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Anthocyanins Present in Selected Tropical Fruits: Acerola, Jambolão, Jussara, and Guajiru

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Many tropical fruits are rich in anthocyanins, though limited information is available about the characterization and quantification of these anthocyanins. The identification and quantification of anthocyanin pigments in four tropical fruits were determined by HPLC-MS/MS. Fruits studied included acerola (*Malphigia emarginata*), jussara (*Euterpe edulis*), jambolão (*Syzygium cumini*), and guajiru (*Chrysobalanus icaco*). All four fruits were found to contain anthocyanin pigments. Anthocyanidin backbones included cyanidin, delphinidin, peonidin, pelargonidin, petunidin, and malvidin. Guajiru contained several acylated forms, while acerola, jussara, and jambolão contained only nonacylated glycosides. These results demonstrate that these tropical fruits are rich in anthocyanins and that the anthocyanins are widely ranging in anthocyanidin backbone, glycosylation, and acylation.

KEYWORDS: *Malpighia emarginata*; *Euterpe edulis*; *Syzygium cumini*; *Chrysobalanus icaco*; acerola; jambolão; jussara; guajiru; anthocyanin

INTRODUCTION

Anthocyanins are brightly colored compounds responsible for much of the red, blue, and purple colors in fruits, vegetables, and ornamental crops. Evidence continues to emerge to show the importance of these compounds for human health. Health benefits associated with anthocyanin intake include reduced risk of coronary heart disease (*1*), protection against obesity and hypoglycemia (*2*), memory enhancement (*3*), and protection of fetal brain tissue (*4*). Anthocyanins are strong antioxidants, which may be related to the health benefits they convey.

Anthocyanidins are flavylium (2-phenylbenzopyrylium) structures with varying hydroxyl or methoxyl substitutions. The anthocyanin forms found in foods are glycosides and acylglycosides of six common aglycon anthocyanidins: pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin. Recently, anthocyanin structures have been identified in fruits and berries (*5*) and in vegetables, nuts, and grains (*6*) that are commonly consumed in the United States.

Many tropical fruits are characterized by bright red or purple pigments, suggesting the possible presence of anthocyanins, though reports of specific anthocyanin contents of tropical fruits are somewhat limited. Acerola (*Malphigia emarginata*) fruits, also known as barbados cherries, are small and round, with bright red or occasionally orange skin and orange-red pulp. This fruit is particularly known for its high vitamin C content and has been used successfully to improve vitamin C serum levels in the elderly (*7*) and in children (*8*). Acerola is consumed mainly as juice, though it is also exported to Japan and the United States as pulp or powder to be used in product formulations. Guajiru (*Chrysobalanus icaco*), also known as abajeru or coco-plum, has been used in traditional medicines, and while fruit consumption is limited, information about content of healthful pigments may increase demand. The fruits are globular to round, and the skin color varies from pinkish to

Table 1. Tropical Fruits: Common and Latin Names

common name	Latin name	other names
acerola	*M. emarginata*	barbados cherry
guajiru	*C. icaco*	abajeru coco-plum
jambolão	*S. cumini*	jamelão jambul jamblon black plum
jussara	*E. edulis*	
açaí	*E. oleracea*	

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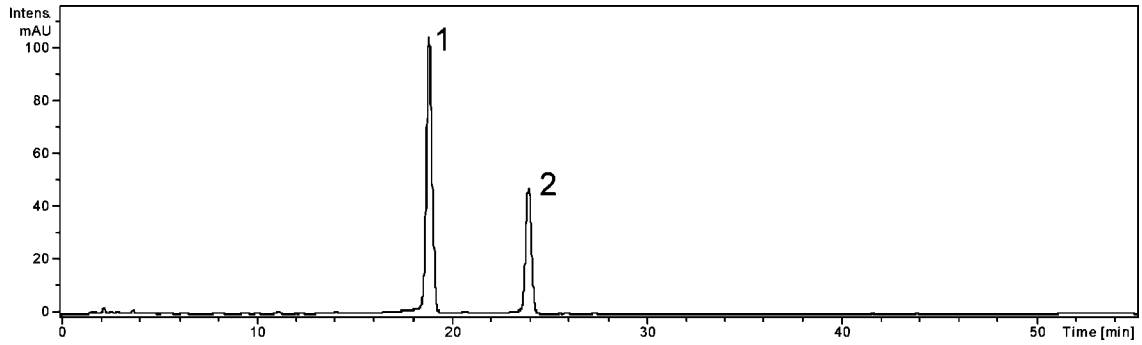

Figure 1. HPLC chromatogram showing the anthocyanin profile for acerola. Refer to **Table 2** for the identification of each peak.

Table 2. Identification of Anthocyanins in Acerola

peak	t_R (min)	$[M]^+$ (*m*/*z*)	MS/MS (*m*/*z*)	anthocyanin	mg/100 g dry weight acerola II47/1	mg/100 g dry weight acerola roxinha
1	18.7	433	287	cyanidin 3-rhamnoside	359	181
2	23.6	417	271	pelargonidin 3-rhamnoside	169	80

purplish black. Jambolão (*Syzygium cumini*), also known as jamelão, jambul, black plum, or jamblon, is the fruit of a well-disseminated tropical tree commonly found in the northeast of Brazil. Jambolão looks like a purple olive and has a sour taste. While underutilized as a food, Jambolão has been used for nonbiologic applications. The use of a natural dye obtained from jambolão extract as the molecular sensitizer of nanostructured TiO_2 films resulted in photoanodes which were employed in photoelectrochemical solar cells (*9*). Jussara, the fruit from South American palm *Euterpe edulis*, is round and has a purple pulp covering a hard seed. Despite its wide distribution in Brazil, jussara is much less commonly eaten than the other well-known palm fruit, açaí (*Euterpe oleracea*). Common and Latin names of these fruits are summarized in **Table 1**.

While these fruits are recognized for their bright colors, limited information is available about their anthocyanin structures and contents. Therefore, in this investigation, we identified and quantified anthocyanins in these tropical fruits: acerola, jambolão, guajiru, and jussara.

MATERIALS AND METHODS

Chemicals. High-performance liquid chromatography (HPLC) grade water and methanol were obtained from Fisher Scientific (Philadelphia, PA). Formic acid (reagent grade) was obtained from Sigma Chemical. Cyanidin 3-glucoside and cyanidin 3-rutinoside were purchased from Indofine Chemical Co. (Somerville, NJ). Delphinidin 3,5-diglucoside was purchased from Aapin Chemicals Ltd. (Abingdon, Oxon, U.K.). Pelargonidin 3-glucoside and peonidin 3-glucoside were purchased from Chromadex (Santa Ana, CA).

Fruits. Acerola cv. roxinha and acerola clone II47/1 were collected at the Embrapa experimental station at Pacajus, CE, Brazil. Jambolão and guajiru were collected at Ceará State, Brazil. Jussara fruits were supplied by Instituto Botânico de São Paulo, São Paulo, Brazil. Fruit pulps were freeze-dried and stored at −80 °C until extraction and analysis.

Fruit Extraction. One hundred grams of each fruit was freeze-dried, and 100 mg of freeze-dried fruit was weighed into a test tube before 2 mL of 10% methanolic formic acid was added. Samples were vortex-mixed for 1 min and sonicated for 10 min prior to centrifugation at 2500*g* for 10 min at 20 °C. The supernatant was decanted to a collection vial, and samples were extracted three more times with 2 mL of 10% methanolic formic acid. The combined extract was diluted to 10 mL with methanol–10% aqueous formic acid (1:9) prior to chromatographic analysis.

LC-MS Conditions. Chromatography was performed on an Agilent HP series 1100 LC MSD trap system with a G1315A diode array detector (DAD), a cooled column compartment (20 °C), and a Zorbax SB-C18 column (3.5 μm, 4.6 × 150 mm). The mobile phase consisted of 10% aqueous formic acid (solvent A) and 10% formic acid in methanol (solvent B). The gradient was linear from 12% solvent B to 25% solvent B over 32 min, then linear to 60% solvent B at 48 min, then linear to 100% solvent B at 50 min and held there for 1 min, and then reduced back to 12% solvent B at 55 min. The flow rate was 1 mL/min. The DAD was set to collect the signal at 530 nm. The MSD source was positive electrospray (ESI), and the following conditions were used: nebulizer pressure set at 15 psi, drying gas (N_2) delivered at 5 L/min, drying temperature set at 325 °C, skimmer set at 40 V, octopole RF amplitude set at 187 Vpp, and capillary exit set at 128.5 V. The mass spectrometer was set to scan from *m*/*z* 50 to *m*/*z* 2200 at a scan resolution of 13000 amu/s, and the ion trap scan was set to scan from *m*/*z* 100 to *m*/*z* 1000.

Quantification was performed on the basis of DAD data. An external standard curve of cyanidin 3-glucoside was used, and concentrations have been expressed as cyanidin 3-glucoside equivalents. Quantification was performed using the Agilent Chemstation software. Compound identification was primarily based on mass spectrometric data for molecular ions and MS-MS product ions and on published observations for anthocyanins in fruits and vegetables (*5*, *6*, *10*). Identifications were confirmed against elution times of authentic standards when available.

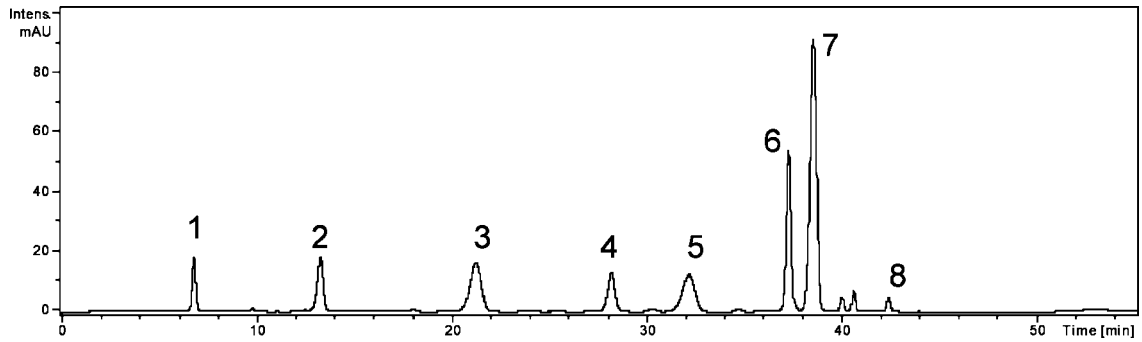

Figure 2. HPLC chromatogram showing the anthocyanin profile for guajiru. Refer to **Table 3** for the identification of each peak.

Table 3. Identification of Anthocyanins in Guajiru

peak	t_R (min)	$[M]^+$ (*m*/*z*)	MS/MS (*m*/*z*)	anthocyanin	mg/100 g dry weight
1	6.9	465	303	delphinidin 3-galactoside or glucoside	44
2a	13.3	479	317	petunidin 3-galactoside or glucoside	74[b]
2b	13.3	507	303	delphinidin 3-(6″-acetoyl)galactoside	
3	21.3	521	317	petunidin 3-(6″-acetoyl)galactoside (?)[a]	121
4	28.3	507	303	delphinidin 3-(6″-acetoyl)glucoside	63
5	32.0	549	303	delphinidin 3-(6″-succinyl)rhamnoside	100
6	37.2	521	317	petunidin 3-(6″-acetoyl)glucoside (?)[a]	164
7	39.5	563	317	petunidin 3-(6″-succinyl)rhamnoside	367
8	42.3	547	301	peonidin 3-(6″-succinyl) rhamnoside	25

[a] Tentative identification. This mass spectrum is also in accord with that of petunidin 3-(6″-oxaloyl)xyloside or petunidin 3-(6″-oxaloyl)arabinoside. [b] Anthocyanin content of this peak is a combination of the two compounds coeluting.

RESULTS AND DISCUSSION

Of available methods for investigating anthocyanin structure, HPLC in tandem with ESI-MS/MS provides particularly valuable information (*6*, *10*). Mass spectrometric analysis of anthocyanins produces easily distinguishable fragment ions from cleavage between the anthocyanidin backbone and glycosidic substitutions (*6*). In this study, HPLC-ESI-MS/MS was used to determine anthocyanin structures in four tropical fruits. We considered the *m*/*z* of the molecular ion and of MS-MS product ions. MS-MS product ions of *m*/*z* 271 suggested pelargonidin, *m*/*z* 287 suggested cyanidin, *m*/*z* 303 suggested delphinidin, *m*/*z* 301 suggested peonidin, *m*/*z* 317 suggested petunidin, and *m*/*z* 331 suggested malvidin. The mass differences between molecular ions and MS-MS product ions were used to identify substitutions on the aglycon backbone. In addition to analysis of mass spectra, regularities in general structural patterns of anthocyanins were considered in assigning compound identifications. For example, in cases where a sugar was found to be connected to a single position of the anthocyanidin, the connection was assumed to occur at position 3 of the pyrylium ring (*6*). Elution order of anthocyanin glycosides using reverse-phase HPLC follows somewhat predictable patterns, and these were also considered in structure identification (*5*). When authentic standards were available, comparisons of retention times were used to confirm identifications. The mass spectra of these chromatographically separated fruit extracts combined with previously published observations and use of standards allowed the characterization of anthocyanins in several tropical fruits for the first time.

A wide distribution of anthocyanins was observed in the tropical fruits studied here. Wu and Prior (*5*) noted that fruits often displayed one of two distribution patterns: (1) sugar-determined, in which different anthocyanidins are present with matching sugar patterns, or (2) anthocyanidin-determined, in which a single anthocyanidin is dominant with other minor anthocyanin components present. Two fruits fell clearly under the sugar-determined category: acerola, with two rhamnosidic anthocyanins, and jambolão, with diglucosidic compounds of five different anthocyanidins. Jussara fit better into the anthocyanidin-determined category, with cyanidin being the major anthocyanidin. Guajiru was more difficult to categorize, with three anthocyanidin backbones present and at least three different sugar groups.

Acerola. Acerola fruit extract exhibited two anthocyanin peaks (**Figure 1**), and these are identified in **Table 2**. These peaks were identified as cyanidin 3-rhamnoside and pelargonidin 3-rhamnoside. Cyanidin 3-rhamnoside and pelargonidin 3-rhamnoside were identified earlier in acerola by NMR (*11*).

Anthocyanin content of acerola was dependent on cultivar (**Table 2**). Cyanidin 3-rhamnoside content was 359 and 169 mg/100 g dry weight in acerola clone II47/1 and roxinha, respectively, and pelargonidin 3-rhamnoside content was 181 and 80 mg/100 g, respectively. Total anthocyanin content of acerola (*M. emarginata*) was shown previously to present large variation depending on the cultivar with values from 3.8 to 47.4 mg/100 g of fruit pulp (*12*).

Acerola antioxidant capacity has been attributed not only to its high vitamin C content but also to phenolic compounds (*13*). Hanamura et al. (*11*) reported that the acerola anthocyanins possessed O_2^- scavenging activity and inhibitory effects on both glucosidase and advanced glycation end product formation *in vitro*. A hepatoprotective effect has also been observed with acerola extract powders from fruit purees, and this effect is attributed to the combination of high vitamin C content, phenolic acids, anthocyanins, and flavonoids. The effect of the water extract powder from fruit purees (100 mg/kg) was moderately stronger than that of ascorbic acid (10 mg/kg) but weaker than that of cyanidin 3-*O*-rhamnoglucoside (13.3 mg/kg) (*14*). Acerola is also considered a good source of provitamin A and contains carotenoids β-carotene, lutein, β-cryptoxanthin, and α-carotene (*15*).

Guajiru. Guajiru fruit presented eight peaks consisting of nine anthocyanin components (**Figure 2**), and most of these components appeared to be acylated (**Table 3**). These eight peaks were compounds representing derivatives of three anthocyanidins: delphinidin, petunidin, and peonidin. Two monoglycosylated peaks were observed as delphinidin 3-hexose and petunidin 3-hexose, with the hexose group being either glucose or galactose. Two delphinidin compounds with acetoyl and hexose substitutions were observed as peaks 2 and 4. The relative elution patterns for galactosides and glucosides reported in the literature (*5*, *16–18*) and also observed in our laboratory suggest that peak 2 represented delphinidin 3-acetoylgalactoside and peak 4 was delphinidin 3-acetoylglucoside. Two petunidin compounds with identical mass spectra were observed as peaks 3 and 6. The mass spectra are in accord with petunidin with

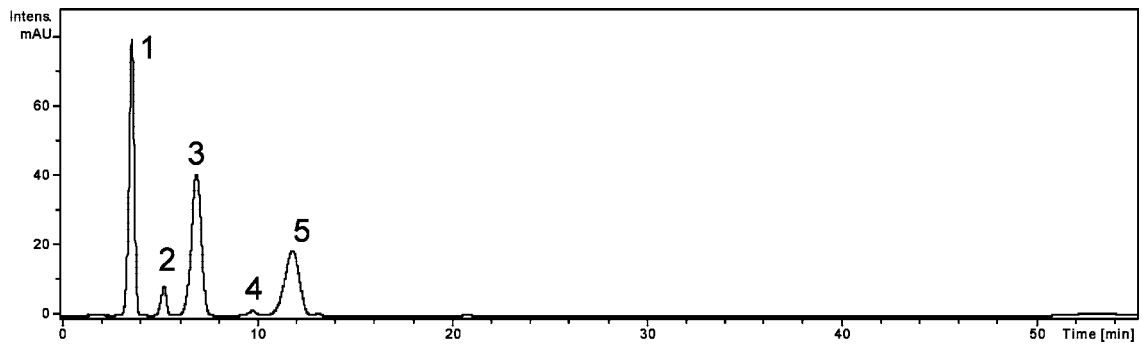

Figure 3. HPLC chromatogram showing the anthocyanin profile for jambolão. Refer to **Table 4** for the identification of each peak.

Table 4. Identification of Anthocyanins in Jambolão

peak	t_R (min)	$[M]^+$ (m/z)	MS/MS (m/z)	anthocyanin	mg/100 g dry weight
1	3.7	627	465/303	delphinidin 3,5-diglucoside	256
2	5.2	611	449/287	cyanidin 3,5-diglucoside	29
3	7	641	479/317	petunidin 3,5-diglucoside	245
4	9.8	625	463/301	peonidin 3,5-diglucoside	75
5	11.9	655	449/331	malvidin 3,5-diglucoside	166

Table 5. Identification of Anthocyanins in Jussara

peak	t_R (min)	$[M]^+$ (m/z)	MS/MS (m/z)	anthocyanin	mg/100 g dry weight
1	8.9	581	449/287	cyanidin 3-sambubioside	13
2	9.5	449	287	cyanidin 3-glucoside	1358
3	11.8	595	449/287	cyanidin 3-rutinoside	1565
4	13.4	433	271	pelargonidin 3-glucoside	8
5	16.6	579	433/271	pelargonidin 3-rutinoside	5
6	18.4	433	287	cyanidin 3-rhamnoside	7

acetoyl and hexose substitutions, suggesting that peak 3 may represent petunidin 3-(6″-acetoyl)galactoside and peak 6 may represent petunidin 3-(6″-acetoyl)glucoside, similarly based on elution patterns for galactosides and glucosides. However, the mass spectra of these peaks are also in accord with petunidin 3-(6″-oxaloyl)xyloside and petunidin 3-(6″-oxaloyl)arabinoside, and we are unable to differentiate between these identities. The remaining peaks were identified as delphinidin 3-succinylrhamnoside, petunidin 3-succinylrhamnoside, and peonidin 3-succinylrhamnoside. When acyl groups were detected, these were assumed to be attached to the glycosylation on position 3, as has been consistently observed previously for acylated anthocyanins (*5*, *6*). The elution order of the anthocyanins observed in guarjiru was in accord with the elution order of the same compounds observed in other fruits as reported by Wu and Prior (*5*), adding support for these identifications.

Petunidin 3-(6″-succinyl)rhamnoside presented the highest concentration (367 mg/100 g dry weight), followed by the peak tentatively identified as petunidin 3-acetoylglucoside (164 mg/100 g), the peak tentatively identified as petunidin 3-(6″-acetoyl)galactoside (121 mg/100 g), delphinidin 3-(6″-succinyl)rhamnoside (100 mg/100 g), delphinidin 3-(6″-acetoyl)glucoside (63 mg/100 g), delphinidin 3-galactoside/glucoside (44 mg/100 g), and peonidin 3-(6″-succinyl)rhamnoside (25 mg/100 g). The unresolved peak containing petunidin 3-galactoside/glucoside + delphinidin 3-(6″-acetoyl)galactoside represented 74 mg/100 g. Anthocyanins acylated with aliphatic acids have a rather widespread occurrence (*5*, *6*), and most reports include malonic or acetic acids as the acyl moieties. Similar acylations with oxalic, malic, or succinic acid have more restricted distributions. An anthocyanin acylated with succinic acid was reported on the flowers of the grass *Phragmatites australis* (*19*).

Jambolão. The jambolão chromatogram presented five peaks (**Figure 3**) and included the widest variety of anthocyanidin groups (**Table 4**): delphinidin, cyanidin, petunidin, peonidin, and malvidin, all presenting as diglucosides. Delphinidin 3,5-diglucoside and cyanidin 3,5-diglucoside identifications were confirmed by comparison of retention times with those of authentic standards. The elution order of anthocyanins identified in jambolão was in accord with the elution order of the same compounds observed in other fruits by Wu and Prior (*5*), adding support for these identifications.

The major compound in jambolão was delphinidin 3,5-diglucoside (256 mg/100 g dry weight), followed by petunidin 3,5-diglucoside (245 mg/100 g), malvidin 3,5-diglucoside (166 mg/100 g), peonidin 3,5-diglucoside (75 mg/100 g), and cyanidin 3,5-diglucoside (29 mg/100 g). In a study of different free radical scavenging systems, it was observed that the fruit skin of jambolão had significant antioxidant activity, which was attributed in part to antioxidant vitamins, phenolics or tannins, and/or anthocyanins (*20*).

Jussara. The jussara chromatogram presented two major peaks (**Figure 4**), which were identified as cyanidin 3-glucoside (1358 mg/100 g dry weight) and cyanidin 3-rutinoside (1565 mg/100 g) (**Table 5**), and the identification of both of these was confirmed by comparison of peak retention times with those of authentic standards. These compounds were reported in jussara previously by Harborne et al. (*21*), who

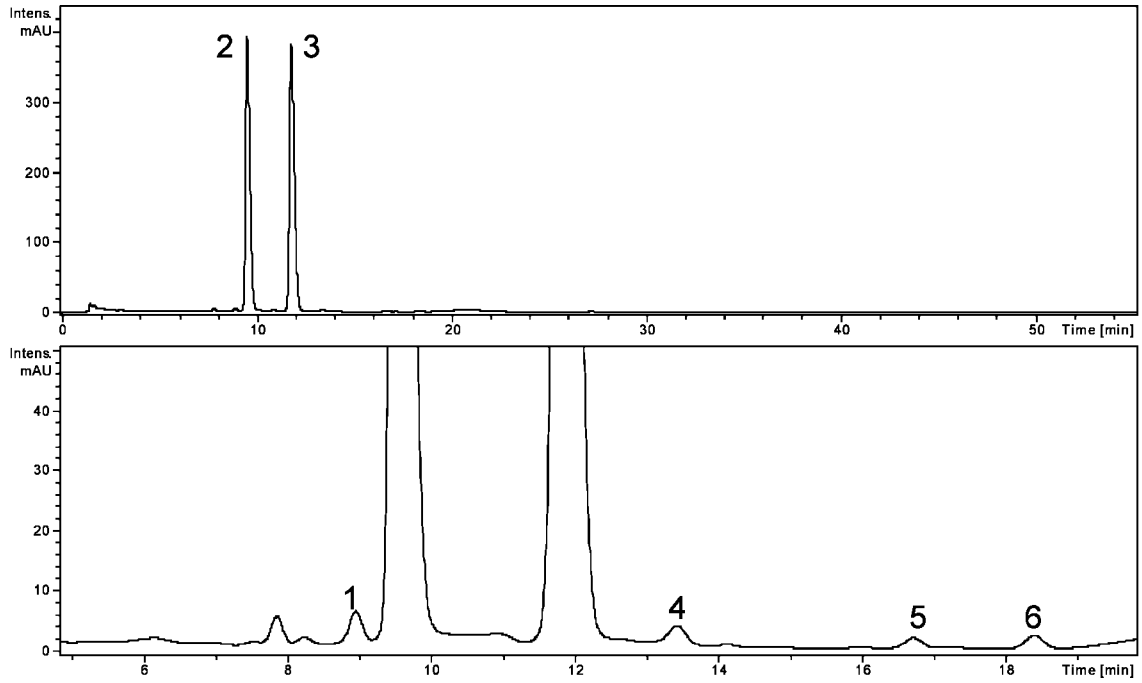

Figure 4. HPLC chromatogram showing the anthocyanin profile for jussara. Refer to **Table 5** for the identification of each peak.

Table 6. Total Anthocyanin Content of Tropical Fruits

fruit	mg/100 g dry weight	mg/100 g fresh weight
acerola II47/1	528	48
acerola roxinha	261	23
guajiru	958	104
jambolao	771	79
jussara	2956	290

compared retention times of jussara anthocyanins to those of anthocyanin standards. Minor compounds were identified as cyanidin 3-sambubioside (12.8 mg/100 g), pelargonidin 3-glucoside (7.8 mg/100 g) (as confirmed against an authentic standard), cyanidin 3-rhamnoside (6.6 mg/100 g), and pelargonidin 3-rutinoside (5.3 mg/100 g). Similar to these findings for jussara, açaí (*E. oleracea*), which is purple in color, was also reported to have two major anthocyanins consisting of cyanidin 3-glucoside and cyanidin 3-rutinoside (*22*, *23*), though Pozo-Insfran et al. (*24*) reported the major anthocyanins in açaí to be cyanidin 3-glucoside and pelargonidin 3-glucoside. Schauss et al. (*23*) also reported minor anthocyanins of cyanidin 3-sambubioside, peonidin 3-glucoside, and peonidin 3-rutinoside. Pozo-Insfran et al. (*24*) also concluded that anthocyanins were the predominant contributors to the antioxidant capacity of açaí, which was considered higher than that of many berries, such as highbush blueberries, strawberries, raspberries, blackberries, cranberries, and muscadine grape juice.

Total content of monomeric anthocyanins in acerola, guajiru, jambolão, and jussara is shown in **Table 6**. Since the anthocyanin contents were determined using an external standard curve of cyanidin 3-glucoside, this is a source of possible error in quantification, especially for the acylated anthocyanins. Content of anthocyanins in fresh material was calculated on the basis of change in mass measured during freeze-drying. The richest source of anthocyanins among the fruits studied here was jussara, with 290 mg of anthocyanins/100 g fresh weight, followed by guajiru with 104 mg/100 g, jambolão with 79 mg/100 g, acerola clone II47/1 with 48 mg/100 g, and acerola roxinha with 23 mg/100 g. All four fruits were similar in anthocyanin content to other fruits considered to be good sources of anthocyanin. For example, anthocyanin contents recently reported for several fruits include the following: strawberry, 21 mg/100 g; red grape, 27 mg/100 g; red raspberry, 92 mg/100 g; cherry, 122 mg/100 g; blackberry, 245 mg/100 g; cultivated blueberry, 387 mg/100 g (*25*).

The results of this investigation have shown that acerola, guajiru, jambolão, and jussara contain substantial amounts of anthocyanins and that these anthocyanins are widely varying in anthocyanidin backbone, acylation, and glycosylation. Besides acerola, these fruits are generally underutilized. However, demonstration of their high content of health-promoting phytonutrients may assist in ultimately bringing these commodities to market and increasing the demand for these items.

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