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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JUNE 2011

Impact Factor: 2.91 · DOI: 10.1021/jf201039r · Source: PubMed

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Phenolic Constituents and Antioxidant Capacity of Four Underutilized Fruits from the Amazon Region

André Gordon,[†] Elvira Jungfer,[†] Bruno Alexandre da Silva,[§] José Guilherme S. Maia,[§] and Friedhelm Marx*[,†]

[†]Department of Nutrition and Food Sciences, University of Bonn, Endenicher Allee 11-13, 53115 Bonn, Germany

[§]Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Universidade Federal do Pará, 66075-900 Belém, PA, Brazil

ABSTRACT: The Amazon region comprises a plethora of fruit-bearing species of which a large number are still agriculturally unimportant. Because fruit consumption has been attributed to an enhanced physical well-being, interest in the knowledge of the chemical composition of underexplored exotic fruits has increased during recent years. This paper provides a comprehensive identification of the polyphenolic constituents of four underutilized fruits from the Amazon region by HPLC/DAD-ESI-MS^n. Araçá (*Psidium guineense*), jambolão (*Syzygium cumini*), muruci (*Byrsonima crassifolia*), and cutite (*Pouteria macrophylla*) turned out to be primarily good sources of hydrolyzable tannins and/or flavonols. Additionally, different flavanonols and proanthocyanidins were identified in some fruits. The antioxidant capacity was determined by using the total oxidant scavenging capacity (TOSC) assay. Cutite showed the highest antioxidant capacity followed by jambolão, araçá, and muruci.

KEYWORDS: tropical fruits, tannins, flavonols, LC-MS, TOSC

INTRODUCTION

The Amazon region is the largest tropical forest area in the world, and its flora bears a plenty of still unexplored or underutilized fruit species. Due to the postulated contribution to an enhanced human well-being and promotion of beneficial health effects against degenerative diseases, interest has arisen in exploiting new and exotic types of fruits during recent years.[1] Promising species may also represent an opportunity for local growers to reach niche markets to increase their revenues.[2] However, many edible fruits have not attained economic importance as they are insufficiently studied with regard to their possibilities of commercialization, crop growing conditions, and chemical composition.[3] As well, scientific information is scarce about the bioactive compounds of the locally popular Brazilian fruits araçá, jambolão, muruci, and cutite.

Psidium guineense Sw. (Myrtaceae), known as araçá, is a shrub or small tree between 4 and 6 m in height. The berry fruit is of spherical to egg-like shape, usually 1−3 cm in diameter, with numerous 2−3 mm stony seeds. The pulp is sweet acetous in taste and is particularly used for preparing jellies, juices, and ice cream.[4] The fruit pericarp of araçá showed antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.[5]

Syzygium cumini (L.) Skeels (Myrtaceae), known as jambolão, is a tree that originates from India and Southeast Asia but is also widespread in some states across Brazil.[6] The edible fruits are of oval shape and 2−3 cm long. The color of the peel is deep purple to black. Jambolão pulp has a grayish white color and embeds a big purple seed. Ripe fruits possess an aromatic sour astringent taste and are either eaten fresh[7] or processed to preserves, jellies, and wine.[8] Fruits as well as bark, seeds, and leaves are traditionally used for diabetes treatment and different gastrointestinal disorders. Additionally, a fruit extract showed antimicrobial and cytotoxic activities and may potentially be used in topical antimicrobial products.[6] In comparison to other nontraditional fruits from Brazil, jambolão showed considerably high antioxidant activity,[9] which can be at least partly ascribed to the phenolic constituents such as anthocyanins,[10] tannins,[8] and flavonols.[11]

Byrsonima crassifolia (L.) Kunth (Malpighiaceae), known as muruci, as well as a number of related species occur in the Amazon basin, suggesting that this may be its center of origin. It is a large shrub to a small tree of 2−6 m in height. Drupes are yellow with a diameter of 1.5−2 cm containing one or, rarely, two to three seeds.[12] The soft pulp develops an exotic, very distinctive cheese-like aroma and is preferably consumed as a juice, jelly, confectionary, or liquor.[13] Compared to six other exotic fruits including the well-known açaí, cashew apple, and acerola, muruci showed a high content of extractable polyphenols, although its radical scavenging capacity was reported to be low.[14]

Pouteria macrophylla (Lam.) Eyma (Sapotaceae), known as cutite, is a small to medium forest tree up to 20−25 m in height. It develops egg-shaped berry fruits up to 6 cm in diameter with a starchy, yellow, soft pulp embedding a long ovoid seed. Cutite is always eaten as a fresh fruit characterized by an agreeable and generally sweet taste that is not always immediately appreciated by those who do not know it. Because of the starch content cutite supplies a reasonable amount of calories.[12]

Only a few studies exist on the phenolic composition of jambolão fruits, and no studies have been published about individual phenolic substances in fruits of araçá, muruci, and cutite. Therefore, the aim of the study was to provide a comprehensive characterization of phenolic constituents in the edible part of the four Amazonian fruits by HPLC/DAD-ESI-MS^n. In addition, the antioxidant capacity was assessed to evaluate their biological activity.

MATERIALS AND METHODS

Chemicals. Ultrahigh quality (UHQ) water was prepared with a Direct-Q 3 system (Millipore, Billerica, MA). HPLC-MS and extraction

Received: March 15, 2011
Revised: June 10, 2011
Accepted: June 11, 2011
Published: June 11, 2011

solvents were obtained from J. T. Baker (Griesheim, Germany). Diethylenetriaminepentaacetic acid (≥99%), α-keto-γ-methiolbutyric acid (KMBA) (≥97%), 2,2′-azobis(2-methylpropionamidine) dichloride (≥97%), 3-morpholinosydnonimine *N*-ethylcarbamide, quercetin (≥98%), myricetin (≥96%), gallic acid (≥99%), and Folin−Ciocalteu's phenol reagent were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Ascorbic acid (≥95%) was purchased from Kraemer & Martin GmbH (Sankt Augustin, Germany).

Fruit Material and Sample Preparation. Fruits were harvested at a particular farm, located in the municipality of São João de Pirabas, northeastern Pará, Brazil, in the months of November and December 2009. The fruits were identified with authentic samples deposited in the Herbarium of Museu Emilio Goeldi, city of Belém, state of Pará, Brazil.

After deseeding, the edible parts of the fruits (peel and flesh) were deep-frozen and freeze-dried immediately subsequent to harvest. Samples were air-shipped to Germany and stored at −30 °C prior to analysis.

Identification of Phenolic Compounds by HPLC/DAD-ESI-MS^n. Polyphenol extraction was carried out by using a modified pressurized liquid extraction method according to Papagiannopoulos et al.[15] Freeze-dried sample (500 mg) was thoroughly ground and extracted with acetone/water/formic acid (70:29:1, v/v/v) in an accelerated solvent extractor (ASE 200, Dionex, Idstein, Germany) at room temperature, for 20 min in two cycles. The following solid-phase extraction (SPE) was performed by using a Gilson ASPEC XL system (Automated Sample Preparation with Extraction Cartridges, Abimed, Langenfeld, Germany). Polyamide (PA) SPE cartridges (500 mg PA, 3 mL cartridge, Macherey Nagel, Düren, Germany) were conditioned with 3 mL of dimethyl sulfoxide/formic acid/trifluoroacetic acid (DMSO/FAc/TFA) (98.7:1:0.3, v/v/v) and washed with 5 mL of UHQ water. Prior to cartridge loading, the sample extract was diluted to contain <15% (v/v) of organic solvent. The cartridge was loaded with sample extract in volumetric steps of 20 mL until exhaustion and washed with 10 mL of water after each load. During elution with DMSO/FAc/TFA solvent, the first 0.5 mL was discarded and the next 1.25 mL collected. Before application to HPLC/DAD-ESI-MS^n, the samples were filtered through a 1.0/0.45 μm syringe filter (Chromafil GF/PET-45/25, Macherey-Nagel).

Analysis of polyphenolic compounds was performed following a HPLC/DAD-ESI-MS^n method according to Papagiannopoulos et al.[15] The liquid chromatograph was a Summit system (Dionex, Germering, Germany) consisting of a P-580 A HPG pump, an ASI-100 T automated sample injector, an STH-585 column oven, and a UVD-340S detector equipped with a capillary cell. Chromeleon software package v6.7 SP2 (Dionex) was used for system control and data evaluation. Separation was carried out with the help of an analytical column Aqua RP 18, 150 mm, 2 mm i.d., 3 μm with a guard column Security Guard, C18, 4 mm, 2 mm i.d. (both Phenomenex, Aschaffenburg, Germany) kept at 25 °C. Solvents were UHQ water with 1% acetic acid (v/v) (mobile phase A) and acetonitrile with 1% acetic acid (v/v) (mobile phase B). The gradient elution program using a flow rate of 0.2 mL/min started with 0.5% B, rose to 40% B after 32 min and to 100% B after 34 min, and was kept at 100% B for 9 min. The column was re-equilibrated for 15 min with initial conditions. For analysis, 20 μL of each sample was injected. An LCQ classic ion trap mass spectrometer (MS) equipped with an electrospray interface was coupled to the HPLC and controlled with Xcalibur software v1.2 (all Thermo Fisher Scientific, Dreieich, Germany). Settings for the negative ionization with MS were as follows: source voltage, −4.0 kV; sheath gas flow, 90; auxiliary gas flow, 60; capillary voltage, −10 V; capillary temperature, 300 °C; tube lens offset, +20 V; first octapole offset, +4 V; interoctapole lens, +30 V; second octapole offset, +10 V; and trap DC offset, +10 V.

The identification of phenolic compounds was performed with authentic standards in the cases of gallic acid, quercetin, and myricetin. All other compounds were tentatively identified by combining characteristic data of HPLC elution order of compounds and UV spectra with those of mass spectrometrical fragmentation analysis. Additionally, compound assignment was supported by comparison with data from the literature when available.

Total Oxidant Scavenging Capacity (TOSC) Assay. The antioxidant capacity of the fruits was determined with the TOSC assay performed as described by Lichtenthäler et al.[16] Briefly, the TOSC assay is based on an ethylene-yielding reaction of KMBA with either peroxyl radicals or peroxynitrite. Antioxidant compounds present in the sample can inhibit the ethylene formation that is recorded in a time course of 1 h using automatically repeated headspace GC analysis (GC-17A, Shimadzu, Tokyo, Japan). Each fruit was analyzed in duplicate. Quantification of generated ethylene results in a kinetic curve of which the area under the curve (AUC) is calculated. Only those data with a variance (standard deviation/arithmetic mean) of the AUC after repeat determination below 5% are further processed. Mean data of a sample are compared to those of an uninhibited reaction with water, which gives rise to the TOSC values. Results of this study indicate the concentration of antioxidants present in the sample in grams per liter that is needed to obtain a radical inhibition of 50%.

For TOSC analysis, freeze-dried sample (1 g) of each fruit was suspended in UHQ water to obtain a total weight of 10 g (w/w). The suspension was sonicated for 10 min and centrifuged for 7 min at 10000 rpm with a Heraeus Biofuge Stratos (Kendro, Langenselbold, Germany). The supernatant of the water extract (WE) was stored until further application at −30 °C.

Total Phenolic Content. Total phenolic content was determined by using the Folin−Ciocalteu assay described by Georgé et al.[17] Briefly, 500 μL of water-diluted Folin−Ciocalteu reagent (9:1, v/v) and 100 μL of the WE were mixed. After incubation for 2 min at room temperature, 400 μL of sodium carbonate (7.5 g/100 mL) was added. The mixture was incubated at 50 °C for 15 min and subsequently photometrically measured (Cary 50, Varian, Walnut Creek, CA) at 760 nm. In total, two water extracts were prepared per fruit and analyzed in duplicate. Seven dilutions (10−100 mg/L) of a gallic acid standard were used to create a calibration curve (r^2 = 0.9980). Results are expressed as gallic acid equivalents in milligrams per 100 g dry matter.

Determination of Ascorbic Acid. Ascorbic acid was determined by HPLC after modification of a method previously described by Gordon et al.[18] The HPLC-DAD system of PRO Star series (Varian) was equipped with an analytical column Synergi 4 μ Hydro RP, 150 mm, 2 mm i.d., and with a guard column Security Guard, C 18, 4 mm, 2 mm i.d. (both Phenomenex, Aschaffenburg, Germany). Separation was performed with acidified UHQ water (1% FAc, v/v) at isocratic condition using a flow rate of 0.3 mL/min. The injection volume was 20 μL. Confirmation of ascorbic acid in the fruits was arranged by standard, retention time, and doping of standard to the sample. A five-point calibration curve (5−100 mg/100 mL, r^2 = 0.9995) was created for quantification with authentic standard. Ascorbic acid was quantified at a wavelength of 260 nm. Two sample extracts were prepared and measured in duplicate.

RESULTS AND DISCUSSION

HPLC/DAD-ESI-MS^n Analysis of Phenolic Compounds. Most of the detected compounds shown in Tables 1−4 can be classified into hydrolyzable tannins (gallotannins, galloylquinic acids, and ellagitannins), condensed tannins (proanthocyanidins), flavonols, and flavanonols. At first, spectral data were used for a distinction of these different compound groups. According to Cantos et al.[19] and Boulekbache-Makhlouf et al.,[20] the obtained UV spectra of the hydrolyzable tannins can generally be arranged into two groups. The first group comprises compounds derived from ellagic acid with two absorption maxima at λ_{max} ∼250 and ∼365 nm. The second group has only one maximum available at λ_{max} ∼275 nm, typically found for galloyl and hexahydroxydiphenoyl

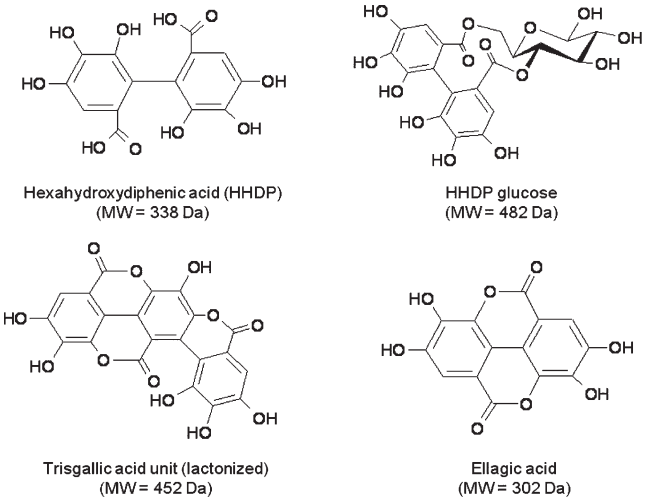

Figure 1. Gallic acid derivatives (according to Hager et al.[24]) occurring in araçá, cutite, or jambolão with corresponding molecular weights (MW).

(HHDP) derivatives. A condensed HHDP molecule gives rise to ellagic acid, for which reason they are also considered to be ellagitannins.[19] Exemplary structures of these compounds are shown in Figure 1. UV spectra of proanthocyanidins are identical with those of catechins showing two maxima at ∼230 and ∼280 nm. Flavonol and flavanonol glycosides come with two absorption maxima derived from the conjugated system of the aglycones. The first maximum of ∼260 nm is attributed to the benzoyl system (ring A) and the second maximum of ∼350 nm to the cinnamoyl system (ring B).[21]

In addition to the UV spectra, mass spectrometrical fragmentation experiments enable at least a tentative identification of the phenolic compounds. MS^n analysis allows the distinction between individual flavonols or flavanonols, the elucidation of proanthocyanidins, and the composition of hydrolyzable tannins.[15]

In the following, all mass spectrometrically identified sugar moieties of gallotannins and HHDP hexosides will be tentatively characterized as glucose due to its predominant abundance within these compound groups.[22]

Araçá (Psidium guineense). The HPLC chromatogram of the araçá extract is shown in Figure 2. According to Table 1, a total of 18 polyphenolic compounds could be at least tentatively identified. All of them were classified as ellagitannins with the exception of peaks 8, 9, and 12, which belong to the gallotannins. Identification of gallic acid in peak 1 was assured by using an authentic standard.

Peaks 8 and 12 were presumably assigned to derivatives of galloyl glucose. Peak 8 provides parental $[M - H]^-$ ions at m/z 483 and MS^2 fragments typically found for digalloylglucose.[23] As peak 12 shows a $[M - H]^-$ ion at m/z 635 and a fragment at m/z 483, the neutral loss of 152 Da gives rise to the presence of an additional esterified galloyl residue conform to a trigalloylglucose. Peak 9 provides a $[M - H]^-$ ion at m/z 453 and gives two MS^2 fragments at m/z 313 and 169. Due to the mass difference of 30 Da, conforming UV data, and the similarity of some fragments in comparison to peak 8, this compound is tentatively assigned to digalloylpentoside.

Mass spectrometric data of peaks 4, 7, and 10 correspond to those of galloyl-HHDP glucose derivatives in grapes[23] and fruits of *Eucalyptus*.[20] Peak 4 shows $[M - H]^-$ ions at m/z 633 and produces daughter ions at m/z 421, 275, and 301 matching with those of HHDP galloylglucose.[20,23] Peaks 7 and 10 correspond to HHDP digalloylglucose isomers having a parental $[M - H]^-$ ion at m/z 785 and characteristic product ions at m/z 633, 483, and 301.[23] Peaks 13 and 14 show $[M - H]^-$ ions at m/z 933 and give among others daughter fragments at m/z 451 and 301. These compounds were tentatively assigned to castalagin/vescalagin isomers as proposed by Hager et al.[24] due to the according fragmentation pattern. On the basis of UV data, the product ion at m/z 301 indicates the presence of a HHDP derivative rather than that of ellagic acid. The neutral loss of 482 Da from the parent ion suggests the existence of a HHDP glucose unit. The resulting fragment ion after this neutral loss at m/z 451 is consistent with that of a trisgalloyl unit (see Figure 1) after undergoing lactonization.[24] In return, the neutral loss of 452 Da accounting for a lactonized trisgalloyl unit is indicated by the daughter ion at m/z 481 in peak 14. The fragment ion at m/z 631 in peak 13 may result from the loss of two galloyl units from the quasi-molecular ion.[24]

The occurrence of di-HHDP glucose derivatives was presumably assessed in peaks 2, 3, 5, 6, and 11. All compounds have a shift in the UV spectrum to λ_{max} ∼260 nm in common. In peaks 2 and 5, a parental $[M - H]^-$ ion at m/z 783 is present, producing fragment ions at m/z 301, 481, and 275 in MS^2 and additionally two fragment ions at m/z 257 and 229 in MS^3. These fragments are characteristic for di-HHDP glucose found also in cork of *Quercus suber*[25] and in blackberries[24] and strawberries.[26] Peaks 3, 6, and 11 show $[M - H]^-$ ions at m/z 951 that yield fragment ions at m/z 907 and 783. Compounds with the same fragmentation pattern were suggested to be trisgalloyl HHDP glucose isomers.[20,27] UV data and the fragment ion at m/z 783 indicate the occurrence of di-HHDP glucose. The fragment $[M - H - 168]^-$ accounts for the presence of an additional galloyl residue but only with a C−C linkage to one of the HHDP molecules. The loss of 44 Da (CO_2) agrees with the presence of a free, unesterified carboxyl group.[27]

UV spectra of peaks 15−18 match with those of ellagic acid.[19] Peak 16 shows $[M - H]^-$ ions at m/z 447 and yields fragment ions at m/z 301 (MS^2) and 257 (MS^3) that were also found for ellagic acid.[23] Due to the neutral loss of 146 Da, peaks 15 and 16 are tentatively assigned to ellagic acid deoxyhexoside isomers. The late retention time is an argument for the occurrence of dimethylated ellagic acid hexoside in peak 17. Parental $[M - H]^-$ ions at m/z 491 and fragmentation pattern (m/z 329, 313) coincide to some extent with that of a dimethylated ellagic acid glucoside described by Boulekbache-Makhlouf et al.[20] Peak 18 shows $[M - H]^-$ ions at m/z 461 and produces MS^n fragment ions at m/z 315, 301, and 300 corresponding to a methylated ellagic acid.[20] Due to the mass difference of 30 Da from peak 17, this compound is tentatively assigned to dimethylellagic acid pentoside.

Jambolão (Syzygium cumini). The HPLC chromatogram of the jambolão extract is shown in Figure 2. According to Table 2, a total of 37 non-anthocyanin polyphenolic compounds could be identified or at least tentatively assigned. They were classified as gallotannins, ellagitannins, flavonols, and flavanonols. Identification of gallic acid in peak 1 was assured by using an authentic standard.

MS^n data of peaks 8−10, 12, 13, 15, 16, 18, 20, 24a, 26a, and 29 agree with those of different gallotannins described by Sandhu et al.[23] Hence, these compounds were tentatively assigned to a series of galloylglucose esters starting from isomers of digalloylglucose ($[M - H]^-$ at m/z 483) to

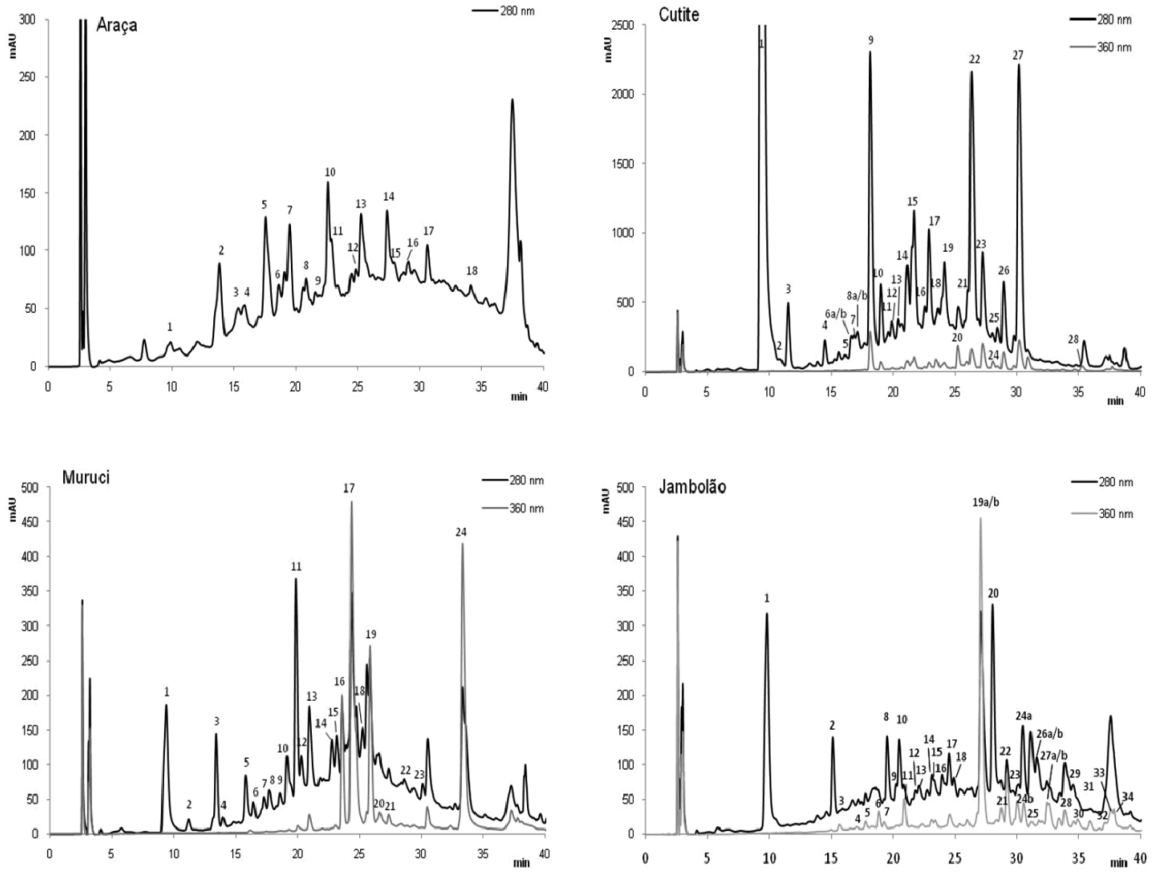

Figure 2. HPLC chromatograms of four different fruits from the Amazon region. The chromatograms of araçá, jambolão, muruci, and cutite correspond to Tables 1, 2, 3, and 4, respectively. The numbered peaks are denoted in the accordant table.

hexagalloylglucose ($[M - H]^-$ at m/z 1091). Peak 14 shows to some extent mass spectrometric attributes of HHDP galloylglucose previously found in grape seeds.[23] However, elucidation of this compound could only conditionally be ascertained due to unutilizable UV data. Peak 17 gives parental $[M - H]^-$ ions at m/z 775 and produces dominating MS^2 fragment ions at m/z 613 and 451. Both fragments indicate the sequential loss of hexosyl units with $[M - H - 162]^-$ and $[M - H - 162 - 162]^-$. The product ion at m/z 451 suggests the existence of a trisgalloyl residue,[24] which was already discussed for ellagitannins occurring in araçá (Figure 1). Hence, this compound is tentatively identified as trisgalloyldiglucoside.

A large number of myricetin-derived compounds were presumptively identified on the basis of UV and mass spectrometric fragmentation data in peaks 19a, 19b, 22, 28, and 33. Myricetin was identified in peak 30 by comparing fragments with those of an authentic standard. Mass spectra obtained from the other myricetin-derived constituents indicate at least the presence of the aglycone at m/z 317. The occurrence of two coeluting myricetin compounds is supposedly revealed in peak 19a/b. Peak 19a yields fragments of $[M - H - 162]^-$ and peak 19b fragments of $[M - H - 176]^-$ corresponding to a myricetin hexoside and myricetin glucuronide, respectively. In previous studies, glucose was the only identified hexoside in jambolão.[11] Hence, peak 19a may be assigned to myricetin glucoside, which was already described by Faria et al.[11] Peak 22 is ascribed to myricetin deoxyhexoside due to the loss of 146 Da from $[M - H]^-$ ions at m/z 463. According to Faria et al.,[11] myricetin rhamnoside (myricitrin) likely occurs in this peak as rhamnose makes up the only deoxyhexoside commonly found in fruits. The loss of 42 Da from $[M - H]^-$ ions at m/z 505 in peak 28 indicates the presence of acylated myricetin deoxyhexoside that was already constituted in jambolão fruits[11] and jambolão leaves.[28] Peak 33 shows parent $[M - H]^-$ ions at m/z 657 and produces dominating fragment ions at m/z 505. These mass data are in agreement with that of acylated galloylmyricetin deoxyhexoside previously found in leaves of jambolão.[28]

Peaks 21, 23, 24b, 25, 26b, 32, and 34 were presumably identified as methylmyricetin derivatives. Peak 23 indicates the presence of methylmyricetin hexoside as the parent ion at m/z 493 results in fragment ions at m/z 331 after neutral loss of 162 Da. The dominating daughter ion at m/z 331 would account for the aglycone methylmyricetin.[28] Because the flavonol mearnsetin (myricetin 4′-methyl ether) was found in jambolão leaves,[28] it likely occurs also in the fruits. As reported by Faria et al.,[11] glucose is the verisimilar occurring hexoside in jambolão. Peak 21 was tentatively identified as galloylmethylmyricetin hexoside showing $[M - H]^-$ ions at m/z 645 and dominating daughter ions at m/z 493. The neutral loss of 132 Da in peaks 24b and 26b as well as the loss of 176 Da in peak 25 would be in agreement with the presence of methylmyricetin pentoside isomers and methylmyricetin glucuronide, respectively. The sequential neutral loss of 42 and 146 Da in peak 32 may account for the occurrence of acylated methylmyricetin deoxyhexoside. This compound could be more precisely ascribed to acylated mearnsetin rhamnoside, which was identified in jambolão leaves.[28] Finally, peak 34 shows $[M - H]^-$ ions at m/z 645 and produces fragments at m/z 331 and 505.

Table 1. UV and Mass Spectrometric Data of Phenolic Constituents Extracted from Araçá (*Psidium guineense*) Fruits

peak	retention time	compound[a,b]	HPLC-DAD λ_{max} (nm)	$[M-H]^-$ m/z	fragments (m/z)
1	9.84	gallic acid	273	169	MS^2 [169]: –
2	13.83	di-HHDP-glucose[24−26]	228, 260	783	MS^2 [783]: 301, 481, 275
					MS^3 [783 → 301]: 257, 229
3	15.34	trisgalloyl HHDP glucose isomer[20,27]	227, 262	951	MS^2 [951]: 907, 783
					MS^3 [951 → 907]: 783
4	15.86	HHDP galloylglucose[20,23]	226, 275	633	MS^2 [633]: 301, 275, 421
5	17.54	di-HHDP glucose[24−26]	228, 260	783	MS^2 [783]: 301, 481, 275
					MS^3 [783 → 301]: 257
6	18.63	trisgalloyl HHDP glucose isomer[20,27]	232, 263	951	MS^2 [951]: 907, 783
					MS^3 [951 → 907]: 783
7	19.47	HHDP digalloylglucose isomer[20,23]	225, 280	785	MS^2 [785]: 301, 633, 275, 483, 615, 419
8	20.83	digalloylglucose[23]	224, 273	483	MS^2 [483]: 439, 313, 271, 331, 169
					MS^3 [483 → 439]: 287, 313
9	21.59	digalloylpentose	224, 280	453	MS^2 [453]: 391, 313, 169
10	22.58	HHDP digalloylglucose isomer[20,23]	225, 276	785	MS^2 [785]: 301, 483, 633, 275
					MS^3 [785 → 301]: 257
11	22.87	trisgalloyl HHDP glucose isomer[20,27]	235, 258	951	MS^2 [951]: 907, 783
					MS^3 [951 → 907]: 783
12	24.83	trigalloylglucose[23]	224, 281	635	MS^2 [635]: 423, 483, 271, 465, 193
					MS^3 [635 → 423]: 271
13	25.26	castalagin/vescalagin isomer[24]	226, 282	933	MS^2 [933]: 451, 631, 301
					MS^3 [933 → 451]: 351, 433, 285, 407, 311
14	27.38	castalagin/vescalagin isomer[24]	225, 289	933	MS^2 [933]: 451, 351, 301, 481
					MS^3 [933 → 451]: 351, 285, 433, 407, 335, 379
15	28.73	ellagic acid deoxyhexoside	252, 371	447	MS^2 [447]: 301
16	29.13	ellagic acid deoxyhexoside	256, 362	447	MS^2 [447]: 301
					MS^3 [447 → 301]: 257
17	30.63	dimethylellagic acid hexoside	249, 368	491	MS^2 [491]: 328, 313, 329, 454, 476
					MS^3 [491 → 328]: 313, 285
18	34.18	dimethylellagic acid pentoside	252, 363	461	MS^2 [461]: 315, 300
					MS^3 [461 → 315]: 300, 301

[a] Superscript numbers indicate the literature in which the compounds were previously described. [b] Gallic acid was identified with authentic standard; all other compounds were tentatively identified.

This corresponds to the loss of a galloyl residue (152 Da) in addition to an acylated pentose unit (42 + 132 Da) tentatively resulting in acylated galloyl ester of methylmyricetin pentoside.

Due to MS^2 (m/z 345) and MS^3 (m/z 330 and 315) data, a dimethylmyricetin is presumptively identified in peak 27a. The loss of 162 Da from $[M-H]^-$ ions at m/z 507 indicates the presence of dimethylmyricetin hexoside. MS^n data of peaks 27b and 31 as well as the mass difference of 30 Da in comparison to peak 27a give rise to the likely occurrence of two dimethylmyricetin pentoside isomers.

All flavanonols in jambolão fruits occur as dihexosides. Mass spectrometric data of the flavanonols are in agreement with those described by Faria et al.[11] Aglycones of methyldihydromyricetin ($[M-H-162-162]^-$ at m/z 333) were tentatively identified in peaks 4 and 6 after neutral loss of two hexose units. Peaks 7 and 11 are presumably assigned to be isomers of dimethyldihydromyricetin dihexoside ($[M-H-162-162]^-$ at m/z 347). Elution time and parental $[M-H]^-$ ions at m/z 643 indicate the presence of dihydromyricetin dihexoside in peak 3. However, peak 3 could only tentatively be denoted as dihydromyricetin dihexoside. Our MS^2 data are significantly in accordance with those previously found by Faria et al.,[11] but lack the presence of the aglycone fragment.

Muruci (Byrsonima crassifolia). The HPLC chromatogram of the muruci extract is shown in Figure 2. According to Table 3, a total of 19 polyphenolic compounds could be at least tentatively identified as gallotannins, quinic acid gallates, proanthocyanidins, and quercetin derivatives. Five compounds could only be specified as gallic acid derivatives. Identification of gallic acid in peak 1 was assured by comparison of the fragments with those of an authentic standard.

As already discussed in the section on araçá, peaks 7 and 8 were tentatively assigned to digalloyl glucose and digalloyl pentose, respectively. Both peaks basically coincide in terms of their fragmentation pattern with these already described compounds. Peaks 2, 4−6, 10, and 11 were presumably found to be a series of quinic acid gallates showing a typical UV spectrum of gallic acid. Peak 2 produces $[M-H]^-$ ions at m/z 343 that yield fragment ions at m/z 169 and 125. The neutral loss of 174 Da corresponds to quinic acid (192 Da − H_2O), accounting for galloylquinic acid. Peak 11 is supposedly assigned to tetragalloylquinic acid producing $[M-H]^-$ ions at m/z 799. Yielded $[M-H-152]^-$ ions of this peak at m/z 647 are also shown in peaks 5 and 10 as parental $[M-H]^-$ ions, suggesting the presence of trigalloylquinic acid. Peaks 4 and 6 suffer from the loss of a galloyl residue

Table 2. UV and Mass Spectrometric Data of Phenolic Constituents Extracted from Jambolão (*Syzygium cumini*) Fruits

peak	retention time	compound[a,b]	HPLC-DAD λ_{max} (nm)	$[M - H]^- m/z$	fragments (m/z)
1	9.78	gallic acid	225, 273	169	MS^2 [169]: 125, 151
2	15.10	gallic acid derivative	226, 277	285	MS^2 [285]: 133, 169,
3	15.65	dihydromyricetin dihexoside[11]	237, 338	643	MS^2 [643]: 463, 481, 283, 355
4	16.68	methyldihydromyricetin dihexoside[11]	224, 277	657	MS^2 [657]: 495, 477, 315, 355
					MS^3 [657 → 495]: 315, 333, 369
5	17.75	unknown compound	225, 277	625	MS^2 [625]: 419, 257, 463, 581
					MS^3 [625 → 419]: 257, 404, 242
6	18.79	methyldihydromyricetin dihexoside[11]	253, 342	657	MS^2 [657]: 495, 315, 477, 333, 297, 355
					MS^3 [657 → 495]: 315, 333, 369
7	19.23	dimethyldihydromyricetin dihexoside[11]	243, 345	671	MS^2 [671]: 509
					MS^3 [671 → 509]: 347, 329
8	19.48	digalloylglucose[23]	224, 278	483	MS^2 [483]: 271, 331, 211, 169
					MS^3 [483 → 271]: 211, 169
9	20.20	trigalloylglucose[23]	227, 291	635	MS^2 [635]: 465, 483, 313, 271
					MS^3 [635 → 483]: 271
10	20.47	digalloylglucose[23]	224, 271	483	MS^2 [483]: 439, 313, 287, 465
					MS^3 [483 → 313]: 169
11	20.87	dimethyldihydromyricetin dihexoside[11]	223, 335	671	MS^2 [671]: 509
					MS^3 [671 → 509]: 347, 371, 329
12	21.76	trigalloylglucose[23]	225, 285	635	MS^2 [635]: 465, 483, 313
					MS^3 [635 → 483]: 271
13	22.04	trigalloylglucose[23]	224, 284	635	MS^2 [635]: 483, 465, 271
					MS^3 [635 → 483]: 423
14	23.13	HHDP galloylglucose[23]		633	MS^2 [633]: 615, 463, 505, 283, 571, 301
					MS^3 [633 → 615]: 463, 505, 571
15	23.40	trigalloylglucose[23]		635	MS^2 [635]: 465, 483, 313, 617
					MS^3 [635 → 465]: 131, 169
16	23.10	trigalloylglucose[23]	222, 278	635	MS^2 [635]: 465, 483, 313, 617
					MS^3 [635 → 465]: 313, 169
17	24.49	trisgalloyldiglucose	242, 267, 359	775	MS^2 [775]: 613, 451, 285
					MS^3 [775 → 613]: 451, 407, 285
18	24.88	tetragalloylglucose[23]	225, 283	787	MS^2 [787]: 635, 617, 465, 447
19a	27.05	myricetin hexoside[11]		479	MS^2 [479]: 317
19b	27.05	myricetin glucuronide		493	MS^2 [493]: 317
					MS^3 [493 → 317]: 179, 151, 194
20	28.03	tetragalloylglucose[23]	224, 280	787	MS^2 [787]: 617, 635, 465, 313
					MS^3 [787 → 617]: 465, 573, 447, 403, 313
21	28.69	galloylmethylmyricetin hexoside	224, 262, 360	645	MS^2 [645]: 493, 331, 479, 316
					MS^3 [645 → 493]: 331
22	29.14	myricetin deoxyhexoside[11]	224, 265, 352	463	MS^2 [463]: 317
					MS^3 [463 → 317]: 179, 272, 151
23	29.99	methylmyricetin hexoside	225, 264, 360	493	MS^2 [493]: 331, 301, 315
					MS^3 [493 → 331]: 315, 301, 179
24a	30.47	pentagalloylglucose[23]	224, 264	939	MS^2 [939]: 769, 787, 617, 599
					MS^3 [939 → 769]: 617, 599
24b	30.47	methylmyricetin pentoside	224, 264, 360	463	MS^2 [463]: 331, 301
					MS^3 [463 → 331]: 301
25	31.10	methylmyricetin glucuronide	224, 287, 352	507	MS^2 [507]: 331, 317
					MS^3 [507 → 331]: 301
26a	31.57	pentagalloylglucose[23]	224, 287	939	MS^2 [939]: 787, 769, 617
					MS^3 [939 → 787]: 617, 635, 465
26b	31.57	methylmyricetin pentoside	224, 287, 352	463	MS^2 [463]: 331, 301
					MS^3 [463 → 331]: 315
27a	32.41	dimethylmyricetin hexoside	225, 260, 355	507	MS^2 [507]: 345
					MS^3 [507 → 345]: 330, 301, 315, 271

Table 2. Continued

peak	retention time	compound[a,b]	HPLC-DAD λ_{max} (nm)	$[M-H]^-$ m/z	fragments (m/z)
27b	32.41	dimethylmyricetin pentoside	225, 260, 355	477	MS^2 [477]: 331, 315
					MS^3 [477 → 331]: 316
28	33.85	acylated myricetin deoxyhexoside[11,28]	223, 267, 354	505	MS^2 [505]: → 316, 463
					MS^3 [505 → 316]: 271, 287, 179
29	34.1	hexagalloylglucose[23]		1091	MS^2 [1091]: 939, 787
					MS^3 [1091 → 787]: 617, 635, 465
30	34.89	myricetin	260, 376	317	MS^2 [317]: 179, 151
31	35.89	dimethylmyricetin pentoside	228, 362	477	MS^2 [477]: 344, 329
32	37.07	acylated methylmyricetin deoxyhexoside[28]	263, 350	519	MS^2 [519]: 315, 331, 477
33	37.56	acylated galloylmyricetin deoxyhexoside[28]	223, 283	657	MS^2 [657]: 505, 317, 597
					MS^3 [657 → 317]: 179, 271
34	37.83	acylated galloyl ester of methylmyricetin pentoside	257, 363	657	MS^2 [657]: 517, 331, 505
					MS^3 [657 → 331]: 316

[a] Superscript numbers indicate the literature in which the compounds were previously described. [b] Gallic acid and myricetin were identified with authentic standard; all other compounds were tentatively identified.

resulting in $[M-H-152]^-$ ions at m/z 343. Hence, these compounds are tentatively assigned to digalloylquinic acid. The presence of galloylquinic acid esters in muruci fruits is supported by Maldini et al.[29] In this paper, 5-*O*-galloylquinic acid, 3-*O*-galloylquinic acid, 3,4-di-*O*-galloylquinic acid, 3,5-*O*-galloylquinic acid, and 3,4,5-tri-*O*-galloylquinic acid were identified in *B. crassifolia* bark by NMR and MS, which assumes the occurrence of these compounds also in the fruit. Mass spectrometric data of galloylquinic acids found in this study are in accordance with those determined in green tea.[30]

Peak 9 was identified as a proanthocyanidin dimer. The typical UV spectrum and fragmentation pattern match with those previously described by Friedrich et al.[31] Data were produced with the same MS instrument. The parent $[M-H]^-$ ion at m/z 729 of peak 13 produces fragment ions at m/z 577, 451, and 407 corresponding to those of peak 9. Due to the neutral loss of 152 Da, this compound was presumably assessed as a galloylproanthocyanidin dimer. Fragments of compounds found in peaks 9 and 13 are also in accordance with those reported by Sandhu et al.[23] Peak 15 suffers from the loss of 152 Da as well. On the basis of peak 13, yielded fragment ions at m/z 729 and 577 give rise to the presence of a digalloylproanthocyanidin dimer. Geiss et al.[32] reported different proanthocyanidins with (+)-epicatechin units occurring in the bark of *B. crassifolia*, which argues for the presence of (+)-epicatechin units also in the fruits.

Peaks 16−21 belong to a series of quercetin derivatives. All of these peaks show in part characteristic fragment ions of a quercetin aglycone (e.g., MS^n data at m/z 301, 300, 271, 255, 179, and/or 151), which were generated from fragmentation of an authentic quercetin standard. Hence, simply quercetin was identified in peak 24. Data of quercetin are consistent with those found by Hvattum and Ekeberg.[33] Peak 16 was tentatively identified as quercetin deoxyhexosylhexoside (m/z 609). The yielded product ion at m/z 300 resulted probably from the homolytic cleavage of the O-glycosidic bond, which gave rise to the formation of a radical aglycone anion.[33] Quercetin hexoside was presumably present in peak 17 (m/z 463), indicated by the neutral loss of 162 Da. Peaks 19 and 20 were assigned to be isomers of quercetin pentoside (m/z 433), which is designated by the neutral loss of 132 Da. Two quercetin gallates were found in peaks 18 and 21, resulting in an additional absorption maximum (∼270 nm) to the distinctive flavonol spectrum. Consequently, peak 18 was tentatively identified as galloylquercetin hexoside after sequential loss of 152 and 162 Da accounting for a galloyl and a hexosyl unit, respectively. The presence of galloylquercetin pentoside is likely in peak 21. The loss of 284 Da may be derived from a galloyl and a pentoside unit (152 Da + 132 Da), resulting into the aglycone ion of quercetin at m/z 301.

Peaks 3, 12, and 14, could not clearly be identified. Nevertheless, these peaks embed characteristics typically found for gallic acid. Peak 3 shows a UV spectrum similar to that of gallic acid. The parent $[M-H]^-$ ions at m/z 285 result in MS^2 data among others at m/z 169 accounting for the presence of gallic acid. Peaks 12 and 14 show interesting parallels. Peak 14 produces $[M-H]^-$ ions at m/z 601 and yields fragment ions (m/z 313, 439) that were previously found in MS^2 data of digalloyl glucoside. MS^3 data of peak 14 indicate a neutral loss of a galloyl residue (m/z 583 to m/z 431) and a neutral loss of a hexoside (m/z 431 to m/z 269). Peak 12 might be a derivative of peak 14. Its parent ions $[M-H]^-$ at m/z 617 suggest the presence of an additional hydroxyl group. MS^2 data also account for an additional hydroxyl group as the same neutral losses occur as in peak 14 but with an increase of 16 Da. The difference between m/z 599 and m/z 447 suggests the loss of a galloyl residue. Eventually, the difference between m/z 447 and m/z 285 assumes the loss of a hexoside. The same substances occur obviously also in cutite in the same elution order (compare peaks 21 and 25 of cutite in Table 4).

Cutite (Pouteria macrophylla). The HPLC chromatogram of the cutite extract is shown in Figure 2. According to Table 4, a total of 22 polyphenolic compounds could be at least tentatively identified as gallotannins, quinic acid gallates, ellagitannins, proanthocyanidins, flavonols, and a flavanonol. Identification of gallic acid in peak 1 was assured by using an authentic standard.

A galloylquinic acid (peak 3) and two digalloylquinic acid isomers (peaks 6b and 8b) were tentatively identified in cutite due to mass spectrometric data that were already discussed for galloylquinic acids in muruci. Findings agree with MS data of quinic acid gallates reported by Clifford et al.[30]

The same accounts for different digalloyl glucoside isomers (peaks 4−6a, 8a) and a trigalloyl glucoside (peak 13). $[M-H]^-$

Table 3. UV and Mass Spectrometric Data of Phenolic Constituents Extracted from Muruci (*Byrsonima crassifolia*) Fruits

peak	retention time	compound[a,b]	HPLC-DAD λ_{max} (nm)	$[M - H]^- m/z$	fragments (m/z)
1	9.35	gallic acid	225, 273	169	MS^2 [169]: 151, 125
2	11.21	galloylquinic acid[30]	226, 276	343	MS^2 [343]: 169, 125
3	13.41	gallic acid derivative	225, 277	285	MS^2 [285]: 169, 133
4	13.96	digalloylquinic acid[30]	225, 276	495	MS^2 [495]: 343, 325, 169
					MS^3 [495 → 343]: 169
5	15.80	trigalloylquinic acid[30]	225, 275	647	MS^2 [647]: 477, 325
					MS^3 [647 → 477]: 325, 169, 307
6	16.40	digalloylquinic acid[30]	226, 279	495	MS^2 [495]: 343, 325, 169
					MS^3 [495 → 343]: 169
7	17.27	digalloylglucose[23]	225, 272	483	MS^2 [483]: 439, 313, 271
					MS^3 [483 → 439]: 313, 287
8	17.67	digalloylpentose	226, 280	453	MS^2 [453]: 313, 327, 285, 169
					MS^3 [453 → 313]: 169
9	18.58	proanthocyanidin dimer[31]	228, 282	577	MS^2 [577]: 425, 407, 451, 289
					MS^3 [577 → 425]: 407
10	19.12	trigalloylquinic acid[30]	226, 277	647	MS^2 [647]: 495, 477, 343
					MS^3 [647 → 495]: 343, 325, 169
11	19.83	tetragalloylquinic acid[30]	227, 277	799	MS^2 [799]: 601, 629, 477, 647,
					MS^3 [799 → 601]: 431, 449, 261
12	20.28	unknown gallic acid derivative	226, 291	617	MS^2 [617]: 285, 313, 599, 447
					MS^3 [617 → 285]: 241
13	20.94	galloylproanthocyanidin dimer[23]	228, 295	729	MS^2 [729]: 407, 559, 577, 451, 603, 289
					MS^3 [729 → 407]: 285
14	22.75	unknown gallic acid derivative	226, 287	601	MS^2 [601]: 583, 269, 313, 439
					MS^3 [601 → 583]: 313, 269, 431
15	23.13	digalloylproanthocyanidin dimer	227, 295	881	MS^2 [881]: 729, 559, 711, 577
					MS^3 [881 → 729]: 407, 577, 559
16	23.59	quercetin deoxyhexosylhexoside	258, 357	609	MS^2 [609]: 300, 271, 343
					MS^3 [609 → 300]: 271, 255, 179, 151
17	24.32	quercetin hexoside	258, 358	463	MS^2 [463]: 301
					MS^3 [463 → 301]: 271, 255, 179, 151
18	24.87	galloylquercetin hexoside	227, 271, 366	615	MS^2 [615]: 301, 463, 313
					MS^3 [615 → 313]: 169
19	25.83	quercetin pentoside	259, 356	433	MS^2 [433]: 301
					MS^3 [433 → 301]: 271, 255
20	26.58	quercetin pentoside	274, 361	433	MS^2 [433]: 301
					MS^3 [433 → 301]: 271, 255
21	27.33	galloylquercetin pentoside	226, 268, 356	585	MS^2 [585]: 301
					MS^3 [585 → 301]: 179, 151
22	28.61	unknown	246, 316	677	MS^2 [677]: 645, 617, 585
					MS^3 [677 → 645]: 489
23	30.07	unknown	248, 316	675	MS^2 [675]: 643, 599
					MS^3 [677 → 643]: 599, 625
24	33.33	quercetin	257, 370	301	MS^2 [301]: 179, 151

[a] Superscript numbers indicate the literature in which the compounds were previously described. [b] Gallic acid and quercetin were identified with authentic standard; all other compounds were tentatively identified.

ions at m/z 483 and m/z 635, respectively, produce characteristic fragment ions that are present in muruci, araçá, and jambolão, too.

Peaks 9, 10, 12, 14, and 16−18 show fragmentation patterns distinctive for ellagitannins.[23] The presence of two HHDP glucose isomers (Figure 2) is indicated in peaks 9 and 10 by the production of $[M - H]^-$ ions at m/z 481 and accordant dominating daughter ions at m/z 301. Peaks 12, 14, and 16−18 yielded fragment ions at m/z 481 accounting for HHDP glucose after loss of a galloyl residue of 152 Da. Hence, these compounds are tentatively ascribed to be isomers of HHDP galloylglucose. The occurrence of the dominating fragment ion at m/z 301 for these compounds is in agreement with the report by Sandhu et al.[23]

Different groups of flavonoids are detectable in cutite. Peaks 7 and 11 were recognized as proanthocyanidins. Both peaks show

Table 4. UV and Mass Spectrometric Data of Phenolic Constituents Extracted from Cutite (*Pouteria macrophylla*) Fruits

peak	retention time	compound[a,b]	HPLC-DAD λ_{max} (nm)	$[M - H]^- m/z$	fragments (m/z)
1	9.35	gallic acid	225, 273	169	MS^2 [169]: 151, 125
2	9.64	gallic acid derivative	229, 274	483	MS^2 [483]: 465, 368, 174, 303, 350, 393, 229
					MS^3 [483 → 368]: 350
3	11.48	galloylquinic acid[30]	226, 276	343	MS^2 [343]: 169, 173
4	14.45	digalloylglucose[23]	226, 278	483	MS^2 [483]: 313, 331, 169, 271
					MS^3 [483 → 313]: 169
5	15.60	digalloylglucose[23]	226, 273	483	MS^2 [483]: 331, 271, 169, 241, 423, 313
					MS^3 [483 → 331]: 169, 271
6a	16.58	digalloylglucose[23]	229, 279	483	MS^2 [483]: 331, 169, 271, 313
					MS^3 [483 → 331]: 169, 271, 241
6b	16.58	digalloylquinic acid[30]	229, 279	495	MS^2 [495]: 343, 191
					MS^3 [495 → 343]: 191, 169
7	16.81	galloyl(epi)gallocatechin dimer	228, 284	761	MS^2 [761]: 423, 609, 575, 305, 405, 287
					MS^3 [761 → 423]: 283, 297
8a	17.10	digalloylglucose[23]	226, 277	483	MS^2 [483]: 331, 169, 241, 271, 313
					MS^3 [483 → 331]: 241, 169, 271
8b	17.10	digalloylquinic acid[30]	226, 277	495	MS^2 [495]: 343, 325, 169
					MS^3 [495 → 343]: 169, 191, 125
9	18.13	HHDP glucose[23]	233, 296	481	MS^2 [481]: 301, 355, 463, 151
					MS^3 [481 → 301]: 257, 215, 283
10	18.96	HHDP glucose[23]	231, 295	481	MS^2 [481]: 301, 355, 463, 151
					MS^3 [481 → 301]: 257, 215, 283
11	19.58	digalloyl(epi)gallocatechin dimer	229, 276	913	MS^2 [913]: 761, 423, 743, 591, 573, 609
					MS^3 [913 → 761]: 609, 591
12	19.86	HHDP galloylglucose[23]	228, 290	633	MS^2 [633]: 507, 301, 481, 271, 331, 615
					MS^3 [633 → 507]: 271, 175, 355
13	20.39	trigalloylglucose[23]	228, 277	635	MS^2 [635]: 465, 483, 617, 313
					MS^3 [635 → 465]: 313, 169, 211
14	21.12	HHDP galloylglucose[23]	231, 296	633	MS^2 [633]: 301, 507, 481, 271, 331
					MS^3 [635 → 507]: 175, 355, 271, 331
15	21.65	unknown compound	233, 295	467	MS^2 [467]: 286, 285, 340, 151
16	22.55	HHDP galloylglucose[23]	229, 293	633	MS^2 [633]: 481, 301, 471, 355, 507, 463
					MS^3 [633 → 481]: 301, 355, 151, 463
17	22.88	HHDP galloylglucose[23]	226, 293	633	MS^2 [633]: 331, 301, 481, 507, 271
					MS^3 [633 → 331]: 271, 169, 211, 193
18	23.60	HHDP galloylglucose[23]	231, 296	633	MS^2 [633]: 331, 301, 481, 507, 271, 215, 355
					MS^3 [633 → 331]: 271, 169, 211, 193
19	24.11	unknown compound		449	MS^2 [449]: 269, 316
					MS^3 [449 → 269]: 225, 151, 197, 183
20	25.24	myricetin deoxyhexoside	268, 355	463	MS^2 [463]: 316
					MS^3 [463 → 316]: 271, 287, 179, 151
21	26.03	unknown gallic acid derivative	235, 295	617	MS^2 [617]: 331, 285, 465, 491, 507
					MS^3 [617 → 285]: 241, 199, 217, 175, 257
22	26.30	dihydroquercetin	295, 337	303	MS^2 [303]: 285, 177, 125, 179, 241, 276
23	27.20	unknown compound	231, 308	263	MS^2 [263]: 219, 191
					MS^3 [263 → 219]: 191
24	28.05	quercetin deoxyhexoside	266, 297, 352	447	MS^2 [447]: 301
					MS^3 [447 → 301]: 179, 271, 255, 151
25	28.41	unknown gallic acid derivative	228, 294	601	MS^2 [601]: 287, 259, 331, 475, 313, 269
					MS^3 [601 → 287]: 259, 243
26	28.89	unknown compound	229, 301	575	MS^2 [575]: 395, 449
					MS^3 [575 → 395]: 367, 243, 449, 269
27	30.14	unknown compound	234, 297	287	MS^2 [287]: 259, 243, 269, 201
					MS^3 [287 → 259]: 215, 173, 125, 241, 151

dx.doi.org/10.1021/jf201039r |*J. Agric. Food Chem.* 2011, 59, 7688–7699

Table 4. Continued

peak	retention time	compound[a,b]	HPLC-DAD λ_{max} (nm)	$[M - H]^- m/z$	fragments (m/z)
28	35.42	quercetin	268, 370	301	MS^2 [301]: 179, 151, 273

[a] Superscript numbers indicate the literature in which the compounds were previously described. [b] Gallic acid and quercetin were identified with authentic standard; all other compounds were tentatively identified.

Table 5. Antioxidant Capacity (TOSC) against Two Different Radicals, Total Phenolic Content, and Ascorbic Acid Content of Four Fruits from the Amazon Region

	peroxyl radicals[a]	peroxynitrite[a]	total phenolic content[b]	ascorbic acid[c]
cutite	0.57	0.83	2915.1 ± 0.0	247.5 ± 23.5
jambolão	1.49	3.13	786.8 ± 6.9	93.5 ± 12.0
araçá	1.58	4.00	754.4 ± 12.5	101.3 ± 9.8
muruci	5.26	10.00	254.7 ± 15.2	nq

[a] Concentration of freeze-dried sample (g/L) that is needed to obtain an inhibition rate of 50%. TOSC values imply a variance <5%. [b] Data expressed as the mean ± standard deviation (n = 4) in mg gallic acid equivalent/100 g dry matter. [c] Data expressed as the mean ± standard deviation (n = 4) in mg/100 g dry matter; nq, not quantifiable.

typical MS^n ions for a (epi)gallocatechin dimer at m/z 609, 423, and 305. Congruent data obtained under the same instrumental conditions were previously published.[31] The parent $[M - H]^-$ ions at m/z 761 of peak 7 and $[M - H]^-$ ions at m/z 913 of peak 11 indicate the likely presence of a galloyl(epi)gallocatechin dimer and a digalloyl(epi)gallocatechin dimer, respectively. This assignment is derived from the neutral loss of a galloyl unit (152 Da) in peak 7 and the sequential loss of two galloyl units in peak 11. Three flavonols were found in peaks 20, 24, and 28. Peak 20 was tentatively identified as myricetin deoxyhexoside and peak 24 as quercetin deoxyhexoside. The neutral loss of 146 Da yielded the particular (radical) aglycone ion (m/z 316 and 301, respectively). Simply quercetin was specified in peak 28 on the basis of an authentic standard. Due to MS^2 data, peak 22 was tentatively identified as the flavanonol dihydroquercetin ($[M - H]^-$ at m/z 303).

Antioxidant Capacity. Table 5 shows the results of the antioxidant capacity measurement of the four Amazonian fruits. Aqueous extracts were determined on their radical scavenging activity against peroxyl radicals (px) and peroxynitrite (pn) by the TOSC assay. TOSC results indicate the concentration of antioxidants present in the sample that is needed to attain a radical inhibition of 50% (IC_{50}). The total phenolic content was measured by Folin−Ciocalteu. The concentration of ascorbic acid was determined in these fruits as ascorbic acid affects results of the total phenolic content[17] and shows a perceivable impact on the antioxidant capacity measured by TOSC.[16] Thus, knowledge of the ascorbic acid content is useful to evaluate more properly the contribution of different bioactive compounds to the overall antioxidant capacity. The results are also given in Table 5.

The highest antioxidant capacity against both radicals was assessed for cutite followed by jambolão, araçá, and muruci. Against px, cutite bore a 9-fold higher antioxidant capacity in comparison to muruci. Against pn, even the 12-fold amount of muruci sample is needed to attain the IC_{50} when compared to cutite. According to both radicals, antioxidant properties between jambolão and araçá were less distinctive. Both fruits showed an approximately 3 times (px) and 4−5 times (pn) lower radical scavenging capacity than cutite.

It becomes obvious that the antioxidant capacity of fruit extracts generally performed higher against px than against pn. By comparing TOSC values against the two radicals, the results indicate differences in the effectiveness of the antioxidants contained in the fruit extracts. The difference between the antioxidant potential of px and pn is less distinctive for cutite in comparison to the other fruits. The 1.5-fold amount of cutite sample is required for the IC_{50} of pn in comparison to px. Double the amount of sample is necessary for jambolão and muruci, and even a 2.5-fold amount of araçá sample is needed. Consequently, the antioxidants present in cutite show the most effective impact against pn, whereas the antioxidants in araçá are least effective against pn when compared to px.

Results of the TOSC assay are interrelated with the total phenolic content. The amounts of determined total phenols of the four fruits give rise to the same ranking as described for px and pn. Hence, the antioxidant properties of each fruit can be ascribed to the total phenolic content in the meaning of the Folin−Ciocalteu test. The lowest phenolic content was found in muruci, being roughly comparable to that of banana pulp.[34] The 12-fold amount of total phenols was constituted in cutite, matching that of tropical highland blackberries.[35]

Results of the ascorbic acid content showed that noticeable amounts were found only in cutite. Jambolão and araçá contained less than half of the concentration present in cutite. Ascorbic acid in muruci could not unambiguously be identified. Besides the phenolic content, ascorbic acid may significantly contribute to the antioxidant behavior of cutite fruits. As described by Lichtenthäler et al.,[16] a similar concentration of ascorbic acid standard is needed to attain a radical inhibition of 50% against both radicals. This could explain the less pronounced difference of the antioxidant capacity of cutite against the two radicals. Jambolão shows a higher antioxidant capacity than araçá, although the content of ascorbic acid is slightly lower. Thus, antioxidant compounds other than ascorbic acid seem to significantly influence the radical scavenging behavior of jambolão. Finally, the comparably weak antioxidant activity of muruci may be explained by the probable absence of ascorbic acid in this fruit in addition to the low total phenolic content.

Up to now only a few studies are known about the antioxidant capacity of the four fruits from the Amazon region. Two different papers reported the free radical scavenging behavior of jambolão and muruci. $DPPH^•$ assay conditions for the determination of both fruits were identical. Results showed a 3-fold higher antioxidant capacity for jambolão in comparison to muruci, which is accordance with our findings.[9,14]

In comparison to other fruits originating from the Amazon basin, the antioxidant properties determined by the TOSC assay of cutite against pn were better than those of açaí pulp. Different harvest years of açaí (1998, 2000, and 2002) require concentrations between 1.17 and 1.72 g/L to attain an inhibition of 50%. In contrast, the radical scavenging potential of cutite against px is less effective than that of açaí (0.39−0.48 g/L).[36] Cutite also shows a lower antioxidant capacity with regard to both radicals than the outstanding fruits of camu-camu,[37] but its antioxidant

capacity was higher when compared to berries of *Clidemia rubra* from Colombia.[18]

In conclusion, a large number of phenolic constituents was detected in the Amazonian fruits. Thereof, 18 compounds were found in araçá, 37 in jambolão, 19 in muruci, and 22 in cutite. The compounds can be ascribed to hydrolyzable tannins, proanthocyanidins, flavonols, and flavanonols. Interestingly, no flavonoids could be found in araçá but only gallic acid derivatives. Cutite and muruci present different galloylquinic acid derivatives, which have rarely been proven in fruits. Studies on the antioxidant capacity revealed the highest bioactive potential for cutite followed by jambolão, araçá, and muruci.

AUTHOR INFORMATION

Corresponding Author

*Phone: +49 228 733713. Fax: +49 228 733757. E-mail: f.marx@uni-bonn.de.

ACKNOWLEDGMENT

Andrea Memmesheimer is gratefully acknowledged for assistance with the analysis of the phenolic content by Folin−Ciocalteu.

ABBREVIATIONS USED

AUC, area under the curve; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DMSO, dimethyl sulfoxide; FAc, formic acid; HHDP, hexahydroxydiphenic acid; IC_{50}, radical inhibition of 50%; KMBA, α-keto-γ-methiolbutyric acid; PA, polyamide; pn, peroxynitrite; px, peroxyl radicals; SPE, solid phase extraction; TFA, trifluoroacetic acid; TOSC, total oxidant scavenging capacity; UHQ, ultrahigh quality; WE, water extract.

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