0347 $[M - H -$ quinic acid + $H_2O]^-$ ($C_9H_7O_4$, 1.7)		
32	18.5	325, 236	355.1016 $[M + H]^+$ ($C_{16}H_{19}O_9$, −3.7)		chlorogenic acid	a−c, e, m, d, f, g, h, i, k, l, n, o
			353.0865 $[M - H]^-$ ($C_{16}H_{17}O_9$, −2.3)	707.1850 $[2M - H]^-$ ($C_{32}H_{35}O_{18}$, 3.8); 389.0642 $[M + Cl]^-$ ($C_{16}H_{18}O_9Cl$, 0.8); 399.0922 $[M - H + HCOOH]^-$ ($C_{17}H_{19}O_{11}$, −1.3); 191.0559 $[M - H -$ caffeoyl group$]^-$ ($C_7H_{11}O_6$, 1.6)		
33	9.3	312, 233		379.0612 $[M + Na]^+$ ($C_{15}H_{16}O_{10}Na$, −7.7); 165.0560 $[M + H -$ glucaric or galactaric acid + $H_2O]^+$ ($C_9H_9O_3$, 4.8)	coumaroylglucaric acid or coumaroyl galactaric acid	f, k, m, n, g, h, i, l, o
			355.0644 $[M - H]^-$ ($C_{15}H_{15}O_{10}$, −5.9)	209.0289 $[M - H -$ coumaric acid + $H_2O]^-$ ($C_6H_9O_8$, −3.8)		
34	12.5	314, 233	357.0798 $[M + H]^+$ ($C_{15}H_{17}O_{10}$, −6.7)	165.0545 $[M + H -$ glucaric or galactaric acid + $H_2O]^+$ ($C_9H_9O_3$, −4.2)	coumaroylglucaric acid or coumaroyl galactaric acid	f, k, m, n, c, g, h, i, l
			355.0654 $[M - H]^-$ ($C_{15}H_{15}O_{10}$, −3.1)	209.0296 $[M - H -$ coumaric acid + $H_2O]^-$ ($C_6H_9O_8$, −0.5)		
35	23.9	311, 234	339.1074 $[M + H]^+$ ($C_{16}H_{19}O_8$, −1.8)	165.0546 $[M + H -$ quinic acid + $H_2O]^+$ ($C_9H_9O_3$, −3.6)	3- or 5-*O*-coumaroylquinic acid	d, a−c, e−o
			337.0924 $[M - H]^-$ ($C_{16}H_{17}O_8$, 0.3)	383.0989 $[M - H + HCOOH]^-$ ($C_{17}H_{19}O_{10}$, 2.9); 675.1942 $[2M - H]^-$ ($C_{32}H_{35}O_{16}$, 2.5)		
36	33.5	308, 250		559.1434 $[M + Na]^+$ ($C_{25}H_{28}O_{13}Na$, 1.1); 519.1446 $[M + H - H_2O]^+$ ($C_{25}H_{27}O_{12}$, −11.0); 357.0953 $[M + H - H_2O -$ hexosyl group$]^+$ ($C_{19}H_{17}O_7$, −5.9); 193.0499 $[M - H - H_2O -$ hexosyl group − coumaric acid$]^-$ ($C_{10}H_9O_4$, −1.0); 165.0544 $[M + H -$ monotropein + $H_2O]^+$ ($C_9H_9O_3$, −4.8)	vaccinoside or andromedoside	d, f, i, j
			535.1474 $[M - H]^-$ ($C_{25}H_{27}O_{13}$, 4.1)	571.1215 $[M + Cl]^-$ ($C_{25}H_{28}O_{13}Cl$, −0.5); 581.1520 $[M - H + HCOOH]^-$ ($C_{26}H_{29}O_{15}$, 2.4)		
37	36.6	312, 247		559.1404 $[M + Na]^+$ ($C_{25}H_{28}O_{13}Na$, −4.3); 519.1509 $[M + H - H_2O]^+$ ($C_{25}H_{27}O_{12}$, 1.2); 357.0955 $[M + H - H_2O -$ hexosyl group$]^+$ ($C_{19}H_{17}O_7$, −5.3); 193.0501 $[M - H - H_2O -$ hexosyl group − coumaric acid$]^-$ ($C_{10}H_9O_4$, 0.0); 165.0549 $[M + H -$ monotropein + $H_2O]^+$ ($C_9H_9O_3$, −1.8)	andromedoside or vaccinoside	d, f, i, j, k, n
			535.1450 $[M - H]^-$ ($C_{25}H_{27}O_{13}$, −0.4)	571.1230 $[M + Cl]^-$ ($C_{25}H_{28}O_{13}Cl$, 2.1); 581.1487 $[M - H + HCOOH]^-$ ($C_{26}H_{29}O_{15}$, −3.3)		
38	81.7		943.5297 $[M + H]^+$ ($C_{48}H_{79}O_{18}$, 3.3)	965.5117 $[M + Na]^+$ ($C_{48}H_{78}O_{18}Na$, 3.2); 781.4758 $[M + H -$ hexosyl group$]^+$ ($C_{42}H_{69}O_{13}$, 2.6); 619.4251 $[M + H - 2 \times$ hexosyl group$]^+$ ($C_{36}H_{59}O_8$, 6.6); 473.3651 $[M + H - 2 \times$ hexosyl group − rhamnosyl group$]^+$ ($C_{30}H_{49}O_4$, 4.2); 455.3525 $[M + H - 2 \times$ hexosyl group − rhamnosyl group − $H_2O]^+$ ($C_{30}H_{47}O_3$, −0.4); 437.3429 $[M + H - 2 \times$ hexosyl group − rhamnosylrhamnosyl group − $2 \times H_2O]^+$ ($C_{30}H_{45}O_2$, 2.1); 409.3485 $[M + H - 2 \times$ hexosyl group − rhamnosyl group $- 2 \times H_2O - CO]^+$ ($C_{29}H_{45}O$, 2.1)	hydroxyursolic acid, hydroxyoleanolic acid, hexosylhexosyl-rhamnoside	e
				977.4931 $[M + Cl]^-$ ($C_{48}H_{78}O_{18}Cl$, 5.5); 987.5186 $[M - H + HCOOH]^-$ ($C_{49}H_{79}O_{20}$, 2.1)		
39	82.5		943.5272 $[M + H]^+$ ($C_{48}H_{79}O_{18}$, 0.6)	965.5063 $[M + Na]^+$ ($C_{48}H_{78}O_{18}Na$, −2.4); 781.4764 $[M + H -$ hexosyl group$]^+$ ($C_{42}H_{69}O_{13}$, 3.3); 619.4272 $[M + H - 2 \times$ hexosyl group$]^+$ ($C_{36}H_{59}O_8$, 10.0); 473.3642 $[M + H - 2 \times$ hexosyl group − rhamnosyl group$]^+$ ($C_{30}H_{49}O_4$, 2.3); 455.3511 $[M + H - 2 \times$ hexosyl group − rhamnosyl group − $H_2O]^+$ ($C_{30}H_{47}O_3$, −3.1); 437.3404 $[M + H - 2 \times$ hexosyl group − rhamnosyl group-$2 \times H_2O]^+$ ($C_{30}H_{45}O_2$, −3.7); 409.3496 $[M + H - 2 \times$ hexosyl group − rhamnosyl group − $2 \times H_2O - CO]^+$ ($C_{29}H_{45}O$, 6.4); 393.3551 $[M + H - 2 \times$ hexosyl group − rhamnosyl group − $2 \times H_2O - COO]^+$ ($C_{29}H_{45}$, 7.6)	hydroxyursolic acid, hydroxyoleanolic acid, hexosylhexosyl-rhamnoside	e, d
				977.4917 $[M + Cl]^-$ ($C_{48}H_{78}O_{18}Cl$, 4.1); 987.5212 $[M - H + HCOOH]^-$ ($C_{49}H_{79}O_{20}$, 4.8)		
40	83.6		797.4744 $[M + H]^+$ ($C_{42}H_{69}O_{14}$, 7.1)	819.4490 $[M + Na]^+$ ($C_{42}H_{68}O_{14}Na$, −2.1); 814.4943 $[M + NH_4]^+$ ($C_{42}H_{72}NO_{14}$, −1.2); 635.4008 $[M + H -$ hexosyl group$]^+$ ($C_{36}H_{59}O_9$, −11.2); 473.3632 $[M + H - 2 \times$ hexosyl group$]^+$ ($C_{30}H_{49}O_4$, 0.2); 455.3523 $[M + H - 2 \times$ hexosyl group − $H_2O]^+$ ($C_{30}H_{47}O_3$, −0.4); 437.3409 $[M + H - 2 \times$ hexosyl group $- 2 \times H_2O]^+$ ($C_{30}H_{45}O_2$, −2.5); 409.3497 $[M + H - 2 \times$ hexosyl group $- 2 \times H_2O - CO]^+$ ($C_{29}H_{45}O$, 6.6)	hydroxyursolic acid, hydroxyoleanolic acid, hexosylhexoside	d, e
				831.4337 $[M + Cl]^-$ ($C_{42}H_{68}O_{14}Cl$, 4.7); 841.4631 $[M - H + HCOOH]^-$ ($C_{43}H_{69}O_{16}$, 5.3)		

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Table 3. continued

no.	RT (min)	UV−vis	$[M]^+$, $[M + H]^+$, or $[M - H]^-$ (MF, ppm)	adduct and fragment ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	tentative identification	plant sources
41	85.3		781.4750 $[M + H]^+$ ($C_{42}H_{69}O_{13}$, 1.5)	619.4233 $[M + H - \text{hexosyl group}]^+$ ($C_{36}H_{59}O_8$, 3.7); 473.3612 $[M + H - \text{hexosyl group} - \text{rhamnosyl group}]^+$ ($C_{30}H_{49}O_4$, −4.0); 455.3480 $[M + H - \text{hexosyl group} - \text{rhamnosyl group} - H_2O]^+$ ($C_{30}H_{47}O_3$, −9.0); 437.3412 $[M + H - \text{hexosyl group} - \text{rhamnosyl group} - 2 \times H_2O]^+$ ($C_{30}H_{45}O_2$, −1.8); 409.3477 $[M + H - \text{hexosyl group} - \text{rhamnosyl group} - 2 \times H_2O - CO]^+$ ($C_{29}H_{45}O$, 1.7)	hydroxyursolic acid, hydroxyoleanolic acid, hexosylrhamnoside	e
				815.4343 $[M + Cl]^-$ ($C_{42}H_{68}O_{13}Cl$, −0.6); 825.4636 $[M - H + HCOOH]^-$ ($C_{43}H_{69}O_{15}$, 0.0)		
42	85.8		781.4742 $[M + H]^+$ ($C_{42}H_{69}O_{13}$, 0.5)	619.4230 $[M + H - \text{hexosyl group}]^+$ ($C_{36}H_{59}O_8$, 3.2); 473.3646 $[M + H - \text{hexosyl group} - \text{rhamnosyl group}]^+$ ($C_{30}H_{49}O_4$, 3.2); 437.3420 $[M + H - \text{hexosyl group} - \text{rhamnosyl group} - 2 \times H_2O]^+$ ($C_{30}H_{45}O_2$, 0.0); 409.3481 $[M + H - \text{hexosyl group} - \text{rhamnosyl group} - 2 \times H_2O - CO]^+$ ($C_{29}H_{45}O$, 2.7)	hydroxyursolic acid, hydroxyoleanolic acid, hexosylrhamnoside	e
				815.4310 $[M + Cl]^-$ ($C_{42}H_{68}O_{13}Cl$, −4.7); 825.4625 $[M - H + HCOOH]^-$ ($C_{43}H_{69}O_{15}$, −1.3)		
43	7.7	242		427.1195 $[M + Na]^+$ ($C_{17}H_{24}O_{11}Na$, −4.9); 422.1640 $[M + NH_4]^+$ ($C_{17}H_{28}NO_{11}$, −5.2); 387.1274 $[M + H - H_2O]^+$ ($C_{17}H_{23}O_{10}$, −4.4); 369.1165 $[M - H - 2 \times H_2O]^-$ ($C_{17}H_{21}O_9$, −5.7)	gardenoside	e, h, i, k, n
			403.1223 $[M - H]^-$ ($C_{17}H_{23}O_{11}$, −4.2)	439.0988 $[M + Cl]^-$ ($C_{17}H_{24}O_{11}Cl$, −4.3); 449.1268 $[M - H + HCOOH]^-$ ($C_{18}H_{25}O_{13}$, −6.0); 241.0697 $[M - H - \text{glucosyl group}]^-$ ($C_{11}H_{13}O_{16}$, −6.2)		
44	14.77	279	291.0856 $[M + H]^+$ ($C_{15}H_{15}O_6$, −4.5)	165.0546 $[M + H - \text{quinic acid} + H_2O]^+$ ($C_9H_9O_3$, −3.6)	catechin	a−e, m, f, g, i, n, o
			289.0721 $[M - H]^-$ ($C_{15}H_{13}O_6$, 3.1)	325.0475 $[M + Cl]^-$ ($C_{15}H_{13}O_6$, −1.2); 335.0764 $[M - H + HCOOH]^-$ ($C_{16}H_{15}O_8$, 0.9)		

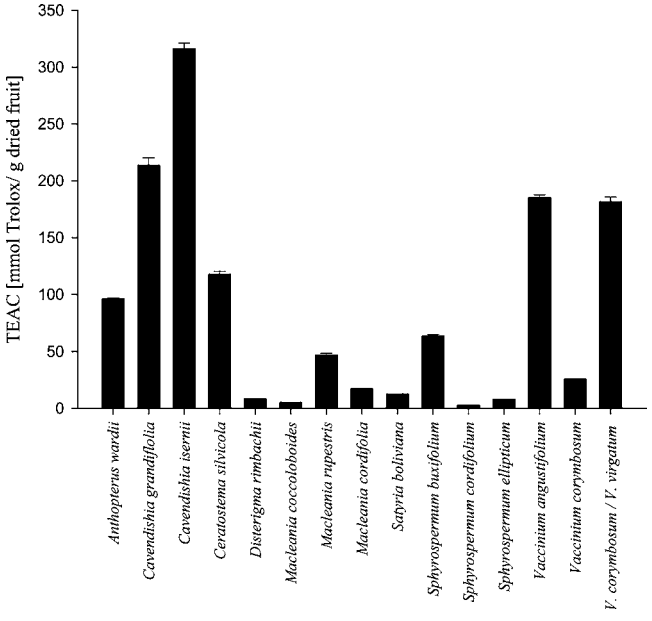

Figure 3. DPPH• scavenging activity of blueberry samples.

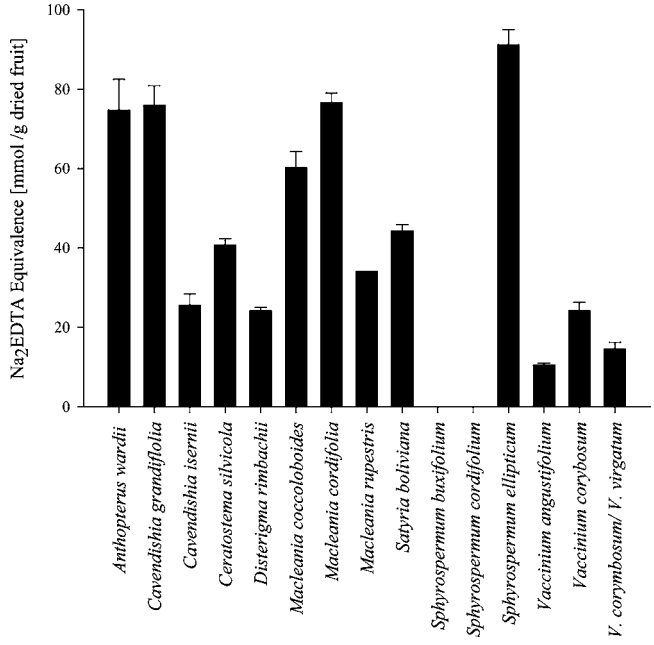

Figure 4. Iron chelation activity of blueberry samples.

samples. The activity of *C. isernii* (315.94 ± 5.35 μmol Trolox/g dried fruit) was far greater than that of any other fruit sample (Figure 3). This was followed by *C. grandifolia* (213.43 ± 6.87 μmol Trolox/g dried fruit), which is the most active fruit sample from the previous study.[10] Therefore, the two *Cavendishia* species showed the strongest DPPH• scavenging properties among the blueberry fruit samples. Six neotropical species and two temperate *Vaccinium* demonstrated significantly higher scavenging activities than the common highbush blueberry *V. corymbosum* ($P < 0.05$). One of the two *Vaccinium* samples, the wild blueberry, *V. angustifolium* (185.09 ± 2.67 μmol Trolox/g dried fruit), was far more active than highbush blueberry, *V. corymbosum* (25.58 ± 0.39 μmol Trolox/g dried fruit).

Iron Chelation. This assay is based on the ability of the samples to chelate transition metal ions, especially iron and copper, thus entrapping metals and preventing them from participating in free radical generation process.[34] This mechanism of antioxidant action is of value in the treatment of diseases mediated by oxidative stress such as cancer, neurodegenerative disorders, diabetes, and COPD.[32,33] Berries of six Ericaceae species, *A. wardii*, *C. grandifolia*, *M. coccoloboides*, *S. buxifolium*, *S. cordifolium*, and *V. corymbosum*, were screened for iron chelation activity in a previous study carried out by our group.[10] In the current study we examined

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Table 4. Correlation of Antioxidant Activities with Marker Ions

peak	RT (min)	exact mass	correlation with activities	tentative identification
		DPPH Scavenging Activities		
1	21.8902	449.1052p	0.6993	cyanidin-3-*O*-glucoside (**5**)
2	22.6137	419.0947p	0.7357	cyanidin-3-*O*-arabinoside (**6**)
3	23.6418	479.1148p	0.7155	petunidin-3-*O*-glucoside (**8**)
4	25.4477	463.1214p	0.9044	peonidin-3-*O*-glucoside (**11**)
5	26.1889	433.1112p	0.8397	peonidin-3-*O*-arabinoside (**12**)
6	26.6490	493.3541p	0.8042	malvidin-3-*O*-glucoside (**14**)
7	27.5636	463.1206p	0.6272	malvidin-3-*O*-arabinoside (**15**)
8	29.534	563.1404n	0.6623	apigenin-pentosyl-hexoside (**19**)
9	30.8526	563.1400n	0.6653	apigenin-pentosyl -hexoside (**21**)
10	18.5402	399.0907n	0.4354	chlorogenic acid (**32**)
11	7.7187	449.1273n	0.6621	gardenoside (**43**)
12	14.6727	335.0714	0.5000	catechin (**44**)
		Iron Chelation		
13	95.3264	279.2244p	0.5797	unidentified
14	92.9523	277.2095p	0.6244	unidentified

berries from nine additional Ericaceae species for their iron chelation properties. The chelating data from the previous study were used for comparing the activities of different fruit samples. All of the Ericaceae samples with the exceptions of *S. buxifolium* and *S. cordifolium* showed iron chelation activity. *S. ellipticum* (91.22 ± 3.82 μmol Na_2EDTA/g dried fruit) had the highest chelation activity (Figure 4), whereas the berries of two other *Sphyrospermum* species, *S. buxifolium* and *S. cordifolium*, did not show any chelation activity. The chelation activity of *S. ellipticum* was followed by those of *A. wardii* (74.74 ± 7.77 μmol Na_2EDTA/g dried fruit) and *C. grandifolia* (75.91 ± 4.95 μmol Na_2EDTA/g dried fruit), the most active samples from the previous study,[10] and *M. cordifolia* (76.54 ± 2.54 μmol Na_2EDTA/g dried fruit) (Figure 4). The berries of *A. wardii* have been reported to contain antioxidant constituents showing efficacy in a cell culture model.[9]

Multivariate Analyses of Marker Compounds Responsible for Antioxidant Activities. In the multivariate analysis, the entire mass data along with the antioxidant screening results were included as variables. The activities included DPPH• scavenging and iron chelation. Both the positive and negative mass data were used for the multivariate analysis. The correlations among activity and mass data variables would indicate contribution of ions to different activities. Once the ions responsible for the activity were detected, they were identified either by fragmentation cleavage analysis or comparison with the authentic standards. On the basis of the multivariate analyses, 12 molecular ions were found to have high correlation with DPPH• scavenging activity. The correlation coefficients of 11 compounds were above 0.6000. Only that of catechin (**44**) was 0.5000. The tentative identification showed that they were seven anthocyanins (cyanidin-3-*O*-glucoside (**5**), cyanidin-3-*O*-arabinoside (**6**), petunidin-3-*O*-glucoside (**8**), peonidin-3-*O*-glucoside (**11**), peonidin-3-*O*-arabinoside (**12**), malvidin-3-*O*-glucoside (**14**), and malvidin-3-*O*-arabinoside (**15**)), three flavonoids (**19**, **21**, and **44**), one hydroxycinnamic acid derivative, chlorogenic acid (**32**), and one iridoid glycoside (**43**). Among them, compounds **11** and **12** had the highest coefficient of correlations, 0.9044 and 0.8397, respectively (Table 4). Anthocyanins, including cyanidin, petunidin, peonidin, and malvidin glycosides, are well-known for their DPPH• scavenging properties. The berries from *Vaccinium* and *Cavendishia* contained high quantities of all seven active anthocyanins, which could explain why they had the highest DPPH• scavenging capacities. Three flavonoids, apigenin pentosylhexosides (**19**, **21**), and catechin (**44**), were also indicated to be responsible for the DPPH• scavenging activity by the PCA. This finding was in agreement with previous reports on apigenin derivatives[35] and catechin.[36]

A hydroxycinnamic acid derivative, chlorogenic acid (**32**), is a well-known antioxidant detected by the multivariate analysis. Gardenoside (**43**) had a correlation coefficient of 0.6621. Although there has been no report of its DPPH• scavenging activity, the compound has been reported to possess antioxidant activity.[37]

Iron chelation data were also included in the multivariate analysis, and only two ions at *m*/*z* 279.2244 (RT 95.3 min) and 277.2095 (RT 92.9 min) were found to have a high correlation with iron chelation activity; their correlation coefficients were 0.5797 and 0.6244, respectively (Table 4). The elucidation of the structures of these two ions is still ongoing.

In this study, we were able to successfully predict antioxidant marker compounds present in the different samples of Ericaceae berries by a novel application of multivariate statistics to data obtained from LC-TOF-MS analysis and antioxidant studies. Altogether, 14 markers were predicted to contribute to the DPPH• scavenging and iron chelation activities, of which 12 were identified. The identified compounds have been reported previously for their antioxidant activities. This information will help in the prioritizing of neotropical species for cultivation on the basis of their bioactive constituents. Another objective of the current study was to investigate differences in chemical composition between North American and neotropical blueberries by applying chemometric analysis to LC-TOF-MS data. A total of 44 marker compounds, including 16 anthocyanins, 15 flavonoids, 7 hydroxycinnamic acid derivatives, 5 triterpene glycosides, and 1 iridoid glycoside, were identified. Discovery and identification of these marker compounds proved to be helpful in explaining the chemical differences between North American and neotropical blueberry species. Some of the markers were also used to investigate differences in metabolite profiles between different genera and species of neotropical blueberries. On the basis of the economic importance of the phytochemicals and their potential health benefits, this information may help to determine which neotropical species will be prioritized for domestication and agricultural development.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures S1A and S1B, total ion current (TIC) chromatogram for the reversed-phase LC-ESI-TOF in positive mode and negative mode, respectively; Figures S2A−S2Z, extracted ion current chromatograms of the 44 marker compounds; Figure S3, EIC chromatograms of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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