Article
pubs.acs.org/JAFC

# Bioactive and Marker Compounds from Two Edible Dark-Colored *Myrciaria* Fruits and the Synthesis of Jaboticabin

Shi-Biao Wu,[†,§] Jing Wu,[‡] Zhiwei Yin,[‡] Jinzhu Zhang,[‡] Chunlin Long,[§] Edward J. Kennelly,[*,†,§] and Shengping Zheng[*,‡]

[†]Department of Biological Sciences, Lehman College, and The Graduate Center, The City University of New York, 250 Bedford Park Boulevard West, Bronx, New York, United States 10468

[‡]Department of Chemistry, Hunter College, and The Graduate Center, The City University of New York, 695 Park Avenue, New York, United States 10065

[§]College of Life and Environmental Sciences, Minzu University of China, 27 Zhong-guan-cun South Avenue, Haidian District, Beijing 100081, China

*Supporting Information*

**ABSTRACT:** Jaboticaba (*Myrciaria cauliflora*) and false jaboticaba (*Myrciaria vexator*) fruits are two pleasant-tasting, dark-colored fruits, native to Brazil. They are rich sources of phenolic compounds, including anthocyanins, flavonoids, phenolic acids, and tannins, as well as less well known polyphenols such as depsides. These two fruits are very similar in morphology, but their taste profiles differ markedly. This study was focused on identifying the marker compounds between them using HPLC-PDA and LC-TOF-MS, combined with principal component analysis. As a result, cyanidin-3-*O*-glucoside was found as the major anthocyanin in *Myrciaria* fruits. Delphinidin-3-*O*-glucoside was found to be the marker compound for jaboticaba, while cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinose were two marker compounds distinguishing false jaboticaba. In addition, two ellagitannins, iso-oenothein C and oenothein C, were isolated and identified from both of these fruits for the first time. Jaboticabin, a minor bioactive depside, occurred in both fruits and, because of its potential to treat chronic obstructive pulmonary disease, was successfully synthesized in the laboratory.

**KEYWORDS:** *jaboticaba, Myrciaria cauliflora, Myrciaria vexaxtor, phenolic compounds, anthocyanins, LC-TOF-MS, PCA, jaboticabin*

## ■ INTRODUCTION

Recently, the interest in edible dark-colored fruits, such as blueberry, pomegranate, and jaboticaba, has been growing due to their comprehensive protective effects, especially their antioxidant and anti-inflammatory activity, for human health.[1,2] *Myrciaria cauliflora* (DC.) O. Berg (jaboticaba) and *Myrciaria vexator* McVaugh (false jaboticaba) are two important edible dark-colored fruits, belonging to the Myrtaceae family. Jaboticaba is native to Brazil and often found in the states of São Paulo, Rio de Janeiro, Minas Gerais, and Espírito Santo,[3] while *M. vexator* is native to Mesoamerica and northern South America.[4] Jaboticaba was previously reported by us to have certain anthocyanins, phenolic acids, flavonoids, and tannins,[5,6] and its strong antioxidant and anti-inflammatory activity makes it a new functional fruit and a promising emerging functional food for smokers trying to lessen the impact of lung damage due to cigarette smoke exposure.[6] These activities were mostly due to the presence of a minor depside, jaboticabin.[5,6] According to jaboticabin's antioxidant and anti-inflammatory activity, our group has explored it and anthocyanins for their effects on chronic obstructive pulmonary disease (COPD).[7] COPD is a complex lung disease characterized by irreversible airflow obstruction due to chronic inflammation. The inflammation and proteolysis observed in COPD is secondary to the normal inflammatory response to cigarette smoke, which is the main etiological factor associated with the disease.[8,9] Also, *M. vexator* fruit was reported to have jaboticabin and some other polyphenolic constituents and was found to inhibit cigarette smoke extract-induced MMP-1 expression *in vitro*.[10] Based on these findings, jaboticaba and *M. vexator* fruits may be of significance in the treatment of COPD. However, these two fruits are very similar in morphology, and it is necessary to distinguish them if they are to become a useful functional food. Both of them are 2−4 cm in diameter and round when mature, with pericarp color ranging from red to purple and black. Generally, *M. vexator* fruit is bigger and darker, has a thicker shell, and considered less palatable than jaboticaba fruit. *M. vexator* fruits can be identified as jaboticaba by mistake, and therefore its common name is "false jaboticaba". Up to now, there are no publications that provide chemical marker profiles to distinguish these two edible dark-colored *Myrciaria* fruits.

In the present study, high-performance liquid chromatography with photodiode array detection (HPLC-PDA), electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS), and untargeted principal component analysis (PCA) were used to evaluate the chemotaxonomic and marker compounds in jaboticaba and false jaboticaba. In addition, two ellagitannins,

Received: January 31, 2013
Revised: March 27, 2013
Accepted: March 28, 2013
Published: April 18, 2013

**Figure 1.** Bioactive and marker compounds isolated or detected from jaboticaba or false jaboticaba. **1**: iso-oenothein C; **2**: oenothein C; **3**: delphinidin 3-*O*-glucoside; **4**: cyanidin 3-*O*-glucoside; **5**: cyanidin 3-*O*-galactoside; **6**: cyanidin 3-*O*-arabinoside; **7**: jaboticabin.

iso-oenothein C (**1**) and oenothein C (**2**), were purified and identified by using an activity-guided approach (Figure 1). An important but minor constituent that contributed to the anti-inflammatory activity and COPD treatment of these two dark-colored fruits, jaboticabin (**7**), has been successfully synthesized in the laboratory. Using synthetic jaboticabin, we plan to further explore this depside's biological activity in animal experiments in the near future. Analogues of jaboticabin are being made as well to explore structure− activity relationships.

## ■ MATERIALS AND METHODS

**Reagents.** 1,1-Diphenyl-2-picrylhydrazyl was purchased from Sigma Chemical-Aldrich (St. Louis, MO, USA). Delphinidin 3-*O*-glucoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-galactoside, and cyanidin-3-*O*-arabinoside standards were bought from WuXi AppTec Inc. (Tianjin, China). 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.** All of the fresh fruits were collected from Fruit and Spice Park in Homestead, FL. The samples of *M. cauliflora*_a and *M. cauliflora*_b were collected in April 2002 and July 2011, respectively; the samples of *M. vexator*_a, *M. vexator*_b, and *M. vexator*_c were collected in February 2002, March 2011, and July 2011, respectively. All fruits were collected when ripe and were stored in a −20 °C freezer before extraction. The whole fruits, including peels, pulp, and seeds, were extracted and used for studies in this project.

**Preparation of Fruit Extracts.** All freeze-dried whole jaboticaba and *M. vexator* fruits (each 100 g) were homogenized with 1000 mL of 70% (v/v) MeOH using a blender followed by ultrasonic extraction for 30 min. Extracts were centrifuged (2500 rpm, 5 min at room temperature), and the supernatants were removed and combined. The marc was extracted and centrifuged two more times. Extracts were combined and concentrated *in vacuo* (45 °C), freeze-dried, and kept at −20 °C for long-term storage.

**HPLC-PDA and LC-TOF-MS Analysis.** HPLC-PDA analyses of the extracts (10 mg/mL) were performed using a Waters (Milford, MA, USA) Alliance 2695 system equipped with a 2695 separation module unit and 2996 PDA detector using a 250 × 4.6 mm, 4 $\mu$m Phenomenex Synergi Hydro-RP 80A column (Torrance, CA, USA) with a 3 × 4.0 mm Phenomenex SecurityGuard guard column. 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 for 45 min, and from 15% to 60% B in 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 $\mu$L, respectively. Both column and sample temperature were 25 °C. HPLC-grade methanol was used to dissolve the freeze-dried samples; each sample was brought up to 2 mg/mL and filtered using a 25 mm syringe filter (0.45 $\mu$m PTFE membrane) prior to injection. The results were monitored using a wavelength range of 210−600 nm.

LC-TOF-MS analysis was recorded by the same Waters Alliance 2695 system equipped with a 2695 separation module unit and a 2998 PDA detector using a 100 × 2.0 mm, 2.5 $\mu$m Phenomenex Synergi Hydro-RP 100A column with a 3 × 4.0 mm Phenomenex SecurityGuard guard column.

The mobile phase consisted of solvents (A) 0.1% aqueous formic acid solution and (B) MeCN. Gradient conditions were performed as follow: from 0% to 6% B in 5 min, from 6% to 22% B in 30 min, from 22% B to 30% B in 40 min, and from 30% to 40% B in 50 min, followed by a final increase to 95% in 2 min. The flow rate and the injection volume were 0.2 mL/min and 10 $\mu$L, respectively. Both column and sample temperature were 25 °C. LC/MS-grade methanol was used to dissolve the freeze-dried samples. Each sample was brought up to 2 mg/mL and filtered using a 25 mm syringe filter (0.45 $\mu$m PTFE membrane) prior to injection. Each sample was injected three times.

High-resolution electrospray ionization time-of-flight mass spectrometry (HR-ESI-TOF-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 $m/z$ 100−1500. The capillary voltages were set at 3000 V (positive mode) and 2700 V (negative mode), respectively, and the cone voltage was 30 V. Nitrogen gas was used both for the nebulizer and in desolvation. The desolvation and cone gas flow rates were 600 and 20 L/h, respectively. The desolvation temperature was 400 °C, and the source temperature was 120 °C.

**Chemometric Data Analysis.** Principal component analysis was performed using Markerlynx XS software. The data analysis used positive mode and the parameters used included a retention time range of 4−52 min, a mass range of 100−1500 Da, and a mass tolerance of 40 mDa. Isotopic peaks were excluded for analysis; noise elimination level was set at 1.00; the intensity threshold (counts) of collection parameters was set at 500; retention time tolerance was set

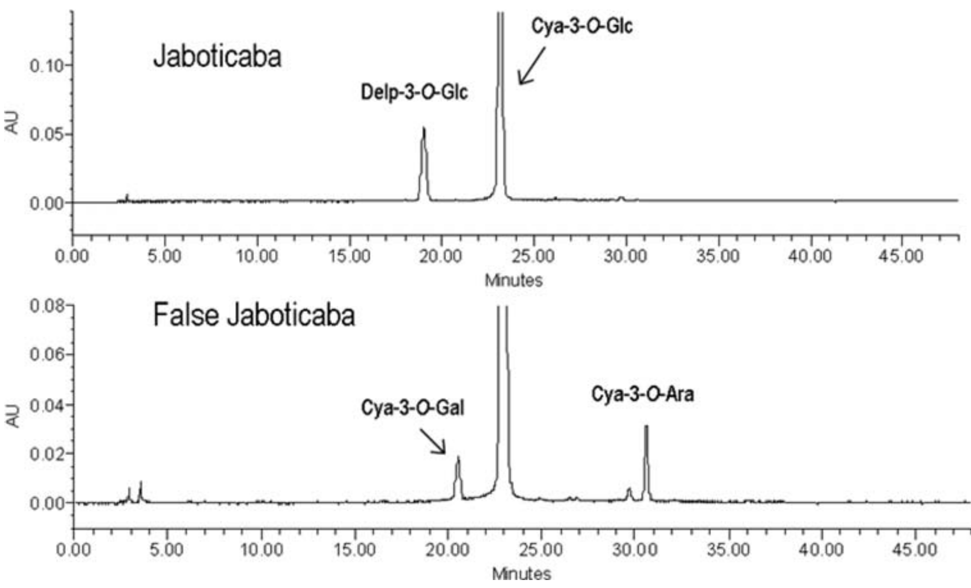


**Figure 2.** HPLC-PDA comparison of jaboticaba and false jaboticaba (*M. vetaxtor*) (520 nm).

**Table 1. LC-MS-TOF Data of the Bioactivity and Marker Compounds Identified from Jaboticaba and False Jaboticaba**

| no. | $t_R$ (min) | UV | $[M]^+$, $[M + H]^+$, or $[M - H]^-$ (M.F., ppm) | adduct and fragmental ion exact masses $[M - X]^+$ or $[M - X]^-$ (M.F., ppm) | identification |
|---|---|---|---|---|---|
| **1** | 11.4 | 230, 256 | 785.0829 $[M + H]^+$ ($C_{34}H_{25}O_{22}$, −1.0) | 807.0619 $[M + Na]^+$ ($C_{34}H_{25}O_{22}Na$, −4.7); 767.0720 $[M + H - H_2O]^+$ ($C_{34}H_{23}O_{21}$, −1.0); 615.0591 $[M + H - H_2O - \text{Galloyal}]^+$ ($C_{27}H_{19}O_{17}$, −1.0); | Iso-oenothein C (NMR) |
| | | | 783.0693 $[M - H]^-$ ($C_{34}H_{23}O_{22}$, 1.5) | 631.0580 $[M - H - H_2O - \text{Galloyal}]^-$ ($C_{27}H_{19}O_{18}$, 1.4) | |
| **2** | 13.6 | 231, 258 | 785.0837 $[M + H]^+$ ($C_{34}H_{25}O_{22}$, 0.0) | 807.0649 $[M + Na]^+$ ($C_{34}H_{25}O_{22}Na$, −1.0); 767.0710 $[M + H - H_2O]^+$ ($C_{34}H_{23}O_{21}$, −2.9); 615.0593 $[M + H - H_2O - \text{Galloyal}]^+$ ($C_{27}H_{19}O_{17}$, 4.7) | Oenothein C (NMR) |
| | | | 783.0691 $[M - H]^-$ ($C_{34}H_{23}O_{22}$, −0.1) | 631.0588 $[M - H - H_2O - \text{Galloyal}]^-$ ($C_{27}H_{19}O_{18}$, 2.7) | |
| **3** | 19.2 | 276, 523 | 465.1035 $[M]^+$ ($C_{21}H_{21}O_{12}$, 0.4) | 303.0487 $[M - \text{Glc}]^+$ ($C_{15}H_{11}O_7$, −3.0); | Delp-3-*O*-Glc (co-injection) |
| | | | 463.0865 $[M - 2H]^-$ ($C_{21}H_{19}O_{12}$, −2.6) | 481.1011 $[M - 2H{+}H_2O]^-$ ($C_{21}H_{21}O_{13}$, 6.0); 509.0909 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{14}$, −4.3); 527.1050 $[M - 2H + HCOOH + H_2O]^-$ ($C_{22}H_{23}O_{15}$, 2.5) | |
| **4** | 21.4 | 280, 517 | 449.1078 $[M]^+$ ($C_{21}H_{21}O_{11}$, −1.3) | 287.0543 $[M - \text{Gal}]^+$ ($C_{15}H_{11}O_6$, −4.5); | Cya-3-*O*-Gal (co-injection) |
| | | | 447.0947 $[M - 2H]^-$ ($C_{21}H_{19}O_{11}$, 4.5) | 465.0985 $[M - 2H + H_2O]^-$ ($C_{21}H_{21}O_{12}$, −10.3); 493.0960 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{13}$, −4.5); 511.1083 $[M - 2H + HCOOH + H_2O]^-$ ($C_{22}H_{23}O_{14}$, −1.0) | |
| **5** | 23.0 | 280, 516 | 449.1070 $[M]^+$ ($C_{21}H_{21}O_{11}$, −3.1) | 287.0535 $[M - \text{Glc}]^+$ ($C_{15}H_{11}O_6$, −7.3); | Cya-3-*O*-Glc (co-injection) |
| | | | 447.0947 $[M - 2H]^-$ ($C_{21}H_{19}O_{11}$, 1.3) | 465.0985 $[M - 2H + H_2O]^-$ ($C_{21}H_{21}O_{12}$, −6.7); 493.0960 $[M - 2H + HCOOH]^-$ ($C_{22}H_{21}O_{13}$, 1.6); 511.1083 $[M - 2H + HCOOH + H_2O]^-$ ($C_{22}H_{23}O_{14}$, 4.9) | |
| **6** | 31.0 | 280, 518 | 419.0971 $[M]^+$ ($C_{20}H_{19}O_{10}$, −1.7) | 287.0544 $[M - \text{Ara}]^+$ ($C_{15}H_{11}O_6$, −4.2); | Cya-3-*O*-Ara (co-injection) |
| | | | 417.0838$[M - 2H]^-$ ($C_{20}H_{17}O_{10}$, 3.8) | 435.0927 $[M - 2H + H_2O]^-$ ($C_{20}H_{19}O_{11}$, 4.4); 463.0862 $[M - 2H + HCOOH]^-$ ($C_{21}H_{19}O_{12}$, −3.2); 481.1028 $[M - 2H + HCOOH + H_2O]^-$ ($C_{21}H_{21}O_{13}$, 9.6) | |
| **7** | 38.7 | 244, 365 | 335.0771 $[M + H]^+$ ($C_{16}H_{15}O_8$, 1.2) | 357.0589 $[M + Na]^+$ ($C_{16}H_{14}O_8Na$, 0.0); 669.1465 $[2M + Na]^+$ ($C_{32}H_{29}O_{16}$, 1.3); 691.1288 $[2M + Na]^+$ ($C_{32}H_{28}O_{16}Na$, 1.9) | Jaboticabin (NMR) |
| | | | 333.0620 $[M - H]^-$ ($C_{16}H_{13}O_8$, 3.0) | 379.0663 $[M - H + HCOOH]^-$ ($C_{17}H_{15}O_{10}$, −0.5); 667.1293 $[2M - H]^-$ ($C_{32}H_{27}O_{16}$, −0.9) | |

at 0.4 min. The retention time and $m/z$ data pair for each peak was determined by the software.

**Isolation of Ellagitannins.** The jaboticaba extract (2.5 g) was fractionated on 500 mg/6 mL Strata X polymeric solid-phase extraction (SPE) tubes (Phenomenex) with a step gradient of 0%, 25%, 50%, and 75% aqueous MeOH (5 mL each). HPLC-PDA analysis showed the 50% fraction was enriched in compounds **1** and **2**. This fraction was evaporated to dryness under $N_2$ gas at room temperature, then dissolved in HPLC-grade menthol and loaded on a semipreparative HPLC (Waters 2695 system with a Phenomenex ODS 25 × 1 cm, 4 $\mu$m column), yielding 3.8 mg of **1** and 4.0 mg of **2**. The method was maintained at 12% MeCN for 30 min, then a linear gradient of MeCN in $H_2O$ (0.1% formic acid) from 12% to 20% for 10 min, followed by 95% MeCN for 10 min (flow rate: 3 mL/min; **1**: $t_R$ = 19.8 min; **2**: $t_R$ = 35.2 min).

**Synthesis of Jaboticabin.** Anhydrous chloroform was distilled from $CaCl_2$, and dichloroethane was distilled from $P_2O_5$, under an atmosphere of argon. All reagents were purchased from commercial sources or synthesized using literature methods.[11] $^1$H and $^{13}$C NMR spectra were recorded on Bruker 500 MHz spectrometers. Chemical shifts ($\delta$ values) were reported in ppm downfield from internal TMS ($^1$H NMR) or $CDCl_3$ ($^{13}$C NMR), respectively. The high-resolution mass spectrometry data were obtained by Dr. Cliff Soll at the Hunter College Mass Spectrometry Facility, taken on an Agilent 6520A Q-TOF using electrospray ionization, and by Dr. Lijia Yang at the City College Mass Spectrometry Facility, taken on an AB Sciex 4000

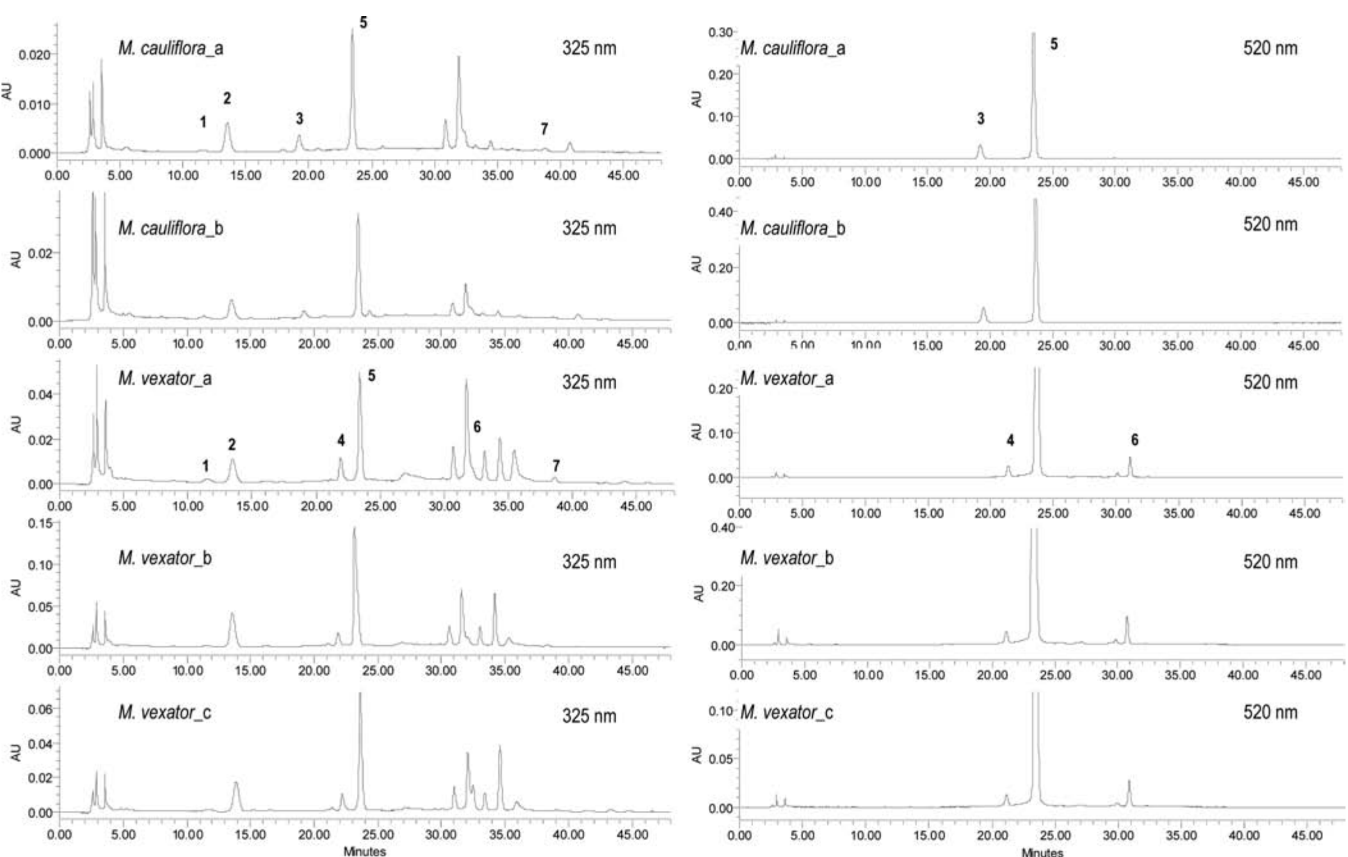


**Figure 3.** HPLC-PDA at 325 and 520 nm of all five samples (collection times include: *M. cauliflora*_a, April 2002; *M. cauliflora*_b, July 2011; *M. vexator*_a, February 2002; *M. vexator*_b, March 2011; and *M. vexator*_c, July 2011). **1**: iso-oenothein C; **2**: oenothein C; **3**: delp-3-*O*-glc; **4**: cya-3-*O*-gal; **5**: cya-3-*O*-glc; **6**: cya-3-*O*-ara; **7**: jaboticabin.

QTRAP LC-MS/MS, to confirm intermediates in the synthesis of jaboticabin. All infrared spectra were taken on a Thermo Nicolet IR100 spectrometer. Column chromatography was performed over silica with a porosity of 60 Å and a particle size of 40−63 $\mu$m.

Synthesis of jaboticabin (**7**) began with the known phloroglucinol triacetate **8**,[11] and direct allylation using Kawamoto's protocol [12] afforded **9** in good yield, presumably through a monodeacetylation/allylation process. Similarly, dibenzylation of **9** led to the known 1-allyloxy-3,5-dibenzyloxybenzene **10** quantitatively.[13] This modified synthesis of **10** led to a clean product without contamination of side products. Claisen rearrangement of **10** was performed under microwave conditions to give substituted phenol **11**. A subsequent esterification was achieved by coupling of phenol **11** with acid chloride **12**, which, in turn, was obtained in three steps from commercially available 3,4-dihydroxybenzoic acid.[14] Ozonolysis followed by Lindgren−Kraus−Pinnick oxidation[15−18] afforded the phenylacetic acid **15**. Treatment of **15** with trimethylsilyldiazomethane[19] followed by global debenzylation[20] provided jaboticabin **7** as an off-white solid, whose $^1$H and $^{13}$C NMR data were in full accord with those reported for the natural product itself.[6]

**1,1-Diphenyl-2-picrylhydrazyl Free Radical (DPPH$^•$) Scavenging.** The DPPH$^•$ scavenging activity was assessed according to the method described by Smith et al.[21] To a 50 $\mu$L aliquot of the sample was added 150 $\mu$L of DPPH solution (400 $\mu$M), 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$^{•+}$) Scavenging.** The determination of ABTS$^{•+}$ scavenging was carried out on the basis of the method of a previous report.[21] The ABTS$^{•+}$ was generated by reacting an ABTS (7 mM) aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 12−16 h, at ambient temperature, and adjusting the absorbance to 0.700 (±0.020) at 734 nm with ethanol. To a 2 $\mu$L aliquot of the sample was added 198 $\mu$L of ABTS$^{•+}$, 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 TEAC ($\mu$mol Trolox/g dry fruit material) values at different time intervals.

## RESULTS AND DISCUSSION

**HPLC-PDA Analysis.** The HPLC-PDA chromatograms (Figure 2) comparing jaboticaba and false jaboticaba fruit extracts (monitored at 520 nm for anthocyanins) shows that cyanidin-3-*O*-glucoside (**5**) was the major anthocyanin detected in both of these fruit extracts. However, delphinidin-3-*O*-glucoside (**3**) can be detected only in jaboticaba fruit extract, while cyanidin-3-*O*-galactoside (**4**) and cyanidin-3-*O*-arabinose (**6**) can be detected only in false jaboticaba fruit extract. These four anthocyanins (**3**−**6**) were identified by LC-TOF-MS analysis and confirmed via co-injection experiments (Table 1). To examine repeatability, extracts from two different jaboticaba fruits and three different false jaboticaba fruits, each collected at different times, were run using the same HPLC method; the

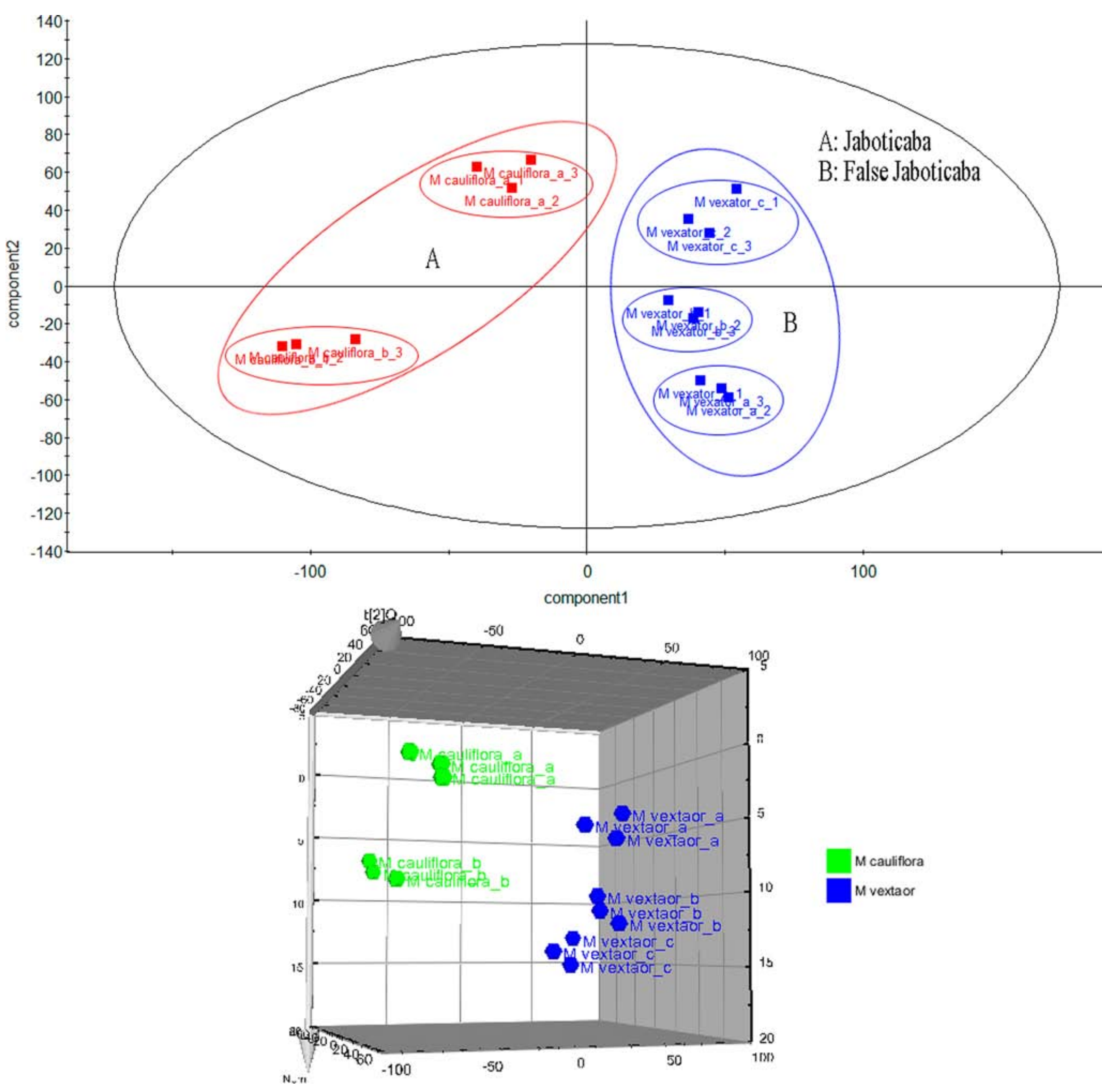


**Figure 4.** Scores of PCA analysis (2D above and 3D below).

resulting chromatograms demonstrated that within each species the major constituents remained the same when examined at both 325 and 520 nm (Figure 3) independent of collection time. However, the chromatograms were significantly different between the two species. The ellagitannins, iso-oenothein C (**1**) and oenothein C (**2**), eluted in both these species at the earlier retention times ($t_R$ range from 12.0 to 13.5 min) owing to their polarity, and jaboticabin (**7**) was also detected in two species at a later retention time of 38.7 min. Jaboticaba fruits were previously reported to have cyanidin-3-*O*-glucoside (**5**) and delphinidin-3-*O*-glucoside (**3**) as the two major anthocyanins.[5,6,22] Also, cyanidin-3-*O*-galactoside (**4**) and cyanidin-3-*O*-arabinose (**6**) were previously reported in false jaboticaba.[10] In our present study, delphinidin-3-*O*-glucoside (**3**) could be tentatively used as a marker compound for jaboticaba species, while cyanidin-3-*O*-galactoside (**4**) and cyanidin-3-*O*-arabinose (**6**) are marker compounds distinguishing false jaboticaba.

**Principal Component Analysis.** PCA analysis is a useful method to distinguish the differences between similar species. In our earlier study, the constituent differences among jaboticaba fruit extracts and their commercial products were also compared by this strategy.[5] Similarly, the same strategy was used to find differences among different ages of ginsengs.[23] In our study, an untargeted PCA analysis based on the positive mode of LC-TOF-MS data was used to find different ions and marker compounds between these two close species. In both two- and three-dimensional score plots based on the data obtained in positive mode, the samples separated well into two clusters: one cluster belonged to the two jaboticaba fruit extracts (*M. cauliflora*_a and *M. cauliflora*_b); the other cluster belonged to three false jaboitcaba fruit extracts (*M. vexator*_a,

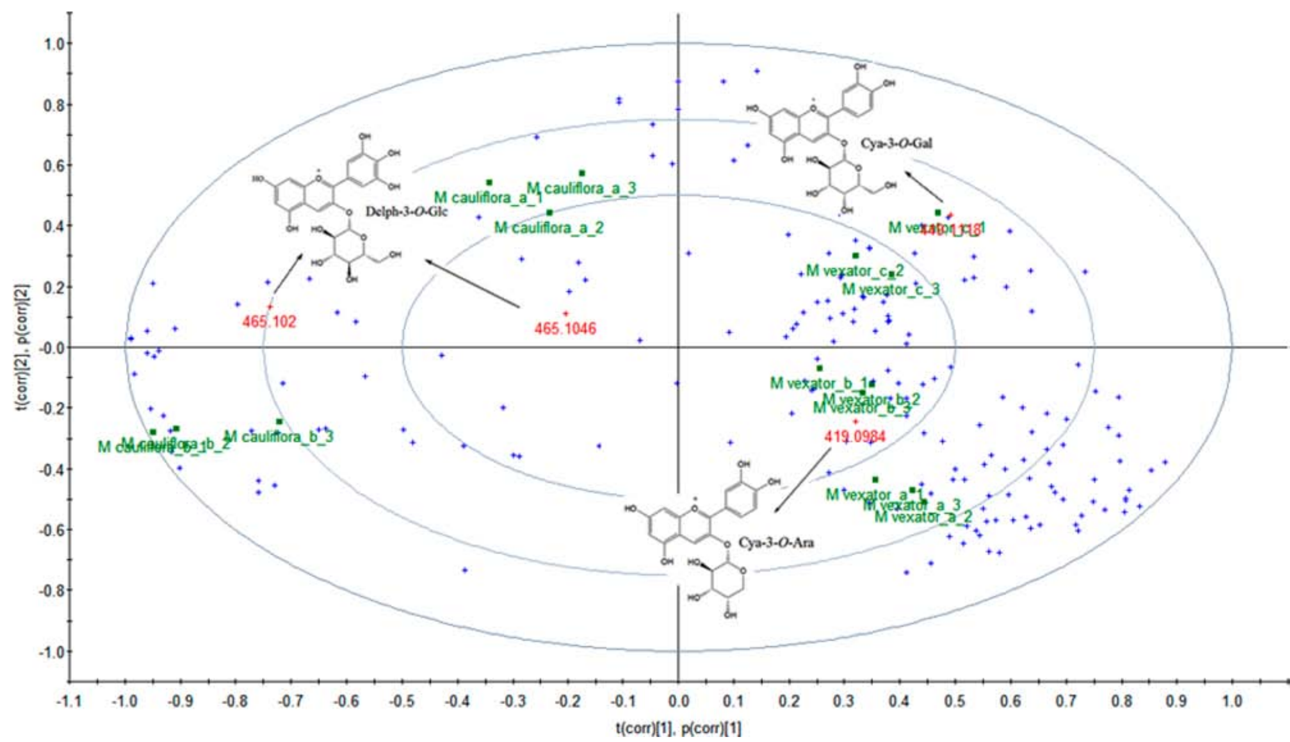


**Figure 5.** Loading biplots of PCA analysis.

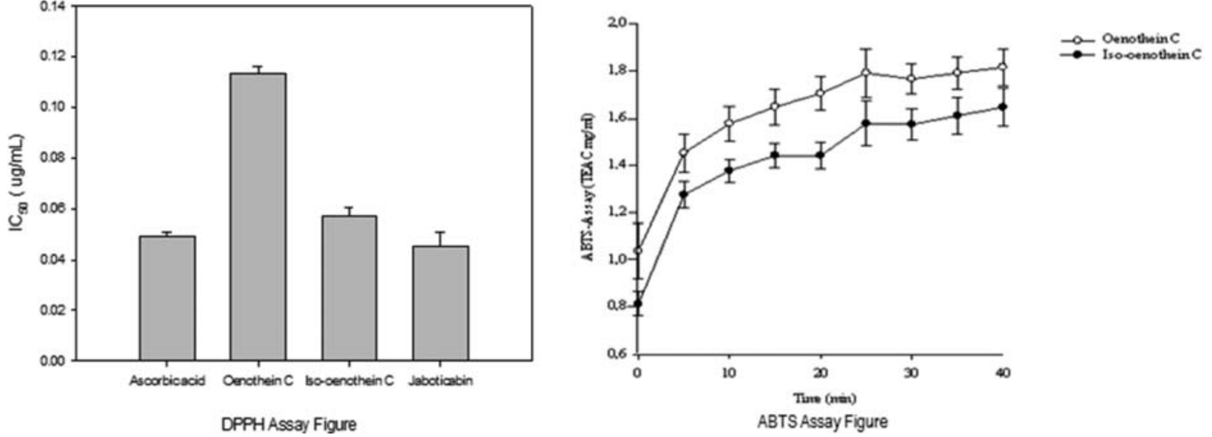


**Figure 6.** DPPH and ABTS assay of the bioactivity of compounds from *Myrciaria* fruits.

*M. vexator*_b, *and M. vexator*_c) after each extract was injected three times (Figure 4), and it seems that these two clusters were dissimilar based on their differences in retention time, exact mass, and intensity of the ions. Figure 5 is a corresponding loading biplot, which shows the correlation among the samples and the metabolites within a single plot; this biplot can help to identify the components that are responsible for their difference. As displayed in Figure 5, an important marker compound with $m/z$ 465.1046 ($C_{21}H_{21}O_{12}$, $t_R$: 19.2 min) was detected only in the jaboticaba fruit extract cluster and was identified as delphinidin 3-*O*-glucoside (**3**). On the other hand, another two marker compounds, $m/z$ 449.1118 ($C_{21}H_{21}O_{11}$ $t_R$: 21.4 min) and $m/z$ 419.0984 ($C_{20}H_{19}O_{10}$ $t_R$: 31.0 min), identified as cyanidin 3-*O*-galactoside (**5**) and cyanidin 3-*O*-arabinoside (**6**), were found only in the false jaboticaba fruit extract cluster. All of these findings were in agreement with the above HPCL-PDA analysis and further confirmed that these three characteristic anthocyanins (**3**, **4**, and **6**) were marker compounds distinguishing these two similar *Myrciaria* fruits. This is the first study on the chemotaxonomic differences and bioactive marker compounds of these two edible dark-colored fruits using LC-TOF-MS combined with an unsupervised PCA analysis strategy. It should be noted that because of shared biosynthetic pathways, detection of these three marker compounds in both of these fruits may be possible using different techniques; however, their quantitative differences should remain strong enough to distinguish these fruits.

Since these two *Myrciaria* fruits do not transport well, fresh fruits are not typically shipped far from where they grow in Central and South America and are seldom imported fresh to the United States. We have worked on fruits that were grown in southern Florida outside of their native range. Therefore, the marker compounds in this study have not yet been compared to those of fresh fruits grown in Central or South America.

**Figure 7.** Method for jaboticabin (7) synthesis.

**Identification of Ellagitannins.** Iso-oenothein C (**1**) and oenothein C (**2**) were isolated from these two *Myrciaria* fruits using SPE and semipreparative HPLC and identified. Although jaboticaba fruits were reported to contain tannins before,[5,24] this is the first report of the isolation and identification of pure ellagitannins from *Myrciaria* fruits. Their $^{1}$H and $^{13}$C NMR data recorded in methanol-$d_4$ were in agreement with the reference.[25] Both of these compounds exist as an inseparable equilibrium mixture of $\alpha$ and $\beta$ forms in solution (methanol-$d_4$), as there are two distinct patterns of proton resonances (1:1) for the sugar and phenolic moieties in their $^{1}$H NMR spectra. Iso-oenothein C (**1**) was reported in 2012 as a new compound isolated from jamun (*Eugenia jambolana*, Myrtaceae) seeds,[25] which showed strong $\alpha$-glucosidase inhibitory activity with $IC_{50}$ 8.2 $\mu$M. Oenothein C (**2**) also showed a moderate inhibitory activity in the same assay.[25] The isolation of these two ellagitannins indicated that these two Myrciaria fruits may also have $\alpha$-glucosidase inhibitory activity and have benefits toward diabetes, which was supported by previous reports.[26,27]

**Biological Activity Analysis.** Anthocyanins, such as delphinidin 3-*O*-glucoside, cyanidin-3-*O*-galactoside (**4**), cyanidin-3-*O*-glucoside (**5**), and cyanidin-3-*O*-arabinose (**6**) in our study, are well known for their strong antioxidant effect.[28] However in this study, the antioxidant activity of the ellagitannins iso-oenothein C (**1**) and oenothein C (**2**) was evaluated by using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays for the first time. As a result, iso-oenothein C (**1**) showed antioxidant activity in the $DPPH^{\bullet}$ assay, which was slightly weaker than the positive control (ascorbic acid), and oenothein C (**2**) also showed moderate antioxidant activity (Figure 6). Similarly, these two compounds showed antioxidant effects in the $ABTS^{\bullet+}$ assay with a TEAC range around 1.6 mg/mL in 20 min; however, in this assay oenothein C (**2**) had stronger activity than iso-oenothein C (**1**) (Figure 6). On the other hand, jaboticabin (**7**) was found to have stronger antioxidant activity compared with ascorbic acid in the $DPPH^{\bullet}$ assay (Figure 6), which was seen in earlier reports.[6]

Jaboticabin (**7**) is an important functional depside, which can inhibit cigarette smoke extract-induced MMP-1 expression and contributed strongly to anti-COPD activity;[6] in the present study, compound **7** can be detected in both these edible dark-colored *Myrciaria* fruits using exact mass full scan function in the TOF-MS Markerlynx software. This indicates these fruits may be important and beneficial to human health, particularly in the treatment of COPD. Considering the broad array of previously reported jaboticaba bioactivity, such as antibacterial,[29,30] improvement of insulin resistance,[27] antiobesity,[27] antiproliferative, and antimutagenic,[31] these two *Myrciaria* fruits may have the potential to be developed as functional foods, although the taste profile of *M. vexator* is considered less pleasant.

**Synthesis of Jaboticabin.** Although jaboticabin (**7**) proved to have strong anti-inflammatory activity and showed potential benefits in COPD,[6] it was shown to be a minor constituent in the fruit extract and sometimes could not be detected in jaboticaba commercial products, such as juice, jam, and wine.[5] After we reported this new compound from jaboticaba in 2006,[6] it has since been reported from kiwi,[32] cranberry,[33] and chokeberry fruits.[34] This depside, together with its derivatives, exist in many important edible fruits. Jaboticabin and its derivatives also exhibit potent antioxidant activity.[34] Therefore, in order to have more of this compound for *in vivo* testing and for use as authentic standard compound, the total synthesis of this compound was undertaken. The synthesis of jaboticabin is outlined in Figure 7 and described in detail in the Materials and Methods section. In summary, jaboticabin was synthesized from commercially available phloroglucinol and 3,4-dihydroxybenzoic acid in nine linear steps. With sufficient supplies of jaboticabin, we are now beginning to plan animal experiments and also to make

synthetic derivatives to study the SAR of this depside in the near future.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

LC-TOF-MS data of all the compounds (Figure 1S); jaboticabin full scan chromatogram (Figure 2S); loading spot of PCA (Figure 3S); detailed synthesis experiments; and NMR spectra are available free of change via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*E-mail: Edward.kennelly@lehman.cuny.edu (E. J. Kennelly). Phone: +1 718 960 1105. Fax: +1 718 960 8236. E-mail: szh0007@hunter.cuny.edu (S. Zheng). Phone: +1 212 396 6322. Fax: +1 212 772 5332.

### Funding

This work was supported by NIH-NHLBI, grant 5SC1HL096016. This project was also supported by the Ministry of Education of China through its 111 and 985 projects for Minzu University of China, grants B08044 and MUC985-9. S.Z. would like to thank the Bertha and Louis Weinstein Research Grant.

### Notes

The authors declare the following competing financial interest(s): The corresponding author (Kennelly) is a co-inventor of a patent for the use of jaboticaba for COPD treatment.

## ■ ACKNOWLEDGMENTS

We would like to thank Chris Rollins, Eric McDonough, and Adam Negrin for collecting jaboticaba and *M. vexator* fruits from the Fruit and Spice Park (Homestead, Florida). The authors also thank Miriam Fredrick for her language assistance.

## ■ ABBREVIATIONS USED

COPD, chronic obstructive pulmonary disease; HPLC-PDA, high-performance liquid chromatography with photodiode array detection; ESI-TOF-MS, electrospray ionization time-of-flight mass spectrometry; PCA, principal component analysis; Q-TOF, quadrupole time-of-flight mass spectrometer; QTRAP-MS, quadrupole-linear ion trap mass spectrometer; DPPH$^{\bullet}$, 1,1-diphenyl-2-picrylhydrazyl free radical; ABTS$^{\bullet+}$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) free radical; Glc, glucoside; Gal, galactoside; Ara, arabinose; SAR, structure−activity relationship; NMR, nuclear magnetic resonance

## ■ REFERENCES

(1) Dastmalchi, K.; Flores, G.; Petrova, V.; Pedraza-Penalosa, P.; Kennelly, E. J. Edible neotropical blueberries: antioxidant and compositional fingerprint analysis. *J. Agric. Food Chem.* **2011**, *59*, 3020−3026.

(2) Flores, G.; Dastmalchi, K.; Dabo, A. J.; Whalen, K.; Pedraza-Penalosa, P.; Foronjy, R. F.; D'Armiento, J. M.; Kennelly, E. J. Antioxidants of therapeutic relevance in COPD from the neotropical blueberry *Anthopterus wardii*. *Food Chem.* **2012**, *131*, 119−125.

(3) Santos, D. T.; Meireles, M. A. A. Jabuticaba as a source of functional pigments. *Pharmacogn. Rev.* **2009**, *3*, 137−142.

(4) Mitra, S. K. *Important Myrtaceae Fruit Crops*. ISHS Acta Horticulturae: Mérida and Aguascalientes, Mexico, 2010.

(5) Wu, S.-B. D., K.; Long, C. L.; Kennelly, E. J. Metabolite profiling of jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. *J. Agric. Food Chem.* **2012**, *60*, 7513−7525.

(6) Reynertson, K. A.; Wallace, A. M.; Adachi, S.; Gil, R. R.; Yang, H.; Basile, M. J.; D'Armiento, J.; Weinstein, I. B.; Kennelly, E. J. Bioactive depsides and anthocyanins from jaboticaba (*Myrciaria cauliflora*). *J. Nat. Prod.* **2006**, *69*, 1228−1230.

(7) D'Armiento, J., Reynertson, K. A., Kennelly, E. J., & Wallace, A. M. *Bioactive depside and anthocyann compounds, compositions, and methods of use*. U.S. Patent PCT/US07/17087, 12/309,804. 2007.

(8) Boschetto, P.; Quintavalle, S.; Miotto, D.; Cascio, N. L.; Zeni, E.; Mapp, C. E. Chronic obstructive pulmonary disease (COPD) and occupational exposures. *J. Occup. Med. Toxicol.* **2006**, *1*, 11−17.

(9) Biswas, S.; Hwang, J. W.; Kirkham, P. A.; Rahman, I. Pharmacological and dietary antioxidant therapies for chronic obstructive pulmonary disease. *Curr. Med. Chem.* **2012**, *19*, PMID: 22963552.

(10) Dastmalchi, K.; Flores, G.; Wu, S.-B.; Ma, C. H.; Dabo, A. J.; Whalen, K.; Reynertson, K. A.; Foronjy, R. F.; D'Armiento, J. M. Edible *Myrciaria vexator* fruits: bioactive phenolics for potential COPD therapy. *Bioorg. Med. Chem.* **2012**, *20*, 4549−4555.

(11) Haines, A. H.; Hughes, D. L. Benzene-1,3,5-triyl triacetate. *Acta Crystallogr.* **2009**, *E65*, O3279.

(12) Kawamoto, H.; Nakatsubo, F.; Murakami, K. *O*-Benzylation of phloroglucinol *via* phloroglucinol triacetate. *Synth. Commun.* **1996**, *26*, 531−534.

(13) Machado, A. H. L.; Sousa, M. A.; Patto, D. S. C.; Azevedo, L. F. S.; Bombonato, F. I.; Correia, C. R. D. The scope of the heck arylation of enol ethers with arenediazonium salts: a new approach to the synthesis of flavonoids. *Tetrahedron Lett.* **2009**, *50*, 1222−1225.

(14) Wang, W.-L.; Chai, S. C.; Ye, Q.-Z. Synthesis and structure-function analysis of Fe(II)-form-selective antibacterial inhibitors of *Escherichia coli* methionione aminopeptidase. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1080−1083.

(15) Lindgren, B. O.; Nilsson, T. Preparation of carboxylic acids from aldehydes (including hydroxylated benzaldehydes) by oxidation with chlorite. *Acta Chem. Scand.* **1973**, *27*, 888−890.

(16) Kraus, G. A.; Taschner, M. J. Model studies for the synthesis of quassinoids. 1. Construction of the BCE ring system. *J. Org. Chem.* **1980**, *45*, 1175−1176.

(17) Kraus, G. A.; Roth, B. Synthetic studies toward verrucarol. 2. Synthesis of the AB ring system. *J. Org. Chem.* **1980**, *45*, 4825−4830.

(18) Bal, B. S.; Childers, W. E.; Pinnick, H. W. Oxidation of $\alpha$, $\beta$-unsaturated aldehydes. *Tetrahedron* **1981**, *37*, 2091−2096.

(19) Kühnel, E.; Laffan, D. D. P.; Lloyd-Jones, G. C.; MartínezdelCampo, T.; Shepperson, I. R.; Slaughter, J. L. Mechanism of methyl esterification of carboxylic acids by trimethylsilydiazomethane. *Angew. Chem., Int. Ed.* **2007**, *46*, 7075−7078.

(20) Mcleod, M. C.; Wilson, Z. E.; Brimble, M. A. Formal synthesis of berkelic acid: a lesson in $\alpha$-alkylation chemistry. *J. Org. Chem.* **2012**, *77*, 400−416.

(21) Smith, R. C.; Reeves, J. C.; Dage, R. C.; Schnettler, R. A. Antioxidant properties of 2-imidazolones and 2-imidazolthiones. *Biochem. Pharmacol.* **1987**, *36*, 1457−1460.

(22) Reynertson, K. A.; Yang, H.; Jiang, B.; Basile, M. J.; Kennelly, E. J. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chem.* **2008**, *109*, 883−890.

(23) Kim, N.; Kim, K.; Lee, D.; Shin, Y.-S.; Bang, K.-H.; Cha, S.-W.; Lee, J.-W.; Choi, H.-K.; Hwang, B.-Y.; Lee, D. Nontargeted metabolomics approach for age differentiation and structure interpretation of age-dependent key constituents in hairy roots of panax ginseng. *J. Nat. Prod.* **2012**, *75*, 1777−1784.

(24) Morton, J. *Fruits of Warm Climates*; Self-published: Winterville, NC, 1987; pp 386−388.

(25) Omar, R.; Li, L.; Yuan, T.; Seeram, N. P. $\alpha$-Glucosidase inhibitory hydrolyzable tannins from *Eugenia jambolana* seeds. *J. Nat. Prod.* **2012**, *25*, 1505−1509.

(26) Alezandro, M. R.; Lajolo, F. M.; Genovese, M. Functional potential of jaboticaba, a Brazilian native fruit. *Pacifichem 2010*,

*International Chemical Congress of Pacific Basin Societies*; Honolulu, HI, United States, 2010.

(27) Lenquiste, S. A.; Batista, Â. G.; Marineli, R. da. S.; Dragano, N. R. V.; Moróstica, M. R., Jr. Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. *Food Res. Int.* **2012**, *49*, 153−160.

(28) Castañeda-Ovando, A.; Pacheco-Hernández, M. de L.; Páez-Hernández, M. E.; Rodríguez, J. A.; Galán-Vidal, C. A. Chemical studies of anthocyanins: a review. *Food Chem.* **2009**, *113*, 859−871.

(29) Haminiuk, C. W. I.; Plata-Oviedo, M. S. V.; Guedes, A. R.; Stafussa, A. P.; Bona, E.; Carpes, S. T. Chemical, antioxidant and antibacterial study of Brazilian fruits. *Int. J. Food Sci. Technol.* **2011**, *46*, 1529−1537.

(30) Souza-Moreira, T. M.; Moreira, R. R. D.; Sacramento, L. V. S.; Pietro, R. C. Histochemical, phytochemical and biological screening of *Plinia cauliflora* (DC.) Kausel, Myrtaceae, leaves. *Braz. J. Pharmacogn.* **2008**, *20*, 48−53.

(31) Leite-Legatti, A. V.; Batista, Â. G.; Dragano, N. R. V.; Marques, A. C.; Malta, L. G.; Riccio, M. F.; Eberlin, M. N.; Machado, A. R. T.; de Carvalho-Silva, L. B.; Ruiz, A. L. T. G.; de Carvalho, J. E.; Pastore, G. M.; Maróstica Júnior, M. R. Jaboticaba peel: antioxidant compounds, antiproliferative and antimutagenic activities. *Food Res. Int.* **2012**, *49*, 596−603.

(32) Wurms, K. V.; Cooney, J. M. Isolation of a new phenolic compound, 3,5-dihydroxy-2(methoxycarbonylmethyl)phenyl 3,4-dihydroxybenzoate, from leaves of *Actinidia chinensis* (kiwifruit). *Asian J. Biochem.* **2006**, *1*, 325−332.

(33) Turner, A.; Chen, S.-N.; Nikolic, D.; van Breemen, R.; Farnsworth, N. R.; Pauli, G. F. Coumaroyl iridoids and a depside from cranberry (*Vaccinium macrocarpon*). *J. Nat. Prod.* **2007**, *70*, 253−258.

(34) Li, J.; Deng, Y.; Yuan, C.; Pan, L.; Chai, H.; Keller, W. J.; Kinghorn, A. D. Antioxidant and auinone reductase-inducing constituents of black chokeberry (*Aronia melanocarpa)* fruits. *J. Agric. Food Chem.* **2012**, *60*, 11551−11559.