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Metabolite Profiling of Jaboticaba (*Myrciaria cauliflora*) and Other Dark-Colored Fruit Juices

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Supporting Information

ABSTRACT: Many dark-colored fruit juices, rich in anthocyanins, are thought to be important for human health. Joboticaba (*Myrciaria cauliflora*) fruits, native to Brazil, have phenolics including anthocyanins and are processed into juice and other products. The phenolic constituents in the fruits of jaboticaba were studied by high-performance liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry. Twenty-two compounds were identified or tentatively determined by detailed analysis of their mass spectral fragmentation patterns; 11 compounds including 7 gallotannins, 2 ellagic acid derivatives, syringin, and its glucoside were detected for the first time in the fruit. The compositional differences among the fruit extracts and their commercial products were also compared by principal component analysis; two anthocyanins, delphinidin 3-*O*-glucoside and cyanidin-3-*O*-glucoside, as well as two depsides, jaboticabin and 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid, present in the fruit extracts were not detected unexpectedly in commercial jaboticaba juice or jam. Therefore, the stability of anthocyanins in jaboticaba fresh fruits and products has been compared directly with that of other dark-colored fruit products made from blueberry and Concord grape, and the same trend of decreasing amounts of anthocyanins was observed in all tested products. The antioxidant activities (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) of jaboticaba fresh fruit extract and commercial samples were also compared. Principal component analysis proved to be a useful way to discern changes between fresh and processed fruits. Jaboticaba is a promising fruit with antioxidant capacity similar to those of other so-called superfruits; however, during processing the levels of some of anthocyanins and other polyphenols decrease significantly, and therefore the capacity of these products to affect human health may vary significantly from that of the fresh fruit.

KEYWORDS: *jaboticaba, Myrciaria cauliflora, LC-TOF-MS, phenolic, anthocyanins, PCA, blueberry, Concord grape*

INTRODUCTION

The jaboticaba tree (*Myrciaria cauliflora* (Mart.) O. Berg.) belonging to the family Myrtaceae, known as the "Brazilian grape tree", is native to Brazil.[1] Jaboticaba fruits grow rapidly within 40−46 days directly from the branches, and when mature, they are round, about 2 cm in diameter, with the pericarp color ranging from red purple to black. Their skin and pulp have a sweet pleasant taste and low acidity. Similar to blueberries, grapes, and other dark-colored fruits, jaboticaba is rich in phenolic constituents, including anthocyanins, flavonoids, and ellagitannins. These compounds possess well-described biological properties including strong antioxidant and anti-inflammatory activities, and some of these are of interest to us for their potential to treat chronic obstructive pulmonary disease (COPD).[2,3] Jaboticaba fruits are consumed in the forms of juices, jams, wines, and liqueurs. Products from dark-colored fruits are very popular in many cultures, and numerous in vitro, in vivo, and clinical studies demonstrate that they are good for human health.[4]

The chemical composition of jaboticaba species has undergone some phytochemical investigation. In our earlier study, a new depside, jaboticabin, and 17 known phenolic compounds were detected and identified from this fruit.[2] Jaboticabin displayed free radical scavenging activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) assay, as well as the ability to reduce IL-8 production in human small airway epithelial (SAE) cells before and after treatment with cigarette smoke extract (CSE), suggesting an important anti-inflammatory action of this compound.[2]

COPD is a complex lung disease characterized by irreversible airflow obstruction due to chronic inflammation. COPD includes chronic obstructive bronchiolitis (fibrosis and obstruction of small airways) and emphysema (permanent enlargement of the airspaces that occurs in the terminal bronchioles accompanied by destruction of lung parenchyma).

In our earlier study, it was demonstrated that jaboticaba depsides and anthocyanins can reduce inflammation caused by exposure to cigarette smoke.[2] Thus, there may be a novel therapeutic role for these compounds in the treatment of COPD.[5] Jaboticaba fruits, rich in certain anthocyanins, phenolic acids, and flavonoids, have high antioxidant activity and may be considered a new "superfruit". Jaboticaba's anti-inflammatory activity also makes it a promising emerging functional food for smokers trying to lessen the impact of lung damage due to cigarette smoke exposure.[2]

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To further characterize the phytochemical constituents from jaboticaba, we have developed a LC-MS-TOF method for the identification of polyphenols from the fruit extracts. Currently there is growing scientific interest in the human health effects of the consumption of dark-colored fruit products.[6−9] The processing of dark-colored fruit into various food products involves variation in a number of parameters, such as temperature, pH, and pressure. Such changes are known to degrade or decompose unstable chemical constituents.[7,8,10] For example, anthocyanin content in grape wine was shown to decrease significantly during storage, although the flavonols, hydroxycinnamic derivatives, and stilbenes did not change much.[7] Another study showed that degradation of anthocyanins was accelerated significantly in blueberries after thermal and high-pressure treatment.[8] The stability of anthocyanins in jaboticaba commercial products has not been studied previously. Therefore, principal component analysis (PCA) was used to determine the compositional differences among fresh and commercial jaboticaba samples. As a point of comparison, anthocyanin stability in blueberry and grape commercial products was examined. The major phenolic compounds were quantified and compared from jaboticaba, blueberry, and Concord grape. The antioxidant capacity of jaboticaba fresh fruit extract and its commercial samples was tested using the DPPH$^{\bullet}$ and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS$^{\bullet+}$) assays.

MATERIALS AND METHODS

Reagents. 1,1-Diphenyl-2-picrylhydrazyl, gallic acid, and ellagic acid were purchased from Sigma Chemical-Aldrich (St. Louis, MO, USA). Isoquercitrin, quercimeritrin, quercitrin, and quercetin were from Chromadex (Irvine, CA, USA). Delphinidin 3-*O*-glucoside and cyanidin-3-*O*-glucoside were from WUXI APPTET (Tianjin) Co., Ltd. (Tianjin, China). Jaboticabin was isolated and determined as a new compound in our earlier study. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt was from TCI-Ace (Tokyo, Japan). HPLC grade MeCN and formic acid were purchased from J. T. Baker (Philipsburg, NJ, USA), and GR grade MeOH was 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 *M. cauliflora* were collected from the Fruit and Spice Park in Homestead, FL, USA, in July 2011. The blueberry fruits, *Vaccinium corymbosum*, cultivar Rubel, were collected from the Philip E. Marucci Center for Blueberry and Cranberry Research, New Jersey, in 2010. The fresh Concord grape fruits, *Vitis labrusca*, were brought from a local food store. The following were purchased from commercial companies or local stores: jaboticaba juice, jam, and wine; highbush blueberry juice and wine; and Concord grape juice, jam, jelly, and wine.

Preparation of Fruit Extracts. *Extract A:* The freeze-dried jaboticaba fruits (8.8 g) were homogenized with 70% (v/v) MeOH using a blender followed by ultrasonic extraction for 30 min. Extracts were filtered, and the marc was extracted two more times. Extracts were combined and concentrated in vacuo (45 °C), freeze-dried, and stored at 4 °C. *Extract B:* The original jaboticaba fruits (10.2 g) were macerated in 100% MeOH for 1 week in the dark, and the extracts were filtered, freeze-dried, and stored at 4 °C. The freeze-dried highbush blueberry fruits (10.0 g) and fresh Concord grapes (10.6 g) were each homogenized with 70% (v/v) MeOH using a blender. The ratio of fruit material to solvent was 1:20 (w/v). Extracts were filtered, and the marc was extracted two more times. Extracts were combined and concentrated in vacuo (45 °C), freeze-dried, and stored at 4 °C.[11] The commercial juices and wines were filtered using 25 mm syringe filter (0.45 μm PTFE membrane) before HPLC injection. Jams and jellies were extracted two times by 100% MeOH, and extracts were filtered and combined, dried under nitrogen, and stored at 4 °C.

HPLC-PDA Analysis. HPLC-PDA analyses of the extracts were performed using a Waters (Milford, MA, USA) Alliance 2695 system equipped with a 2695 separation module unit and a 2996 PDA detector using a 250 × 4.6 mm, 4 μm, Phenomenex Synergi Hydro-RP 80A column (Torrance, CA, USA). The mobile phase consisted of solvents (A) 10% aqueous formic acid solution and (B) MeCN. Gradient conditions were performed as follows: from 0 to 5% B in 10 min, from 5 to 15% B until 30 min, maintained at 15% B to 45 min, and from 15 to 60% B to 55 min, followed by a final increase to 100% in 5 min. The flow rate and the injection volume were 1 mL/min and 10 μL, respectively. Each sample was injected three times. The results were monitored using a wavelength range of 210−800 nm.

LC-TOF-MS Analysis. The HPLC conditions were the same as above, substituting 0.1% aqueous formic acid in place of 10% aqueous formic acid in solution B. The flow rate and the injection volume were 1 mL/min and 10 μL, respectively. Each sample was injected three times. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed using an LCT premier XE TOF mass spectrometer (Waters) equipped with an ESI interface and controlled by MassLynx V4.1 software. Mass spectra were acquired in both 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 for both 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, Steinheim, Germany) was infused by a secondary reference probe at 200 pg/mL in acetonitrile/water (1:1) containing 0.1% formic acid with a second LC pump (Waters 515 HPLC pump). 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. The full chromatograms were recorded at two different aperture voltages. The most intense fragmental ions and molecular ions could be obtained when the aperture voltages were set at 60 and 0 V, respectively. V-optics mode was used for increased intensity.

Chemometric Data Analysis. Principal component analysis used Markerlynx v4.1 software. The parameters used included a retention time range of 4−70 min, a mass range of 100−1000 Da, and a mass tolerance of 50 mDa. Isotopic peaks were excluded for analysis; the noise elimination level was set at 1.00, the intensity threshold (counts) of collection parameters was set at 500; retention time tolerance was set at 0.4 min. The retention time and m/z data pair for each peak were determined by the software.

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.[12] with minor modifications, including longer incubation time (30 min), lower DPPH concentration (400 μM), and different sample to reagent ratio (3: 1). To a 50 μL aliquot of the sample was added 150 μL of DPPH solution (400 μM), and the absorbance at 515 nm was recorded after an incubation period of 30 min 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, and the IC_{50} values were calculated using linear regression analysis.

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

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonate) Free Radical (ABTS$^{\bullet+}$) Scavenging. The determination of ABTS$^{\bullet+}$ scavenging was carried out on the basis of the method of Re et al.[12] with minor modifications, including long measurement interval (5 min), longer measurement period (40 min), lower sample to reagent ratio (1:99), and lower ABTS to $K_2S_2O_8$ ratio (1:0.0176). The ABTS$^{\bullet+}$ was generated by reacting an ABTS (7 mM) aqueous solution with $K_2S_2O_8$

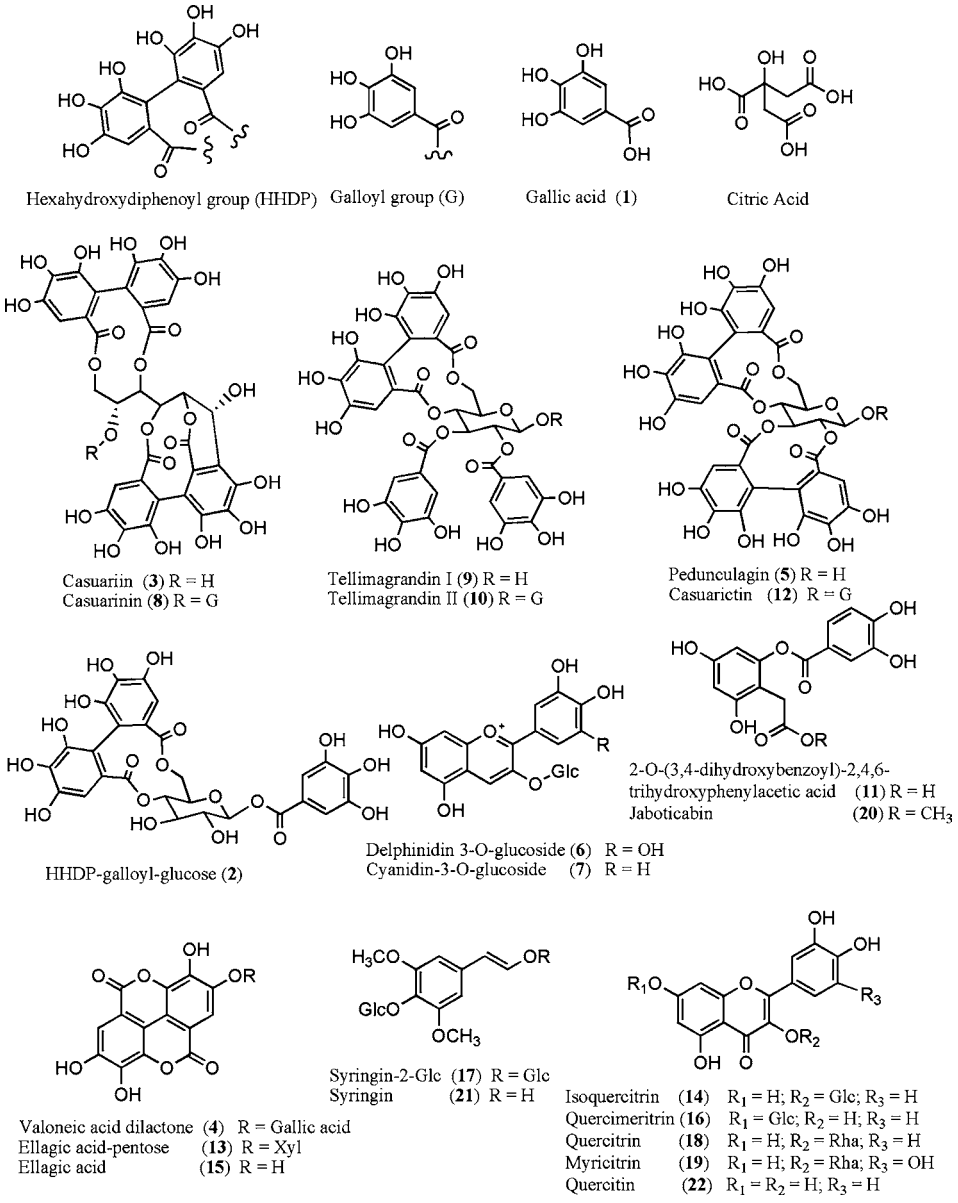

Figure 1. Chemical structures of phenolic compounds detected in jaboticaba (*M. cauliflora*) fruits.

(2.45 mM) in the dark for 12−16 h, at ambient temperature, and adjusting the $Abs_{734\ nm}$ to 0.700 (±0.020) with ethanol. To a 2 μL aliquot of the sample was added 198 μL of $ABTS^{\bullet+}$, 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$_{max}$ microplate reader. The results were expressed as the TEAC (μmol Trolox/g dry fruit material) values at different time intervals.

RESULTS AND DISCUSSION

Identification of Jaboticaba Constituents. A method based on HPLC coupled with both PDA and HR-ESI-TOF-MS has been developed to determine the phenolic composition of fruits of jaboticaba. Twenty-two compounds, including gallic acid (**1**), 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid (**11**), ellagic acid (**15**), delphinidin-3-*O*-glucoside (**6**), cyanidin-3-*O*-glucoside (**7**), isoquercitrin (**14**), quercimeritrin (**16**), quercitrin (**18**), myricitrin (**19**), jaboticabin (**20**), and quercitin (**22**) were identified, and 7 gallotannins (**2**, **3**, **5**, **8**−**10**, and **12**), 2 ellagic derivatives (**4** and **13**), and syringin and its glucoside (**17** and **21**) were tentatively identified (Figure 1). Their retention times, UV spectra, and exact mass spectral fragmental ions in positive and negative modes are shown in Table 1. Among them, HHDP-galloyl-glucose (**2**), casuariin (**3**), valoneic acid dilactone (**4**), pedunculagin (**5**), casuarinin (**8**), tellimagrandins I (**9**) and II (**10**), casuarictin (**12**), ellagic acid-pentose (**13**), syringin-2-glc (**17**), and syringin (**21**) were detected and identified from this fruit for the first time.

Gallic Acid and Gallotannins. Compound **1** was determined to be gallic acid, identified by comparison of its retention time, UV−visible spectrum, and MS signal at *m*/*z* 171.0294 $[M + H]^+$ in positive mode and 169.0114 $[M - H]^-$, 339.0342 $[2M - H]^-$ as well as 215.0166 $[M - H + HOOH]^-$ in negative mode with those of an authentic standard.

Compounds **2**, **3**, **5**, **8**−**10**, and **12** were identified as gallotannins, which was deduced by the observation of their characteristic fragmentation pathways (Figure 2) and comparison with the literature.[13−15] The LC-MS chromatogram of **2**

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Table 1. Structure Identification of Compounds from Jaboticaba

no.	RT (min)	UV	$[M]^+$, $[M + H]^+$, or $[M - H]^-$ (MF, ppm)	other fragmental ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	identification	note
1	6.73	216, 271	171.0294 $[M + H]^+$ ($C_7H_7O_5$, 0.6);		gallic acid (co-injection)	reported earlier in *Myrciaria* species[2]
			169.0114 $[M - H]^-$ ($C_7H_5O_5$, −1.6)	339.0342 $[2M - H]^-$ ($C_{14}H_{11}O_{10}$, −2.9); 215.0166 $[M - H + HOOH]^-$ ($C_8H_7O_7$, −12.1)		
2	9.60	226, 283	635.0828 $[M + H]^+$ ($C_{27}H_{23}O_{18}$, −8.8);	617.0758 $[M + H - H_2O]^+$ ($C_{27}H_{21}O_{17}$, −3.4); 652.1122 $[M + H + NH_3]^+$ ($C_{27}H_{26}O_{18}N$, −4.3); 465.0651 $[M + H -$ gallic acid$]^+$ ($C_{20}H_{17}O_{13}$, −3.9); 333.0822 $[M + H -$ HHDP$]^+$ ($C_{13}H_{17}O_{10}$, 1.8); 321.0247 $[M + H -$ galloyl group − glucosyl group$]^+$ ($C_{14}H_9O_9$, −1.9);	HHDP-galloylglucose	reported earlier in Myrtaceae family.[13,14] detected for first time in this fruit
			633.0706 $[M - H]^-$ ($C_{27}H_{21}O_{18}$, −3.5)	481.0630 $[M - H -$ galloyl group$]^-$ ($C_{20}H_{17}O_{14}$, 2.5)		
3	15.82	207, 258	785.0834 $[M + H]^+$ ($C_{34}H_{25}O_{22}$, −0.4);	802.0872 $[M + H + NH_3]^+$ ($C_{34}H_{28}O_{22}N$, 0.9); 767.0714 $[M + H - H_2O]^+$ ($C_{34}H_{23}O_{21}$, 2.3); 483.0764 $[M + H -$ HHDP$]^+$ ($C_{20}H_{19}O_{14}$, 2.3)	casuariin	reported earlier in Myrtaceae family;[13,14] detected for first time in this fruit
			783.0691 $[M - H]^-$ ($C_{34}H_{23}O_{22}$, 1.3)	829.0747 $[M - H + HOOH]^-$ ($C_{35}H_{25}O_{24}$, 1.3); 801.0775 $[M - H + H_2O]^-$ ($C_{34}H_{25}O_{23}$, −1.5)		
4	18.21	222, 254, 365	471.0167 $[M + H]^+$ ($C_{21}H_{11}O_{13}$, −8.3);	453.0103 $[M + H - H_2O]^+$ ($C_{21}H_9O_{12}$, 2.0)	valoneic acid dilactone	reported earlier in Myrtaceae family;[16] detected for first time in this fruit
			469.0045 $[M - H]^-$ ($C_{21}H_9O_{13}$, 0.4)			
5	22.31	231, 256	785.0804 $[M + H]^+$ ($C_{34}H_{25}O_{22}$, −4.2);	802.0919 $[M + H + NH_3]^+$ ($C_{34}H_{28}O_{22}N$, 6.7); 767.0710 $[M + H - H_2O]^+$ ($C_{34}H_{23}O_{21}$, −2.9); 483.0780 $[M + H -$ HHDP$]^+$ ($C_{20}H_{19}O_{14}$, 1.0); 465.0662 $[M + H -$ HHDP $- H_2O]^+$ ($C_{20}H_{17}O_{13}$, −1.5);	pedunculagin	reported earlier in Myrtaceae family;[13,14] detected for first time in this fruit
			783.0693 $[M - H]^-$ ($C_{34}H_{23}O_{22}$, 1.5);	829.0735 $[M - H + HOOH]^-$ ($C_{35}H_{25}O_{24}$, −0.1); 801.0784 $[M - H + H_2O]^-$($C_{34}H_{25}O_{23}$, −0.4)		
6	23.71	519, 274	465.1036 $[M]^+$ ($C_{21}H_{21}O_{12}$, 1.3)	303.0496 $[M -$ glucosyl group$]^+$ ($C_{15}H_{11}O_7$, −3.0);	delphinidin 3-*O*-glucoside (co-injection)	reported earlier in *Myrciaria* species[2]
			463.0877 $[M - 2H]^-$ ($C_{21}H_{19}O_{12}$, 0.9)	481.0935 $[M - 2H + H_2O]^-$ ($C_{21}H_{21}O_{13}$, 9.8); 509.0996 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{14}$, −6.9)		
7	26.43	516, 279	449.1071 $[M]^+$ ($C_{21}H_{21}O_{11}$, −2.9)	287.0515 $[M -$ glucosyl group$]^+$ ($C_{15}H_{11}O_6$, 14.3);	cyanidin-3-*O*-glucoside (co-injection)	reported earlier in *Myrciaria* species[2]
			447.0935 $[M - 2H]^-$ ($C_{21}H_{19}O_{11}$, 1.8)	465.1038 $[M - 2H + H_2O]^-$ ($C_{21}H_{21}O_{12}$, 2.5); 493.0958 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{13}$, −4.7); 511.1088 $[M - 2H + HCOOH + H_2O]^-$ ($C_{22}H_{23}O_{14}$, −0.2)		
8	27.31	225, 265	937. 0868 $[M + H]^+$ ($C_{41}H_{29}O_{26}$, −8.4)	954.1016 $[M + H + NH_3]^+$ ($C_{41}H_{32}O_{26}N$, 4.4); 919.0833 $[M + H - H_2O]^+$ ($C_{41}H_{27}O_{25}$, 0.9); 617.0731 $[M + H - H_2O -$ HHDP$]^+$ ($C_{27}H_{21}O_{17}$, −7.8); 767.0730 $[M + H - H_2O -$ galloyl group$]^+$ ($C_{34}H_{23}O_{21}$, −0.3);	di-HHDP-galloylglucose (casuarinin)	reported earlier in Myrtaceae family'[13] detected for first time in this fruit

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Table 1. continued

no.	RT (min)	UV	$[M]^+$, $[M + H]^+$, or $[M - H]^-$ (MF, ppm)	other fragmental ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	identification	note
				635.0892 $[M + H - HHDP]^+$ ($C_{27}H_{23}O_{18}$, 1.3); 785.0879 $[M + H -$ galloyl group$]^+$ ($C_{34}H_{25}O_{22}$, 5.3);		
			935. 0782 $[M - H]^-$ ($C_{41}H_{27}O_{26}$, 1.0)	783.0692 $[M - H -$ galloyl group$]^-$ ($C_{34}H_{23}O_{22}$, 1.4); 633.0721 $[M - H - HHDP]^-$ ($C_{27}H_{21}O_{18}$, 1.1)		
9	28.13	255	787.1023 $[M + H]^+$ ($C_{34}H_{27}O_{22}$, 3.7);	769.0887 $[M + H - H_2O]^+$ ($C_{34}H_{25}O_{21}$, −0.1); 617.0681 $[M + H - H_2O -$ galloyl group$]^+$ ($C_{27}H_{21}O_{17}$, −6.5)	HHDP-digalloylglucose (tellimagrandin I)	detected for first time in this genus
			785.0818 $[M - H]^-$ ($C_{34}H_{25}O_{22}$, −2.4)	633.0729 $[M - H -$ galloyl group$]^-$ ($C_{27}H_{21}O_{18}$, 0.2)		
10	31.90	256, 364		956.1189 $[M + H + NH_3]^+$ ($C_{41}H_{34}O_{26}N$, 0.4); 961.1135 $[M + Na]^+$ ($C_{41}H_{30}O_{26}Na$, 0.7); 769.0797 $[M + H - H_2O -$ galloyl group$]^+$ ($C_{34}H_{25}O_{21}$, −11.8)	HHDP-trigalloylglucose (tellimagrandin II)	reported earlier in Myrtaceae family;[13] detected for first time in this fruit
			937. 0957 $[M - H]^-$ ($C_{41}H_{29}O_{26}$, 1.1)	785.0847 $[M - H -$ galloyl group$]^-$ ($C_{34}H_{25}O_{22}$, 1.3); 635.0776 $[M - H - HHDP]^-$ ($C_{27}H_{23}O_{18}$, 1.3); 633.0728 $[M - H -$ 2galloyl group$]^-$ ($C_{27}H_{21}O_{18}$, 0.0); 319.0088 $[M - H -$ 3galloyl group − glucosyl group$]$ ($C_{14}H_7O_9$, −0.6)		
11	32.80	245, 365	321.0620 $[M + H]^+$ ($C_{15}H_{13}O_8$, 3.1);	663.0962 $[2M + Na]^+$ ($C_{30}H_{24}O_{16}$, 0.0);	2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphen ylacetic acid (co-injection)	reported earlier in *Myrciaria* species[2]
			319.0428 $[M - H]^-$ ($C_{15}H_{11}O_8$, −8.1)	365.0447 $[M - H + HOOH]^-$ ($C_{16}H_{13}O_{10}$, −17.0)		
12	33.56	236, 256		954.0980 $[M + H + NH_3]^+$ ($C_{41}H_{32}O_{26}N$, 0.6); 919.0878 $[M + H - H_2O]^+$ ($C_{41}H_{27}O_{25}$, 4.0); 617.0737 $[M + H - H_2O - HHDP]^+$ ($C_{27}H_{21}O_{17}$, −6.8); 767.0757 $[M + H - H_2O -$ galloyl group$]^+$ ($C_{34}H_{23}O_{21}$, 3.3); 635.0879 $[M + H - HHDP]^+$ ($C_{27}H_{23}O_{18}$, 0.8); 785.0859 $[M + H -$ galloyl group$]^+$ ($C_{34}H_{25}O_{22}$, 2.8);	di-HHDP-galloylglucose isomer (casuarictin)	reported earlier in Myrtaceae family;[13,14] detected for first time in this fruit
			935.0793 $[M - H]^-$ ($C_{41}H_{27}O_{26}$, 0.2)	783.0675 $[M - H -$ galloyl group$]^-$ ($C_{34}H_{23}O_{22}$, −0.8); 633.0718 $[M - H - HHDP]^-$ ($C_{27}H_{21}O_{18}$, 1.6)		
13	34.70	252, 373	435.0544 $[M + H]^+$ ($C_{19}H_{15}O_{12}$, −4.6)	303.0134 $[M + H -$ pentosyl unit$]^+$ ($C_{14}H_7O_8$, −2.3);	ellagic acid-pentose	detected for first time in this genus
			433.0379 $[M - H]^-$ ($C_{19}H_{13}O_{12}$, −6.5);	300.9995 $[M - H -$ pentosyl unit$]^-$ ($C_{14}H_5O_8$, 3.7); 867.0874 $[2M - H]^-$ ($C_{38}H_{27}O_{24}$, −2.1)		
14	38.32	254, 353	465.1078 $[M + H]^+$ ($C_{21}H_{21}O_{11}$, 9.7)	487.0860 $[M + Na]^+$ ($C_{21}H_{20}O_{12}Na$, 1.6); 303.0468 $[M + H -$ glucosyl group$]^+$ ($C_{15}H_{11}O_7$, −12.2)	isoquercitrin (co-injection)	reported earlier in *Myrciaria* species[2]
			463.0866 $[M - H]^-$ ($C_{21}H_{19}O_{12}$, −2.4)	509.0907 $[M - H + HCOOH]^-$ ($C_{22}H_{21}O_{14}$, −4.7); 927.1840 $[2M - H]^-$ ($C_{42}H_{39}O_{24}$, 1.0)		

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Table 1. continued

no.	RT (min)	UV	$[M]^+$, $[M + H]^+$, or $[M - H]^-$ (MF, ppm)	other fragmental ion exact masses $[M - X]^+$ or $[M - X]^-$ (MF, ppm)	identification	note
15	41.82	253, 366	303.0148 $[M + H]^+$ ($C_{14}H_7O_8$, 2.3)		ellagic acid (co-injection)	reported earlier in *Myrciaria* species[2]
			300.9974 $[M - H]^-$ ($C_{14}H_5O_8$, −3.3)	603.0034 $[2M - H]^-$ ($C_{28}H_{11}O_{16}$, −2.2)		
16	45.26	254, 355	465.1037 $[M + H]^+$ ($C_{21}H_{21}O_{11}$, 0.9)	487.0852 $[M + Na]^+$ ($C_{21}H_{20}O_{12}Na$, 4.3); 303.0468 $[M + H - \text{glucosyl group}]^+$ ($C_{15}H_{11}O_7$, −12.2);	quercimeritrin (co-injection)	reported earlier in *Myrciaria* species[2]
			463.0869 $[M - H]^-$ ($C_{21}H_{19}O_{12}$, −1.7)	509.0922 $[M - H + HCOOH]^-$ ($C_{22}H_{21}O_{14}$, −1.8)		
17	47.23	249, 276	535.1962 $[M + H]^+$ ($C_{30}H_{31}O_9$, −1.1);	557.1779 $[M + Na]^+$ ($C_{30}H_{30}O_9$, −1.6);	syringin-2-Glc	detected for first time in this genus
			533.1816 $[M - H]^-$ ($C_{30}H_{29}O_9$, 0.8)	371.1279 $[M - H - \text{glucosyl group}]^-$ ($C_{24}H_{19}O_4$, −1.1); 209.0693 $[M - H - 2\ \text{glucosyl group}]^-$ ($C_{11}H_{13}O_4$, −6.5)		
18	50.06	255, 355	449.1075 $[M + H]^+$ ($C_{21}H_{21}O_{11}$, −2.0)	471.0903 $[M + Na]^+$ ($C_{21}H_{21}O_{11}Na$, 10.0); 303.0505 $[M + H - \text{deoxyhexosyl unit}]^+$ ($C_{15}H_{11}O_7$, −1.3)	quercitrin (co-injection)	reported earlier in *Myrciaria* species[2]
			447.0891 $[M - H]^-$ ($C_{21}H_{19}O_{11}$, −8.1);	493.0991 $[M - H + HCOOH]^-$ ($C_{20}H_{21}O_{13}$, 1.8); 895.1933 $[2M - H]^-$ ($C_{42}H_{39}O_{22}$, 1.6)		
19	51.58	252, 372	465.1033 $[M + H]^+$ ($C_{21}H_{21}O_{12}$, 0.6);	957.1711 $[2M + H]^+$ ($C_{42}H_{40}O_{24}Na$, −10.1)	myricitrin (co-injection)	reported earlier in *Myrciaria* species[2]
			463.0856 $[M - H]^-$ ($C_{21}H_{19}O_{12}$, −4.5)	509.0934 $[M - H + HCOOH]^-$ ($C_{22}H_{21}O_{14}$, 0.6);		
20	52.14	244, 365	335.0745 $[M + H]^+$ ($C_{16}H_{15}O_8$, −6.6)	357.0587 $[M + Na]^+$ ($C_{16}H_{14}O_8Na$, 0.3);	jaboticabin (co-injection)	reported earlier in *Myrciaria* species[2]
			333.0591 $[M - H]^-$ ($C_{16}H_{13}O_8$, −5.7)	379.0624 $[M - H + HCOOH]^-$ ($C_{17}H_{15}O_{10}$, −10.8)		
21	53.13	249	373.1476 $[M + H]^+$ ($C_{17}H_{25}O_9$, −6.2);		syringin	reported earlier in Myrtaceae family;[29] detected for first time in this fruit
			371.1326 $[M - H]^-$ ($C_{17}H_{23}O_9$, −4.3)	209.0693 $[M - H - \text{glucosyl group}]^-$ ($C_{11}H_{13}O_4$, −57.9)		
22	55.90	254, 365	303.0478 $[M + H]^+$ ($C_{15}H_{11}O_7$, −8.9)		quercitin (co-injection)	reported earlier in *Myrciaria* species[2]
			301.0291 $[M - H]^-$ ($C_{15}H_9O_7$, −18.9)	603.0739 $[2M - H]^-$ ($C_{30}H_{19}O_{14}$, −6.0); 347.0407 $[M - H + HCOOH]^-$($C_{16}H_{11}O_9$, 1.2)		

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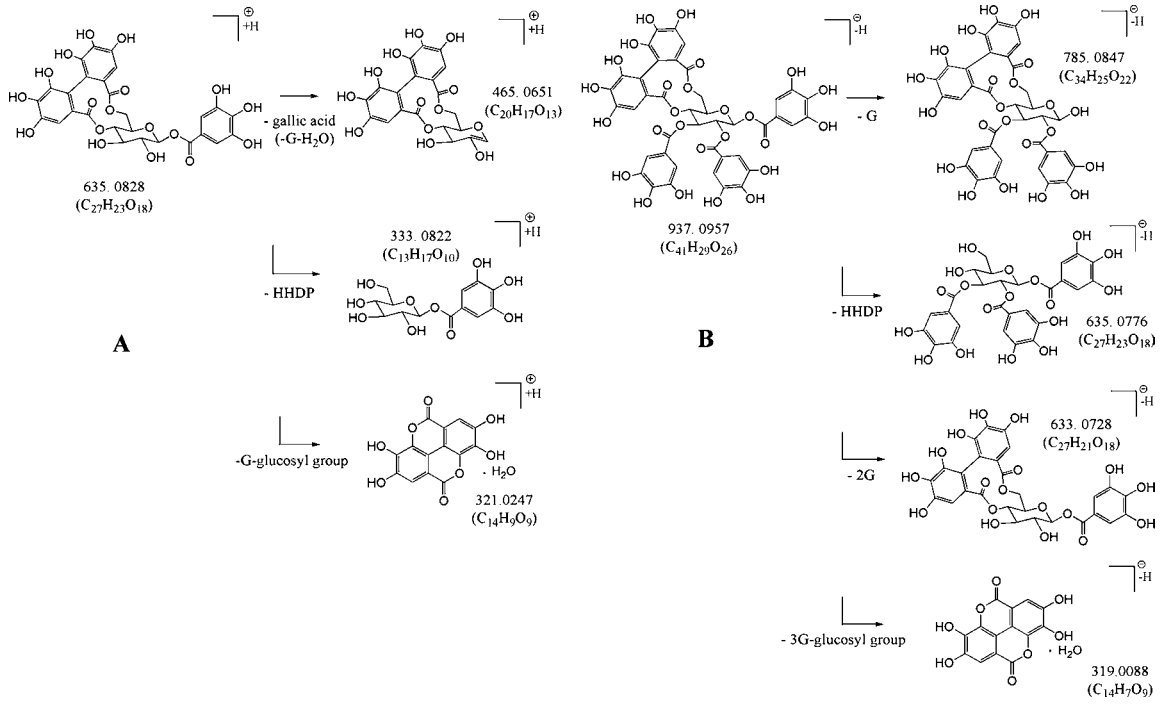

Figure 2. Proposed fragmentation pathway of (A) HHDP-galloyl-glucose (**2**) in positive mode and (B) HHDP-trigalloyl-glucose (**10**) in negative mode.

showed two strong ions at m/z 652.1122 $[M + NH_4]^+$ in positive mode and m/z 633.0706 $[M - H]^-$ in negative mode. When the aperture voltage was set at 60 V, three cleavage fragmental ions at 465.0651 $[M + H -$ gallic acid$]^+$, 333.0822 $[M + H -$ HHDP$]^+$, and 321.0247 $[M + H -$ galloyl group (G) − glucosyl group$]^+$ with losses of 170, 302, and 314 (152 + 162) Da from the protonated molecular ion at m/z 635.0828 $[M + H]^+$ were generated for **2** (Figure 2 and Figure 1S in the Supporting Information), and this is very similar to the characteristic fragmentation pathways of an isomer of galloyl-HHDP-glucose.[14] Therefore, compound **2** was tentatively identified as HHDP-galloyl-glucose, which was detected previously in another Myrtaceae species.[13,14]

In the positive mode, compound **10** displayed adduct ions at 956.1189 $[M + NH_4]^+$ and 961.1135 $[M + Na]^+$. In the negative mode, when the aperture voltage was increased to 60 V, the molecular ion $[M - H]^-$ at m/z 937.0957 of **10** fragmentation included 785.0847 $[M - H - G]^-$, 635.0776 $[M - H -$ hexahydroxydiphenoyl (HHDP)$]^-$, 633.0728 $[M - H - 2G]^-$, and 319.0088 $[M - H - 3G -$ glucosyl group$]^-$. These losses helped to confirm the presence of G, HHDP, 2G, and 3G + glucosyl groups in **10** (Figure 2 and Figure 2S in the Supporting Information). Therefore, compound **10** was tentatively determined as HHDP-trigalloyl-glucose (tellimagrandin II), which has been found in the same family previously.[13] Similarly, compounds **5**, **9**, and **12** were also found to have the same characteristic fragmentation pathways, depending on their MS/MS fragmentation ions and if they can lose galloyl or HHDP groups; their structures were tentatively determined as pedunculagin (**5**), tellimagrandin I (**9**), and casuarictin (**12**), respectively. Compounds **3** and **8** are isomers of pedunculagin (**5**) and casuarictin (**12**), respectively. However, two pairs of isomers (**3** and **5**; **8** and **12**) could be distinguished by their retention times. Compound **3** elutes 7 min prior to its structural isomer **5** (Table 1) because it has an open glucose ring structure and therefore an additional hydroxyl group; likewise, **8** elutes 5 min prior to **11** for the same reason. Thus, **3** and **8** were tentatively determined as casuariin and casuarinin, respectively. All of the above compounds, except for **8**, have been previously detected from Myrtaceae species,[13,14] but are being reported for the first time from *Myrciaria* species.

Anthocyanins. Two anthocyanins, delphinidin 3-*O*-glucoside (**6**) and cyanidin-3-*O*-glucoside (**7**), were detected in jaboticaba fruits by co-injection; these two compounds have been reported previously from jaboticaba fruits.[2]

Ellagic Acid and Derivatives. Compound **15** was identified as ellagic acid by its UV−visible spectrum (λ_{max} 253 and 366 nm), retention time (41.82 min) with the authentic standard, and HR-MS data at m/z 300.9974 $[M - H]^-$ and 603.0034 $[2M - H]^-$ in its negative mode. Compound **4** was tentatively identified as valoneic acid dilactone by its UV−visible sepectrum (λ_{max} 222, 254, and 365 nm) and the molecular ions at m/z 471.0167 $[M + H]^+$ in positive and 469.0045 $[M - H]^-$ in negative mode. Compound **13** also had a UV−visible spectrum (λ_{max} 252 and 373 nm) characteristic of an ellagic acid derivative. In the MS of compound **13**, besides the ions at m/z 435.0544 $[M + H]^+$, 433.0379 $[M - H]^-$, and 867.0874 $[2M - H]^-$, upon increasing the aperture voltage to 60 V additional ions at m/z 303.0134 $[M + H -$ pentosyl$]^+$ in positive and m/z 300.9995 $[M - H -$ pentosyl$]^-$ in negative modes were detected (Table 1). The loss of a pentosyl unit by these ions indicates this structure has a pentosyl unit. Therefore, compound **13** was tentatively determined as ellagic acid-pentose. Compound **15** has been reported previously in jaboticaba fruits,[2] and **4** was detected in another Myrtaceae species.[16] Compound **13** is being reported for the first in this genus.

Flavonoids and Other Constituents. Flavonoids were another important class of constituents detected from

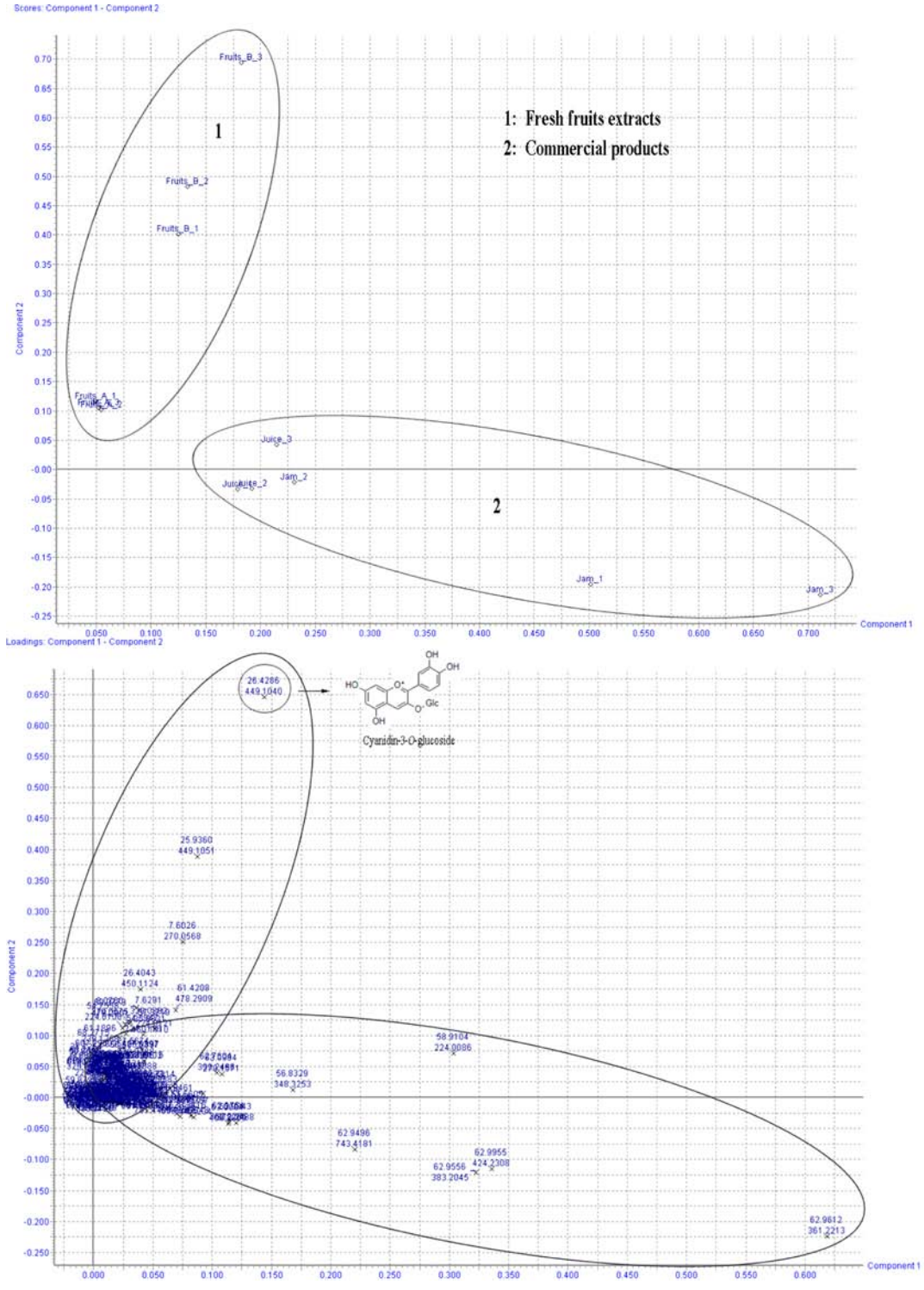

Figure 3. Score and loading plots of jaboticaba fruit extracts and jaboticaba commercial products (juice and jam).

jaboticaba fruits. Isoquercitrin (**14**), quercimeritrin (**16**), quercitrin (**18**), myricitrin (**19**), and quercetin (**22**) were detected in this fruit. All of these flavonoids were determined by their UV−visible spectrum, retention time, and comparison with the reference standards and have all been reported in this species previously.[2]

Compounds **21** and **17** were identified as syringin and its glucose derivative, respectively, using mass spectral fragmentation patterns. In the negative mode, compound **21** displayed a fragmental ion at m/z 209.0693 [M − H − glucosyl group]$^-$, indicating the loss of one glucose group. Likewise, in the negative mode, compound **17** displayed fragmental ions at m/z 371.1326 [M − H − glucosyl group]$^-$ as well as 209.0693 [M − 2H − glucosyl group]$^-$, demonstrating that **17** has two glucoses. Thus, syringin (**21**) and syringin-2-glc (**17**) were identified. The depsides 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid (**11**) and jaboticabin (**20**) were

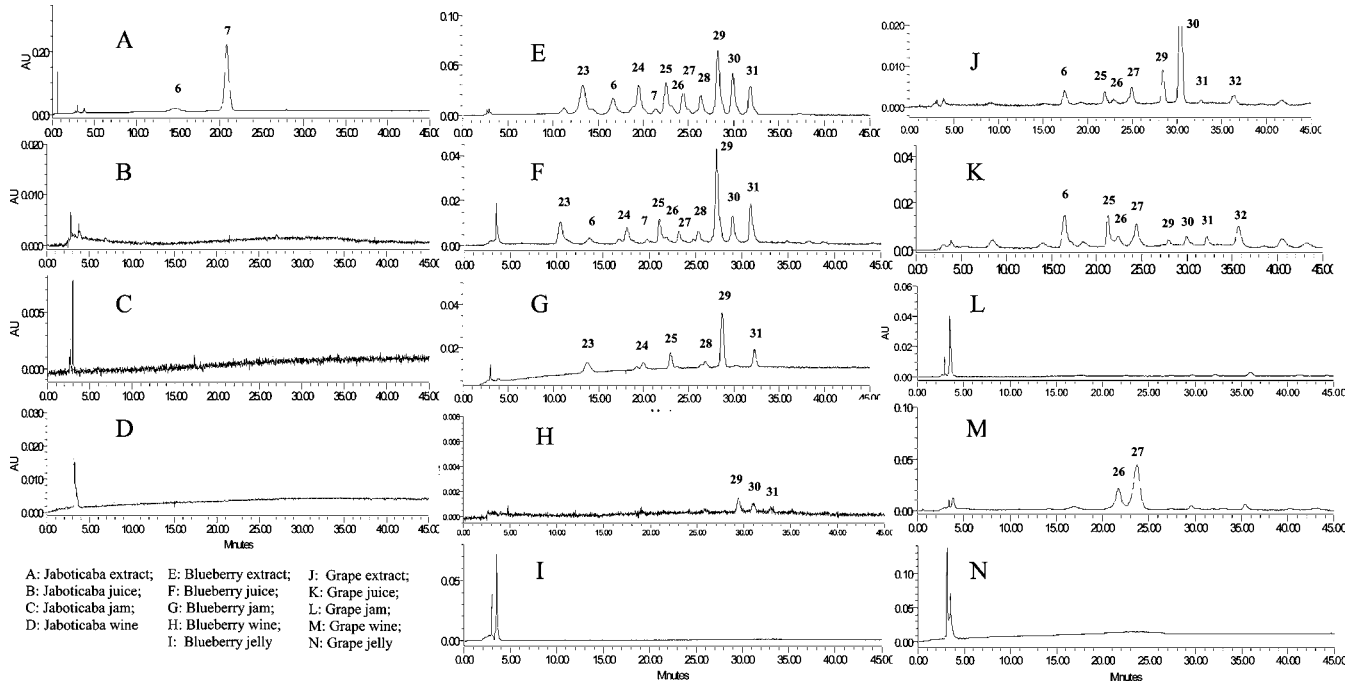

Figure 4. HPLC-PDA analysis (all at 520 nm) of jaboticaba, blueberry, and Concord grape fruit extracts and their commercial products. Anthocyanins identified as **6**, delphinidin-3-*O*-glucoside; **7**, cyanidin-3-*O*-glucoside; **23**, delphinidin-3-*O*-galactoside; **24**, delphinidin-3-*O*-arabinoside; **25**, petunidin-3-*O*-galactoside; **26**, cyanidin-3-*O*-arabinoside; **27**, petunidin-3-*O*-glucoside; **28**, petunidin-3-*O*-arabinoside; **29**, malvidin-3-*O*-galactoside; **30**, malvidin-3-*O*-glucoside; **31**, malvidin-3-*O*-arabinoside; **32**, delphinidin-3-*O*-acetylglucoside.

Table 2. Some Marker Compound's LC-TOF-MS Intensity of the PCA Data

compd	RT	$[M]^+/[M + H]^+/[M - H]^-$	chem formula	fruit extract A	fruit extract B	juice	jam
CA[a]	4.22	191.0020[b]	$C_6H_7O_7$	0.0000	0.0000	366.6565	1025.6953
6	23.71	465.1036[c]	$C_{21}H_{21}O_{12}$	8.0021	26.6412	0.0000	0.0000
7	26.43	449.1041[c]	$C_{21}H_{21}O_{11}$	44.4220	733.6481	0.0000	0.0000
11	32.80	321.0591[b]	$C_{15}H_{13}O_8$	1.5956	1.6609	0.0000	0.0000
20	52.14	333.0743[c]	$C_{16}H_{13}O_8$	4.3202	4.7983	0.0000	0.0000

[a]Citric acid. [b]Detected in negative mode. [c]Detected in positive mode.

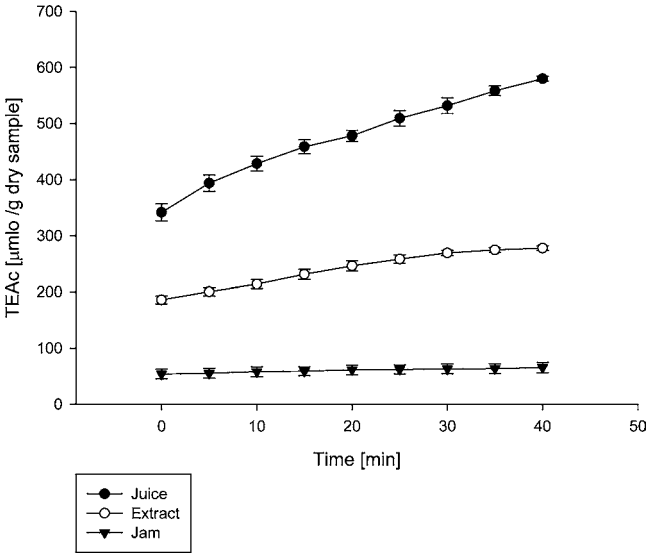

Figure 5. $ABTS^{\bullet+}$ scavenging assessment of jaboticaba extract A, juice, and jam.

identified using their positive and negative fragmental ions (Table 1) and a co-injection experiment using standards.[2]

Many species of Myrtaceae are rich sources of anthocyanins, flavonoids, phenolic acids, and tannins, which are well-known antioxidants and inflammatories and are believed to play an important role in the prevention of certain diseases.[17] Flavonoids, such as quercetin (**22**) and fisetin, were reported to reduce in vivo inflammation associated with pulmonary inflammatory diseases such as COPD.[18] Also, jaboticabin (**20**), a depside with anti-inflammatory activity in vitro, and the anthocyanins delphinidin-3-*O*-glucoside (**6**) and cyanidin-3-*O*-glucoside (**7**) may be useful for the treatment of COPD.[2] With these important bioactive phenolic constituents, including compounds unique to this species detected and identified from the jaboticaba fruit extract, we believe this fruit has potential to be developed as a functional food to promote pulmonary health.

Principal Component Analysis. Jaboticaba fruits do not transport well. Growers in Brazil report that these fruits spoil easily and begin to ferment (personal communication) Therefore, fresh fruits are not typically shipped far from where they grow in Brazil and are seldom imported to the United States. We therefore have worked on jaboticaba that was grown in southern Florida. Jaboticaba processed food products are available in limited distribution in Brazil as well as the United States; the compositional differences between fresh

Table 3. Major Compounds Quantified from Dark-Colored Fruit and Juice Extract (Milligrams per 10 g, Mean ± SD)[a]

	jaboticaba		blueberry		Concord grape	
compd	fruit extract	juice	fruit extract	juice	fruit extract	juice
gallic acid[b]	5.07 ± 0.48	0.41 ± 0.10	0	0	0.40 ± 0.07	0.23 ± 0.04
valoneic acid dilactone[b]	2.01 ± 0.18	2.05 ± 0.42	0	0	0	0
ellagic acid[b]	1.54 ± 0.16	1.56 ± 0.27	0	0	0.13 ± 0.02	0.08 ± 0.00
ellagic acid-pentoside[b]	2.85 ± 0.45	0.81 ± 0.20	0	0	0	0
HHDP-galloyl-glucose[b]	0.88 ± 0.14	0.52 ± 0.11	0	0	0	0
casuariin[b]	0.59 ± 0.16	0.41 ± 0.04	0	0	0	0
casuarinin[b]	3.43 ± 0.75	2.49 ± 0.09	0	0	0	0
casuarictin[b]	3.44 ± 0.74	7.08 ± 0.30	0	0	0	0
pedunculagin[b]	1.13 ± 0.20	0.39 ± 0.03	0	0	0	0
tellimagrandin I[b]	2.17 ± 0.27	0.73 ± 0.07	0	0	0	0
tellimagrandin II[b]	3.05 ± 0.17	0.97 ± 0.07	0	0	0	0
syringin-2-glucose[c]	0.22 ± 0.09	0.10 ± 0.02	0	0	0	0
syringin[c]	0.24 ± 0.05	0.01 ± 0.00	0	0	0	0
isoquercitrin[d]	4.20 ± 0.32	1.14 ± 0.07	4.41 ± 0.18	6.11 ± 0.31	8.16 ± 0.14	4.82 ± 0.20
quercitin[d]	11.57 ± 0.66	0.33 ± 0.14	1.51 ± 0.10	1.28 ± 0.09	0.97 ± 0.07	0.87 ± 0.09
quercitrin[d]	3.88 ± 0.18	1.11 ± 0.33	0	0	0	0
quercimeritrin[d]	0.49 ± 0.06	0.35 ± 0.06	0.06 ± 0.01	0.01 ± 0.00	0	0
myricitrin[d]	1.80 ± 0.2	0.97 ± 0.08	0	0	0	0
myricitrin-3-*O*-hexoside[d]	0	0	1.07 ± 0.10	0.84 ± 0.03	6.48 ± 0.30	5.26 ± 0.47
quercitin-3-*O*-pentoside[d]	0	0	0.86 ± 0.07	1.26 ± 0.10	0	0
orientin[d]	0	0	0.48 ± 0.05	0.44 ± 0.03	2.46 ± 0.09	0.55 ± 0.11
syringetin-3-*O*-hexoside[d]	0	0	1.01 ± 0.08	1.29 ± 0.12	0	0
catechin[d]	0	0	0.27 ± 0.03	0.25 ± 0.04	1.93 ± 0.05	1.20 ± 0.17
caffeoylquinic acid[c]	0	0	3.95 ± 0.07	5.79 ± 0.15	0	0
jaboticabin[c]	0.93 ± 0.05	0	0	0	0	0
2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-phenylacetic acid[c]	0.12 ± 0.04	0	0	0	0	0
delphinidin 3-*O*-glucoside[e]	7.36 ± 0.64	0	3.48 ± 0.07	0.74 ± 0.05	2.44 ± 0.11	0.90 ± 0.10
cyanidin-3-*O*-glucoside[e]	29.80 ± 1.73	0	0.56 ± 0.02	0.22 ± 0.02	0	0
delphinidin-3-*O*-galactoside[e]	0	0	5.17 ± 0.13	3.26 ± 0.10	0	0
delphinidin-3-*O*-arabinoside[e]	0	0	3.25 ± 0.29	1.81 ± 0.13	0	0
petunidin-3-*O*-galactoside[e]	0	0	3.65 ± 0.09	1.92 ± 0.12	1.60 ± 0.10	0.79 ± 0.03
cyanidin-3-*O*-arabinoside[e]	0	0	0.17 ± 0.01	0.17 ± 0.03	0.35 ± 0.02	0.10 ± 0.01
petunidin-3-*O*-glucoside[e]	0	0	2.80 ± 0.17	0.85 ± 0.04	2.32 ± 0.07	0.80 ± 0.10
petunidin-3-*O*-arabinoside[e]	0	0	1.43 ± 0.04	0.89 ± 0.03	0	0
malvidin-3-*O*-galactoside[e]	0	0	11.15 ± 0.65	10.06 ± 0.63	4.85 ± 0.27	2.10 ± 0.17
malvidin-3-*O*-glucoside[e]	0	0	7.04 ± 0.24	2.41 ± 0.17	23.95 ± 1.78	3.23 ± 0.20
malvidin-3-O-arabinoside[e]	0	0	5.13 ± 0.11	4.34 ± 0.21	0.49 ± 0.08	1.61 ± 0.12
delphindin-3-*O*-acetylglucoside[e]	0	0	0	0	1.93 ± 0.09	3.49 ± 0.34

[a]Results correspond to the average value of samples analyzed in triplicate. [b]Calibrations curve used: gallic acid (after correction).[13] [c]Calibrations curve used: jaboticabin. [d]Calibrations curve used: quercitin. [e]Calibrations curve used: cyanidin-3-*O*-glucoside.

jaboticaba fruit extracts and the commercial products were unknown. Two different fresh jaboticaba fruit extracts together with their juice and jam products were compared by applying PCA to LC-TOF-MS data. In the score plot based on the data obtained in positive mode, the samples were separated into two well clusters; one cluster belonged to the fresh fruit extracts (Fruits_A_1−A_3 and Fruits_B_1−B_3); the other cluster belonged to commercial products (Juice_1−3 and Jam_1−3) (Figure 3). In the corresponding loading plots (Figure 4) an important marker compound at 26.43 min was detected only in the fresh fruit extracts and was identified as cyanidin-3-*O*-glucoside (**7**). Detailed marker compound analysis in the positive mode allowed us to find that there are four marker compounds (**6**, **7**, **11**, and **20**), which were present in the fresh fruit extracts that were not detected from commercial juice and jam (Table 2). Similarly, the score and loading plots from negative mode data detected citric acid (retention time of 4.22 min) (Figures 3S and 4S in the Supporting Information), which as a food additive was in the commercial juice and jam but not found in fresh jaboticaba fruit extracts. In addition, a common processed food contaminant, tributyl citrate ($C_{18}H_{33}O_7$, $[M + H]^+$, ppm −3.6), was also detected from commercial juice and jam. Therefore, PCA proved to be a good strategy to identify compounds that change in the fruit during processing, as well as compounds that have been added purposely or via contamination.

Anthocyanin Contents Comparison (HPLC-PDA Analysis). Differences in the anthocyanin contents from jaboticaba fruit extract and commercial products (juice and jam) were monitored by HPLC-PDA at 510−520 nm. As shown in Figure 4, two strong peaks, delphinidin 3-*O*-glucoside (**6**) and cyanidin-3-*O*-glucoside (**7**), were observed in the chromatogram of the fresh fruits; however, unexpectedly neither was detected in commercial juice, jam, and wine (Figure 4).

Anthocyanins are not stable during storage.[7,10] Their stability is affected by several factors such as pH, temperature, light, and oxygen.[19] Any or all of these conditions may change during juice and jam production and thereby degrade the anthocyanins. Mulero et al. reported that the contents of anthocyanins in the conventional and organic red wine decreased during 6 months of storage.[7] Maier reported grape pomace processed with pectin and gelatin gels had the most significant effect on the phenolic contents, resulting in total losses up to 24.6%.[10] Also, anthocyanin degradation was accelerated significantly in the blueberry juice after thermal and high-pressure treatment.[8] Landbo and Meyer found freshly pressed black currant juice can contain 134−322 mg anthocyanins/100 mL; however, the anthocyanin content of commercial juice varied from 17 to 133 mg/100 mL, with a mean value of 53 ± 33 mg/100 mL.[20] Ferrari et al. reported that a small concentration of dissolved oxygen could cause the degradation of the anthocyanins.[21] All of the above products were dark-colored fruit drinks, which are a potentially rich source of many dietary anthocyanins and are believed to play an important role in the prevention of many oxidative and inflammatory diseases.[19] Therefore, identifying a method to optimize the anthocyanin content of these dark-colored fruit drinks may be desirable for human health as well as product shelf life reasons. Earlier studies reported that under many storage conditions the monomeric anthocyanin content in commercial grape pomace decreases, whereas the proportion of polymeric anthocyanins increased.[10] The anthocyanin contents of these same grape samples stored in the dark were up to 4 times higher than of those stored under light, thus clearly demonstrating that light is a key factor promoting pigment degradation during storage.[10] Most of the commercial juice, jam, wine, and jelly products we analyzed were in clear glass containers. To maximize color retention of anthocyanins and extend their shelf life, companies may consider using an opaque glass container to store the anthocyanin-enriched products.

To compare anthocyanin stability in jaboticaba extracts and commercial products with those in other popular dark-colored commercial fruit juices and food products, we analyzed blueberry fruit (*Vaccinium carymbosum*) extract with its commercial juice, jam, and jelly, as well as Concord grape (*Vitis labrusca*) fruit extract with its juice, wine, jam, and jelly by HPLC-PDA analyses. On the basis of the LC-TOF-MS data and our earlier studies, 12 anthocyanin compounds (**6**, **7**, **23**−**32**, Figure 4A−N) were identified. The HPLC profiles of blueberry fruit extract (Figure 4E) and juice (Figure 4F) were similar; however, the quantities of some anthocyanin glucosides were different. For example, in blueberry juice both petunidin-3-*O*-glucoside (**27**) and malvidin-3-*O*-arabinoside (**30**) were less than those in blueberry fruit, which was supported by comparing the relative amounts of **27** to **28** and **30** to **31**, respectively. In the chromatogram of jam (Figure 4G), besides the two aforementioned peaks (**27** and **30**), anthocyanin glucoside **7** was not detected and **24** decreased significantly. These trends suggest that anthocyanins linked to glucosides are more easily degraded than those linked to galactoside and arabinoside. This is consistent with our results from Concord grape fruit extract and its products. Malvidin-3-*O*-arabinoside (**30**) was a major constituent of Concord grape fruit extract; however, the amount of **30** decreased in Concord grape juice (Figure 4K), wine (Figure 4L), and jam (Figure 4M). On the basis of the HPLC chromatograms, we can conclude that the blueberry and Concord grape juices that we tested retain their anthocyanins better than jaboticaba juice. The anthocyanins in jaboticaba juice may be less stable due to their glucoside sugars. Also, the anthocyanin- linked glucosides in blueberries and Concord grape jams and wines were more easily degraded than those linked with galactosides and arabinosides. In addition, there was no anthocyanin detected in all of these three dark-colored fruit jellies.

Comparison of Other Phenolic Composition. To compare the phenolic composition of the extract and juice of jaboticaba, blueberry, and Concord grape, we have quantified 38 marker compounds using AUC of the ions scan of LC-TOF-MS (Table 2). Although jaboticaba has fewer anthocyanin constituents when compared with other dark-color fruits, jaboticaba fruit extract and juice contain large quantities of gallic acid, ellagic acid, ellagic acid derivatives, and related ellagitannins, all of which are seldom detected in blueberries and Concord grapes. Earlier studies reported that ellagic acid, detected from *Eucalyptus globulus* leaf, may be responsible for its antioxidant activity concerning COPD.[22] Ellagic acid is a well-known antioxidant compound with strong free radical scavenging and metal chelating properties.[23] The compound has been reported for various biological and pharmacological activities of relevance in the treatment of diseases such as asthma and COPD.[24−26] In foods, ellagic acid is mainly found as polymeric ellagitannins, which can be easily detected and quantified.[4] Tannins are reported to possess biologically multifunctional properties including antioxidant, antibacterial, antiviral, anti-inflammatory, and other activities.[27] Gil et al. reported that the main antioxidant constituents in pomegranate juice are hydrolyzable tannins.[9] The presence of large quantities of ellagic acid and ellagitannins in jaboticaba fruits and juices shows that the fruit can be considered as a rich dietary source of phenolics beneficial to health.[4] However, how to maintain the anthocyanins and other unique constituents, such as jaboticabin, in the juices and other products has become a challenge to commercial producers. Factors such as sterilization, light, acidity, and temperature can affect these products.

Antioxidant Activity Comparison. Most earlier studies showed that there is a very weak correlation between antioxidant activity and anthocyanins in red wine.[7] However, some other investigations indicated that the anthocyanin content of wine correlated with the antioxidant activity of red wine.[28] In our study, we have compared the antioxidant activities of commercial jaboticaba samples with those of fresh fruit extract using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays. The activity data showed that the $DPPH^{\bullet}$ value of fresh fruit extract was higher than those of the commercial juice and jam (IC_{50} = 0.282 ± 0.009 mg/mL in fruit extract, 0.453 ± 0.000 mg/mL in juice, and 0.618 ± 0.023 mg/mL in jam). However, the $ABTS^{\bullet+}$ value of the juice is stronger than those of fruit extracts and jam products (Figure 5). The difference in the activity trend between the two assays can be due to several reasons. In the $ABTS^{\bullet+}$ assay, unlike the $DPPH^{\bullet}$, there is no absorption interference from phytochemical constituents such as anthocyanins due to the wavelength, 734 nm, at which the meseaurements are taken. Another feature of the $ABTS^{\bullet+}$ assay is that the scavenging properties of both lipophilic and hydrophobic constituents can be assessed. The presence of larger quantities of the ellagitannin casuarictin (**12**), which is medium in polarity, in jaboticaba juice compared to the fruit extract (Table 3) may be responsible for the strong $ABTS^{\bullet+}$ activity in the jaboticaba juice. However, the jam in both assays shows the least activity, indicating its low phenolic content. On

the basis of the large number of biologically active phenolic constituents identified in the fruit, including some with limited distribution in nature, such as jaboticabin, and others more widely known and appreciated, such as cyanidin-3-*O*-glucoside and ellagic acid, jaboticaba and its products may be considered as a functional food, but proper processing is important to retain these active constituents in food products.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): CUNY is one of the patent holders for constitutents from jaboticaba (anthocyanins and depsides) for the treatment of COPD, and Kennelly is listed as one of the inventors. This patent is cited in the paper.

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