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Phenolic compounds are highly correlated to the antioxidant capacity of genotypes of *Oenocarpus distichus* Mart. fruits

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ABSTRACT

This research aimed to evaluate 32 genotypes of *Oenocarpus distichus* fruits regarding the contents of total phenolic compounds, flavonoids, flavanols, monomeric anthocyanins, antioxidant capacity (ABTS and DPPH assays), and the phenolic compound profiles of the five genotypes that presented the highest yields of bioactive compounds. The genotypes were harvested in three different locations in Pará State, Northern Brazil, (Belém, São João do Araguaia and Marabá). Among the 32 genotypes, the highest bioactive compound contents and antioxidant capacity were found for three genotypes harvested in Belém (B-3, B-7 and B-8) and two harvested in São João do Araguaia (SJ-1 and SJ-4), and the total phenolic compounds varied from 131.97 to 363.01 mg gallic acid equivalent/100 g, total flavonoids from 24.23 to 38.19 mg quercetin equivalent/100 g, total flavanols from 72.29 to 259.18 mg catechin equivalent/100 g, and monomeric anthocyanins from 21.31 to 67.76 mg cyanidin 3-rutinoside/100 g. The main phenolic compounds tentatively identified in the five selected genotypes were cyanidin 3-*O*-rutinoside (48.47 to 196.51 μg/g), which could be identified and quantified as the major phenolic compound in *Oenocarpus distichus* fruits, for the first time, followed by chlorogenic acid (0.71 to 64.56 μg/g) and rutin (13.98 to 56.76 μg/g).

1. Introduction

The Amazon hosts a great number of palm tree species whose fruits are consumed by the local population and widespread in the local economy at street markets, but mostly unknown to the general population. Among the wide diversity of native and exotic palm trees in the Amazonian region, the genus *Oenocarpus* spp. is one of the most explored by the regional extractivism due to the great economic, ecologic, and dietary potential of the species of this genus (Moscoso, Albernaz, & Salomão, 2010; Pereira, Alves, Sousa, & Costa, 2013). Six species of this genus are accepted as native to Brazil, but not endemic, and among them, four species are popularly called *bacabeiras* (bacaba trees): *O. distichus* Mart., *O. bacaba* Mart., *O. minor* Mart., and *O. mapora* H. Karten, and one called *patauazeiro* (patawa tree): *O. pataua* Mart. (Leitman, Henderson, Noblick, & Martins, 2015).

Bacaba-de-leque is the Brazilian name given to *Oenocarpus distichus* Mart. and it is widely used in the Amazonian region to prepare a nutritive energy drink popularly known as “bacaba wine,” which has promising commercial potential, similar to the beverage prepared from açaí (*Euterpe oleracea*) (Balick, 1979; Henderson, 1995; Lorenzi, Kahn, Noblick, & Ferreira, 2010).

Due to its high dietary potential, researches on domestication of *Oenocarpus distichus* Mart. have been developed through genetic improvement programs aiming to maintain the sustainably use of this important natural resource. To accomplish such purpose, between the 1980s and 1990s, the Brazilian Agricultural Research Corporation (EMBRAPA), through Embrapa Eastern Amazon Research Center, implemented the Active Germplasm Bank (*Banco Ativo de Germoplasma* - BAG) for bacaba trees, comprising a huge number of genotypes from natural populations and genetic improvement, which is the only bacaba tree repository in Brazil (Oliveira & Rios, 2012).

Despite the socioeconomic, cultural, and nutritional potential of bacaba-de-leque fruits, information on bioactive compound contents and profiles available in the literature are still scarce for the species, which limits the knowledge on its nutritional value and exploitation potential, as well as genetic improvement programs. Thus, the bioactive compounds characterization allied to genetic improvement represents an important step forward for genetic improvement programs on palm

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trees of genus *Oenocarpus*, resulting in a database for the selection of promising genotypes to develop new cultivars with health-related attributes.

The phenolic compound composition of fruits of species in the genus *Oenocarpus* spp. is quite broad (Carvalho, Silveira, Sousa, Moraes, & Godoy, 2016; Finco et al., 2012; Rezaire et al., 2014). In a recent study Carvalho et al. (2016) investigated the phenolic compound profile of *O. distichus* Mart fruits and pointed out that main phenolic compounds identified were ferulic acid (4,77–10,8 μg/g), rutin (12,5–56,8 μg/g) and epicatechin (15,5–21,2 μg/g). Studies comprising the phytochemical composition of fruits might contributes to valuing native species to Amazon, which may greatly contribute to the availability of natural antioxidant compounds in the diet.

Therefore, this study aimed to determine the contents of different phenolic compound classes of 32 genotypes of *Oenocarpus distichus* Mart ripe fruits from different locations and to assess the influence of those bioactive compounds on their antioxidant capacity. All data were analyzed by Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) aiming to classify the 32 genotypes according to the bioactive compound contents. Moreover, the phenolic compound profiles of the most promising genotypes (with high bioactive compound contents and antioxidant capacity values) were determined by high-performance liquid chromatography coupled to diode array detector (HPLC-DAD).

2. Material and methods

2.1. Chemicals

The standards 3,4-dihydroxybenzoic acid, chlorogenic acid, cyanidin 3-*O*-rutinoside, syringic acid, ferulic acid, rutin, gallic acid, quercetin, catechin, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Sigma-Aldrich (St. Louis, USA). Methanol (analytical grade) was purchased from Dinâmica (São Paulo, Brazil) and formic acid, acetonitrile, and methanol, all chromatographic grade, were obtained from Tedia® (Fairfield, USA). Water was purified in a Milli-Q system (Millipore Co., Bedford, USA). For chromatographic analysis, samples and solvents were filtered using, respectively, membranes of 0.22 and 0.45 μm, (Chromafil®, Duren, Germany).

2.2. Oenocarpus distichus samples and bioactive compounds extraction

Ripe fruits of 32 genotypes of *Oenocarpus distichus* Mart were collected in December 2015 from plants from the BAG of Embrapa Eastern Amazon, located in Belém, Pará State (PA), Brazil, and from naturally occurring plants in the cities of Marabá and São João do Araguaia, both located in PA, Brazil. The fruits were coded according to the location of harvesting, with 11 genotypes from Belém (B-1, B-2, B-3, B-4, B-5, B-6, B-7, B-8, B-9, B-10, and B-11), 09 from São João do Araguaia (SJ-1, SJ-2, SJ-3, SJ-4, SJ-5, SJ-6, SJ-7, SJ-8, and SJ-9), and 12 from Marabá (MB-1, MB-2, MB-3, MB-4, MB-5, MB-6, MB-7, MB-8, MB-9, MB-10, MB-11, and MB-12).

About 2 kg of completely ripe fruits, collected from each genotype, were transported to the Laboratory of Food Processing of Embrapa Eastern Amazon (Belém, PA, Brazil), where they were selected and the ones with signs of spoilage or with some type of physical damage were discarded. After the selection, the fruits were washed in running water and submitted to water at 60 °C for 15 min to soften the hard shell of the fruit and facilitate the depulping process (traditional artisanal way of obtaining the pulp). Mechanical depulping was carried out at a 2:1 ratio (fruit:water, w/v) in a vertical stainless steel machine (METVISA® DG.10, Belém, Brazil) previously sanitized with a sodium hypochlorite aqueous solution at 200 mg/L and the seeds were discarded.

The obtained pulps were packed in polyethylene bags (HDPE) and immediately frozen at −18 °C in a cold chamber. After freezing, the material were directed to the freeze-drying process at −55 °C in vacuum for 48 h using a benchtop lyophilizer (Liotop, L101, São Paulo, Brazil) and the samples were stored in polypropylene bags (BOPP) protected from light at 10 °C until analyses.

The bioactive compounds of each genotype of *Oenocarpus distichus* were extracted from the lyophilized material with methanol/water (60:40 v/v), according to the method proposed by Carvalho et al. (2016). The obtained bioactive compound extracts were used in all the assays.

2.3. Bioactive compound determination in O. distichus fruits

2.3.1. Total phenolic compounds

The content of total phenolic compounds was determined by the Folin-Ciocalteu colorimetric assay, as described by Singleton and Rossi (1965) and modified by Georgé, Brat, Alter, and Amiot (2005). Quantification was performed with an UV–Vis spectrophotometer (Thermo Scientific, Evolution 300, San Jose, USA) at 760 nm using five-point analytical curves (duplicate) of gallic acid, with concentrations varying from 20 to 100 mg/L ($r^2 = 0.99$). All the results ($n = 3$) were expressed as mg of gallic acid equivalents (GAE) per 100 g of freeze-dried sample (dry basis - d.b.).

2.3.2. Total flavonoids

Total flavonoids were quantified using the colorimetric assay with aluminum chloride ($AlCl_3$), described by Meda, Lamien, Romito, Millogo, and Nacoulma (2005). The compounds were quantified by spectrophotometry at 415 nm using seven-point analytical curves (duplicate) of quercetin, with concentrations varying from 5 to 35 mg/L ($r^2 = 0.99$). The results ($n = 3$) were expressed as mg of quercetin equivalent (QE) per 100 g of freeze-dried samples (d.b.).

2.3.3. Total flavanols

Total flavanols were determined by the colorimetric vanillin-HCl assay, described by Julkunen-Tiitto (1985). The quantification was performed by spectrophotometry at 500 nm using five-point analytical curves (duplicate) of catechin, with concentrations varying from 100 to 500 mg/L ($r^2 = 0.99$). The results ($n = 3$) were expressed as mg of catechin equivalent (CE) per 100 g of freeze-dried samples (d.b.).

2.3.4. Total monomeric anthocyanins

Total monomeric anthocyanins were quantified according to the spectrophotometric method of differential pH, described by Giusti and Wrolstad (2001). Absorbance was read in the spectrophotometer at wavelengths of 510 and 700 nm. The results (n = 3) were calculated as cyanidin 3-rutinoside equivalent (molar extinction coefficient 28,840 $L \cdot cm^{-1} \cdot mol^{-1}$ and molecular weight of 594 $g \cdot mol^{-1}$) and the final contents were expressed as mg cyanidin 3-rutinoside/100 g of freeze-dried samples (d.b.).

2.4. In vitro scavenging capacity by chemical assays

2.4.1. TEAC assay

The TEAC assay was based on a method developed by Miller et al. (1993) with modifications. $ABTS^{\cdot +}$ was generated by reacting 7 mM ABTS stock solution with 145 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot +}$ solution was diluted with methanol to an absorbance of ≈0.70 at 734 nm. After the addition of 30 μL of *O. distichus* extract or Trolox standard to 3 mL of diluted $ABTS^{\cdot +}$ solution, the absorbance spectra were recorded after 6 min mixing. Methanolic solutions of Trolox were used to build the analytical curves (0.01 to 0.20 mg/mL) and the results ($n = 3$) were expressed as μmol Trolox equivalent (TE) per g of freeze-dried sample (d.b.).

Table 1
Bioactive compound contents (n = 3, dry basis) of fruits from 32 genotypes of *Oenocarpus distichus* from different locations in the Amazonian region (Pará State, Brazil).

Locations	*Oenocarpus distichus* genotypes	Total phenolic compounds (mg GAE/100 g)	Total flavonoids (mg QE/100 g)	Total flavanols (mg CE/100 g)	Total monomeric anthocyanins (mg cyanidin 3-rutinoside/100 g)
Belém	B-1	90.90 ± 4.25^{l}	16.86 ± 1.60^{d}	82.55 ± 6.97^{h}	10.56 ± 0.58^{i}
	B-2	167.38 ± 2.46^{g}	34.19 ± 2.84^{a}	162.64 ± 17.40^{d}	11.40 ± 0.31^{i}
	B-3	267.63 ± 5.86^{c}	25.73 ± 3.06^{c}	257.66 ± 18.81^{a}	67.76 ± 1.85^{a}
	B-4	181.50 ± 22.70^{f}	16.56 ± 2.22^{d}	178.48 ± 8.71^{c}	12.97 ± 0.67^{h}
	B-5	133.19 ± 2.18^{h}	28.28 ± 1.97^{b}	95.23 ± 5.44^{g}	5.80 ± 0.36^{l}
	B-6	106.02 ± 1.58^{j}	30.11 ± 2.56^{b}	67.40 ± 9.03^{i}	10.23 ± 0.69^{i}
	B-7	363.01 ± 24.40^{a}	37.44 ± 4.35^{a}	194.55 ± 9.34^{b}	61.40 ± 1.36^{b}
	B-8	319.98 ± 19.49^{b}	24.23 ± 2.67^{c}	259.18 ± 10.58^{a}	38.54 ± 0.12^{c}
	B-9	224.94 ± 2.46^{d}	35.25 ± 3.50^{a}	208.99 ± 19.33^{b}	20.19 ± 1.27^{f}
	B-10	118.67 ± 1.57^{i}	26.60 ± 3.33^{c}	65.89 ± 6.95^{i}	36.58 ± 0.46^{d}
	B-11	134.77 ± 3.72^{h}	28.97 ± 2.58^{b}	112.65 ± 10.01^{f}	18.05 ± 0.24^{g}
São João do Araguaia	SJ-1	131.97 ± 4.60^{h}	34.75 ± 3.51^{a}	105.02 ± 7.65^{g}	21.31 ± 1.27^{f}
	SJ-2	106.07 ± 1.20^{j}	14.81 ± 1.38^{e}	101.53 ± 2.90^{g}	14.65 ± 1.25^{h}
	SJ-3	123.60 ± 0.98^{i}	25.07 ± 2.78^{c}	32.36 ± 2.82^{j}	7.46 ± 0.44^{j}
	SJ-4	221.57 ± 14.37^{d}	38.19 ± 3.79^{a}	72.29 ± 9.31^{i}	25.58 ± 0.25^{e}
	SJ-5	102.11 ± 9.14^{j}	15.92 ± 2.45^{d}	86.89 ± 5.53^{h}	13.51 ± 0.39^{h}
	SJ-6	81.86 ± 5.65^{l}	18.27 ± 1.95^{d}	78.50 ± 5.92^{h}	16.13 ± 2.11^{g}
	SJ-7	104.58 ± 2.75^{j}	19.80 ± 2.26^{d}	100.08 ± 2.34^{g}	8.37 ± 0.35^{j}
	SJ-8	96.02 ± 4.85^{j}	17.91 ± 1.25^{d}	68.48 ± 5.53^{i}	13.51 ± 0.50^{h}
	SJ-9	107.96 ± 1.90^{j}	17.15 ± 2.4^{d}	99.39 ± 3.84^{g}	7.48 ± 0.44^{j}
Marabá	MB-1	106.80 ± 2.33^{j}	25.18 ± 2.76^{c}	88.56 ± 2.85^{g}	17.16 ± 0.44^{g}
	MB-2	96.67 ± 1.15^{j}	18.14 ± 2.54^{d}	76.70 ± 3.84^{h}	6.27 ± 0.44^{j}
	MB-3	115.80 ± 1.70^{i}	24.58 ± 2.13^{c}	116.49 ± 12.34^{f}	20.51 ± 1.28^{f}
	MB-4	82.19 ± 0.11^{l}	16.67 ± 2.31^{d}	84.30 ± 7.71^{h}	12.04 ± 0.55^{h}
	MB-5	195.06 ± 1.36^{e}	29.25 ± 3.40^{b}	140.38 ± 11.23^{e}	5.00 ± 0.30^{l}
	MB-6	102.30 ± 10.45^{j}	10.81 ± 1.41^{e}	88.28 ± 4.85^{h}	3.05 ± 0.32^{l}
	MB-7	105.78 ± 6.48^{j}	14.30 ± 2.19^{e}	78.62 ± 2.01^{h}	9.25 ± 0.86^{i}
	MB-8	193.28 ± 5.82^{e}	9.53 ± 1.65^{e}	109.23 ± 11.52^{f}	24.41 ± 1.23^{e}
	MB-9	89.78 ± 5.85^{l}	14.05 ± 1.02^{e}	87.41 ± 4.70^{h}	18.24 ± 0.87^{g}
	MB-10	178.99 ± 4.50^{f}	26.84 ± 2.31^{c}	68.74 ± 5.55^{i}	4.70 ± 0.45^{l}
	MB-11	196.02 ± 53.90^{e}	12.36 ± 1.07^{e}	153.88 ± 10.60^{d}	25.38 ± 0.70^{e}
	MB-12	117.64 ± 1.34^{i}	19.52 ± 2.36^{d}	99.45 ± 8.21^{g}	11.20 ± 0.53^{i}

All data are means ± standard deviation (n = 3, dry basis). Means in the same column with the same lowercase letter are not statistically different at 95% significance by the Scott-Knott test.

2.4.2. DPPH· assay

Antioxidant capacity was determined using the modified DPPH· method (Brand-Williams, Cuvelier, & Berset, 1995). A methanol solution containing 0.06 mM DPPH· was prepared. After adjusting the blank with methanol, an aliquot of 100 µL of *O. distichus* extract was added to 3.9 mL of this solution. The decrease in absorbance at 515 nm was monitored by spectrophotometry at 1 min intervals during the first 10 min and then at 5 min intervals until stabilization (60 min, as determined by a preliminary study). The antioxidant capacity was expressed as the concentration of antioxidant required to reduce the original amount of DPPH· by 50% (EC_{50}), and the results (n = 3) were expressed as g of freeze-dried sample per g DPPH·.

2.5. HPLC-DAD profile of phenolic compounds

The phenolic compound profiles of the five most promising *O. distichus* genotypes, chosen based on the high contents of bioactive compounds, were determined in an HPLC system (Thermo Scientific, Finnigan Surveyor CA 95134, San Jose, USA) equipped with a quaternary pump (Surveyor LC Pump Plus 500201), diode array detector (DAD, UV–Vis 450176-PDA-5P), column oven, and an auto-sampler (Surveyor auto-sampler Plus ASP, San Jose, USA). The *O. distichus* extracts were diluted in ultrapure water, filtered, and injected into the chromatographic system.

The phenolic compounds were separated on a Synergi Hydro C_{18} column (Phenomenex, USA, 4 µm, 250 × 4.6 mm). A linear gradient of water with 0.5% formic acid (eluent A) and acetonitrile with 0.5% formic acid (eluent B) was used from A:B 99:1 to 50:50 in 50 min, then from 50:50 to 1:99 in 5 min and this final ratio (1:99) was kept for an additional 5 min (Chisté & Mercadante, 2012). The flow rate was 0.9 mL/min and the column temperature was set at 29 °C. The injection volume for the chromatographic system was 20 µL. The phenolic compounds were tentatively identified based on the following information: elution order on a C_{18} column, retention time and UV–Visible features as compared to authentic standards analyzed under the same experimental conditions, and data available in the literature (Carvalho et al., 2016; Finco et al., 2012). The compounds were quantified by external standards using seven-point analytical curves (duplicate) and for all the compounds, the limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the parameters of the analytical curves (standard deviation and the slope) (ICH, 2005): 3,4-dihydroxybenzoic acid (1.56 to 50 µg/mL, r^2 = 0.99, LOD = 0.12 µg/mL and LOQ = 0.64 µg/mL), chlorogenic acid (1.56 to 50 µg/mL, r^2 = 0.99, LOD = 0.15 µg/mL and LOQ = 0.62 µg/mL), cyanidin 3-*O*-rutinoside (1.56 to 50 µg/mL, r^2 = 0.99, LOD = 0.16 µg/mL and LOQ = 0.68 µg/mL), syringic acid (1.56 to 50 µg/mL, r^2 = 0.99, LOD = 0.11 µg/mL and LOQ = 0.66 µg/mL), rutin (1.56 to 50 µg/mL, r^2 = 0.99, LOD = 0.12 µg/mL and LOQ = 0.67 µg/mL), and ferulic acid (1.56 to 50 µg/mL, r^2 = 0.99, LOD = 0.10 µg/mL and LOQ = 0.61 µg/mL). The phenolic compound contents were expressed as µg/g of freeze-dried samples (d.b.), considering three independent extraction procedures (n = 3).

2.6. Statistical analysis

All the determinations were performed in triplicate and the results were expressed as mean ± standard deviation (n = 3). In order to check for differences, the averages of the results were submitted to analysis of variance (ANOVA) and, when significant, they were compared by Scott-Knott test at 95% probability using Assistat software

version 7.7. Furthermore, Pearson correlation was employed to assess the association between pairs of variables at 95% probability using Statistica® software (StatSoft, Inc., Tulsa, USA).

Two multivariate exploratory techniques, namely, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were applied for the classification of 32 different genotypes of *O. distichus* from different locations using Statistica 7.0 software. In the PCA, total phenolic compounds, total flavonoids, total flavanols, and total monomeric anthocyanins were used as active variables in the derivation of the principal components, and the supplementary variable (scavenging capacity against ABTS$^{\cdot+}$ and DPPH·) was projected onto the factor space. For HCA, the hierarchical tree was obtained considering the same active variables applied to PCA and the 32 genotypes of *O. distichus* were joined by unweighted pair-group average as the linkage rule, considering the Euclidian distances as the coefficient of similarity.

3. Results and discussion

3.1. Bioactive compounds of O. distichus genotypes

Table 1 presents the mean values of total phenolic compounds, total flavonoids, total flavanols, total monomeric anthocyanins, and the origin of the *O. distichus* genotypes assessed in this study.

Significant differences were observed among the genotypes for total phenolic compounds, with mean values varying from 81.86 to 363.01 mg GAE/100 g (d.b.). The highest concentration of total phenolic compounds was observed for genotype B-7 (363.01 mg GAE/100 g), originally from Belém, which was significantly higher than the values obtained for the other genotypes. Genotypes B-3 (267.63 mg GAE/100 g) and B-8 (319.98 mg GAE/100 g), both from Belém, also can be highlighted by their total phenolic compound contents. The contents of total phenolic compounds of the *O. distichus* genotypes were close to those reported in the literature for other traditionally consumed palm trees native to Amazon, such as buriti (*Mauritia flexuosa*) (310 mg GAE/100 g d.b.), maripa palm (*Attalea mapira* (Aubl) Mart) (109.75 mg GAE/100 g d.b.), and tucuma (*Astrocaryum vulgare* Mart) (127.27 mg GAE/100 g d.b.) (Santos, Mamede, Rufino, Brito, & Alves, 2015). However, the contents observed in the present study were lower than the values reported for other species of the Arecaceae family, such as bacaba (*Oenocarpus bacaba* Mart) (1622.41 mg GAE/100 g d.b.) (Santos et al., 2015), carnauba (*Copernicia prunifera*) (830 mg GAE/100 g d.b.), and açaí (*Euterpea oleraceae*) (3268 mg GAE/100 g d.b.) (Rufino et al., 2010).

The total flavonoid contents showed a wide variation among the genotypes, with values varying from 9.53 mg QE/100 g to 38.19 mg QE/100 g (d.b.) (Table 1). The highest average was found for genotype SJ-4, originally from São João do Araguaia, which is statistically different ($p < 0.05$) from the others, except for genotypes B-7 (37.44 mg QE/100 g), B-9 (35.25 mg QE/100 g), B-2 (34.19 mg QE/100 g), and SJ-1 (34.75 mg QE/100 g), which did not differ among themselves ($p < 0.05$). According to the literature, fruits of some palm trees from the Arecaceae family have higher total flavonoid contents than those observed for *O. distichus* fruits in the present study, such as buriti (246.84 to 567 mg QE/100 g d.b.) (Koolen, Silva, Gozzo, Souza, & Souza, 2013). On the other hand, a similar value to the highest ones observed for *O. distichus* Mart fruits was found for maripa palm (*Attalea mapira* Mart) fruits (34.14 mg/100 g d.b.) (Santos et al., 2015).

Concerning the total flavanol contents (Table 1), significant differences ($p < 0.05$) were also found among several genotypes studied, from 32.36 to 259.18 mg CE/100 g (d.b.). Genotypes B-8 (259.18 mg CE/100 g) and B-3 (257.66 mg CE/100 g) had the highest averages and were statistically equal to each other, although different from other genotypes assessed. As far as our knowledge is concerned, there are no published studies reporting the contents of flavanols in *O. distichus* fruits. However, the highest values observed in the present study were lower than the contents reported by Freitas et al. (2016) for fruits of camu-camu (*Myrciaria dubia*), whose total flavanol concentrations varied from 852.68 to 1929.26 mg CE/100 g (d.b.). Importantly, flavanols are efficient natural antioxidants and they are related to the astringency of catechins and other flavanols present in fruits (Pietta, 2000).

The total monomeric anthocyanins also showed a wide variation among the *O. distichus* genotypes, with contents from 3.05 to 67.76 mg cya 3-rut/100 g (d.b.) (Table 1). Genotypes B-3 (67.76 mg cya 3-rut/100 g), B-7 (61.40 mg cya 3-rut/100 g), B-8 (38.54 mg cya-3-rut/100 g), and B-10 (36.58 mg cya-3-rut/100 g), all of them from Belém, were highlighted by the highest contents. Despite the broad variation among *O. distichus* genotypes, the results are close to those for bacaba fruits (*Oenocarpus bacaba* Mart) (60.96 mg cya 3-glu/100 g, d.b.) (Finco et al., 2012) and for bacaba-de-leque (*Oenocarpus distichus* Mart) fruits (10.5 to 25.8 mg cya 3-glu/100 g) (Carvalho et al., 2016). On the other hand, high contents of total monomeric anthocyanins were already reported for *O. bacaba* Mart. fruits (139.65 mg cya 3-glu/100 g (d.b.) (Santos et al., 2015), which is much higher than those reported in our study. According to Taiz and Zeiger (2006), extrinsic factors such as light incidence, temperature, and rainfall in the cultivation area lead to variations in the biosynthetic pathway and concentration of those phenolic pigments. For example, the decrease by 15% in solar radiation decreased up to 60% the total anthocyanins contents in grapes (Mazza & Miniati, 1993). These authors stated that the decrease in the activity of phenylalanine ammonia lyase, the main enzyme involved in the synthesis of anthocyanins and other flavonoids, whose activity is strongly dependent on light exposure, may explain the variations in anthocyanin contents of fruits from different locations.

Genotypes B-3, B-7, B-8, SJ-1, and SJ-4 (Table 1) were the ones with the highest bioactive compound contents that can be indicated as possible participants in genetic improvement programs to obtain varieties with high natural antioxidant contents. Furthermore, the great variation in the bioactive compound contents among the 32 genotypes supports the evidence that the composition of *O. distichus* Mart fruits is influenced by factors such as the genetic variability of the species, as well as by cultivation and edaphoclimatic conditions, which, according to Diamanti et al. (2012), may cause variations in fruit composition.

3.2. In vitro antioxidant capacity of O. distichus genotypes

The antioxidant capacity of the *O. distichus* Mart fruit extracts showed that all genotypes were effective in scavenging ABTS$^{\cdot+}$. However, this action differed among the genotypes (Table 2) and varied from 18.77 μM Trolox/100 g to 77.99 μM Trolox/100 g (d.b.). Such difference may be related to the variation in the bioactive compound contents found in the different genotypes. It is widely known that phenolic compounds are efficient scavengers of reactive oxidant species, which is directly related to antioxidant capacity of any food or derived product (Pietta, 2000; Karabin, Hudcová, Jelinek, & Dostálek, 2015).

Among the assessed genotypes, B-7 and B-3 exhibited high contents of total phenolic compounds, as well as the highest antioxidant capacity, according to the ABTS$^{\cdot+}$ assay (77.99 μM Trolox/g and 63.94 μM Trolox/g, respectively), which indicates the association between phenolic compounds and antioxidant capacity of *O. distichus* Mart fruits. In comparison to other palm trees from the Amazonian region, the antioxidant capacity of *O. distichus* Mart fruits was close to those reported for buriti (92.8 μM Trolox/g, d.b.) (Cândido, Silva, & Agostini-Costa, 2015), bacaba (57.9 μM Trolox/g, d.b) (Finco et al., 2012), and açaí fruits (64.5 μM Trolox/g, d.b.) and carnauba (*Copernia prunifera*) fruits (16.4 μM Trolox/g, d.b.) (Rufino et al., 2010).

In relation to the DPPH· assay (Table 2), the *O. distichus* Mart genotypes also proved to be effective in scavenging DPPH·, with values varying from 1510.48 g of freeze-dried sample/g DPPH· to 6721.47 g of freeze-dried sample/g DPPH· (d.b.). Among the assessed genotypes, B-3 (1680.70 g of freeze-dried sample/g DPPH·, d.b.) and B-7 (1510.48 g of

Table 2
Antioxidant capacity of 32 genotypes of *Oneocarpus distichus* fruits from different locations in the Amazonian region (Pará State, Brazil).

Locations	*Oenocarpus distichus* genotypes	TEAC (μM Trolox/g)	DPPH· EC_{50} (g fruit/g DPPH·)
Belém	B1	18.77 ± 1.83^{n}	5311.33 ± 72.75^{e}
	B2	48.18 ± 1.01^{d}	2586.34 ± 75.24^{j}
	B3	63.94 ± 0.51^{b}	1680.70 ± 0.09^{m}
	B4	25.28 ± 0.06^{J}	6216.64 ± 13.20^{b}
	B5	32.41 ± 1.13^{i}	6504.11 ± 21.28^{a}
	B6	47.94 ± 1.11^{d}	4173.00 ± 1.83^{h}
	B7	77.99 ± 1.35^{a}	1510.48 ± 2.58^{n}
	B8	58.79 ± 0.86^{c}	2305.75 ± 19.74^{l}
	B9	45.94 ± 0.55^{e}	2320.62 ± 7.74^{i}
	B10	42.55 ± 1.16^{f}	4141.64 ± 47.82^{h}
	B11	56.60 ± 1.13^{c}	2618.06 ± 16.11^{j}
São João do Araguaia	SJ-1	49.80 ± 2.10^{d}	2852.24 ± 20.04^{i}
	SJ-2	35.15 ± 0.85^{h}	5187.67 ± 259.66^{e}
	SJ-3	25.75 ± 2.21^{j}	6518.43 ± 114.21^{a}
	SJ-4	56.27 ± 0.54^{c}	2239.49 ± 7.77^{l}
	SJ-5	48.17 ± 1.38^{d}	4113.56 ± 13.06^{h}
	SJ-6	24.33 ± 1.14^{l}	4922.89 ± 95.32^{f}
	SJ-7	40.92 ± 0.06^{g}	4453.90 ± 9.17^{g}
	SJ-8	23.06 ± 1.09^{l}	5832.86 ± 4.55^{c}
	SJ-9	23.96 ± 0.26^{l}	5103.40 ± 23.39^{e}
Marabá	MB-1	47.10 ± 0.22^{e}	3933.19 ± 57.16^{h}
	MB-2	43.81 ± 0.53^{f}	6140.27 ± 11.08^{b}
	MB-3	19.73 ± 0.16^{n}	5367.67 ± 10.15^{e}
	MB-4	42.00 ± 0.46^{f}	6502.26 ± 181.66^{a}
	MB-5	42.30 ± 0.57^{f}	2215,94 ± 54.83^{l}
	MB-6	19.19 ± 1.01^{n}	6679.33 ± 25.40^{a}
	MB-7	25.63 ± 0.59^{j}	4766.55 ± 53.48^{f}
	MB-8	24.16 ± 1.16^{l}	6721.47 ± 73.96^{a}
	MB-9	25.26 ± 0.95^{j}	5726.20 ± 18.16^{c}
	MB-10	39.79 ± 1.31^{g}	4528.47 ± 23.66^{g}
	MB-11	21.89 ± 1.27^{m}	5556.56 ± 25.71^{d}
	MB-12	40.10 ± 0.92^{g}	2708.89 ± 31.32^{j}

All data are means ± standard deviation (n = 3, dry basis). Means in the same column with the same letter are not statistically different at 95% significance by the Scott-Knott test.

freeze-dried sample/g DPPH·, d.b.), both from Belém, presented the lowest EC_{50} values, and therefore, those are the genotypes with the highest DPPH· scavenging capacity. Similar values were found for açaí and patawa (*Oenocarpus patauа* Mart) fruits with EC_{50} values at 2447 g of freeze-dried fruit/g DPPH· (d.b.) for açaí and 2292 g of freeze-dried fruit/g DPPH· (d.b.) for patawa (Rezaire et al., 2014).

Overall, considering the antioxidant capacity of 32 genotypes of *O. distichus*, the most promising genotypes concerning their protective effect against reactive oxidant species were B-3, B-7, B-8, SJ-1, and SJ-4. As previously mentioned, these genotypes also exhibited high phenolic compound contents and they might be further explored by genetic improvement programs to obtain varieties and cultivars with high antioxidant potential.

3.3. Phenolic compounds profile of O. distichus genotypes by HPLC-DAD

Among the 32 genotypes of *O. distichus*, five genotypes were selected to be used in phenolic compounds profile determination based on that samples with the highest total phenolic compounds contents (B-3, B-7, B-8, SJ-1, and SJ-4) (Table 1), which were the same genotypes with the highest antioxidant capacity (Table 2).

Four phenolic acids were separated (Fig. 1) and identified in the extracts of *O. distichus* Mart fruits: 3,4-dihydroxybenzoic acid, chlorogenic acid, syringic acid, and ferulic acid, along to with two flavonoids: rutin and cyanidin 3-*O*-rutinoside (Table 3). All of these compounds were positively confirmed with co-elution with authentic standards.

Peak 1 was assigned as 3,4-dihydroxybenzoic acid and the contents found in all the five *O. distichus* genotypes were lower than those

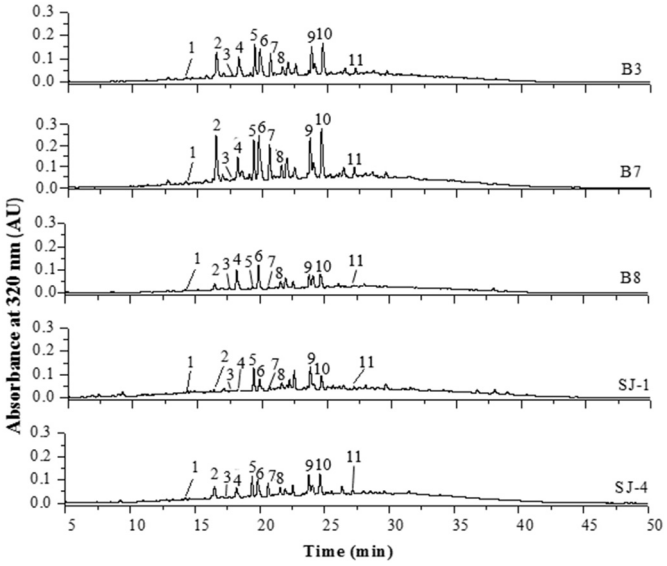

Fig. 1. HPLC-DAD chromatogram at 320 nm of *Oenocarpus distichus* Mart. extracts. Peaks: 1) 3,4-dihydroxybenzoic acid; 2) chlorogenic acid; 3) cyanidin derivative; 4) cyanidin 3-*O*-rutinoside; 5) not identified 1; 6) not identified 2; 7) not identified 3; 8) syringic acid; 9) rutin; 10) flavonol derivative; 11) ferulic acid.

reported for *Oenocarpus distichus* Mart fruits (2.66 to 7.61 μg/g) (Carvalho et al., 2016). However, the content of 3,4-dihydroxybenzoic acid found for genotype B-7 was similar to açai fruits (1.77 μg/g (d.b.) (Pacheco-Palencia, Talcott, Safe, & Talcott, 2008). Peak 2 was identified as chlorogenic acid and genotype B-7 was highlighted by the highest content among the selected genotypes (Table 4). Notwithstanding, the values found in our study were in the same range that those contents reported for jussara (*Euterpe edulis*) (13.3 μg/g, d.b.) (Bicudo, Ribani, & Beta, 2014) and açaí (*Euterpe oleracea*) (0.2 μg/g to 16.4 μg/g, d.b.) (Gordon et al., 2012). Chlorogenic acid is a derived compound from caffeic acid and it was reported to exhibit high antioxidant capacity, decreasing the occurrence of reactions responsible for several steps of self-oxidation (Marinova, Toneva, & Yanishlieva, 2009).

Peak 3 presented absorptions at 280 and 514 nm in the UV–visible spectrum, which is similar to that found for anthocyanin compounds and was tentatively identified as cyanidin derivative with contents varying from 17.35 μg/g to 1.80 μg/g (d.b.). Peak 4, the major phenolic compound in the *O. distichus* genotypes, was positively identified as cyanidin 3-*O*-rutinoside (Fig. 2), through co-elution with authentic standard, with mean variations between 48.47 μg/g (SJ-1) and 196.51 μg/g (B-7) (d.b.) (Table 4). Cyanidin 3-*O*-rutinoside was also identified in other Amazonian fruits, such as açaí (Garzón, Narváez-Cuenca, Vincken, & Gruppen, 2017), jussara (Bicudo et al., 2014), patawa (Rezaire et al., 2014), and bacaba (Finco et al., 2012). The variation observed among the five genotypes matches the fact that the environmental characteristics under which the fruits were cultivated strongly impact the synthesis and concentration of the compounds responsible for their pigmentation; although the nature and relative concentrations of those substances follow a genetic pattern related to the plant itself (Taiz & Zeiger, 2006).

Peaks 5, 6 and 7 could not be identified by the applied technique, but they presented similar UV–Visible spectra with absorption at 270–273 and 331–335 nm and their contents varied from 40.45 μg/g to 1.26 μg/g (d.b.) (peak 5), 20.21 μg/g to 3.85 μg/g (d.b.) (peak 6) and 14.31 μg/g to 1.03 μg/g (d.b.). Peak 8 was identified as syringic acid and showed concentrations from 7.13 μg/g (SJ-1) to 8.43 μg/g (B-7) (d.b.) (Table 4) for the *O. distichus* Mart genotypes, with no significant differences among themselves and higher than those reported by Carvalho et al. (2016) for *O. distichus* fruits (1.94 μg/g to 3.53 μg/g).

Table 3
Chromatographic and spectroscopic characteristics (HPLC-DAD) and contents of phenolic compounds of 32 genotypes of *Oenocarpus distichus* from different locations in the Amazonian region (Pará State, Brazil).

Peak	t_R (min)[A]	λ_{max} (nm)[B]	Compound[C]	*Oenocarpus distichus* genotype (μg/g)[D]				
				B-3	B-7	B-8	SJ-1	SJ-4
1	14.1	298	3,4-dihydroxybenzoic acid[1]	0.98 ± 0.09^c	1.65 ± 0.16^a	1.19 ± 0.12^b	0.55 ± 0.03^d	0.90 ± 0.08^c
2	16.6	326	Chlorogenic acid[2]	28.29 ± 1.51^b	64.56 ± 2.75^a	5.44 ± 0.77^d	0.71 ± 0.03^e	16.18 ± 0.16^c
3	17.6	280, 514	Cyanidin derivative[3]	10.29 ± 0.08^b	17.35 ± 0.90^a	10.55 ± 0.10^b	1.80 ± 0.06^d	2.57 ± 0.05^c
4	18.3	280, 516	Cyanidin 3-*O*-rutinoside[3]	76.39 ± 1.63^c	196.51 ± 2.73^a	90.95 ± 2.22^b	48.47 ± 1.55^e	62.59 ± 1.24^d
5	19.4	273, 333	Not identified 1[4]	2.0.61 ± 0.38^b	40.45 ± 0.54^a	1.26 ± 0.06^e	7.65 ± 0.61^d	9.29 ± 0.21^c
6	19.7	270, 331	Not identified 2[4]	18.22 ± 1.26^b	20.21 ± 0.31^a	7.35 ± 0.27^c	3.85 ± 0.04^e	5.45 ± 0.24^d
7	20.6	272, 335	Not identified 3[4]	14.31 ± 0.31^b	17.65 ± 0.29^a	1.03 ± 0.06^e	1.98 ± 0.09^d	3.22 ± 0.22^c
8	21.6	275	Syringic acid[4]	7.52 ± 0.92^b	8.43 ± 0.22^a	7.22 ± 0.29^b	7.13 ± 0.27^b	8.42 ± 0.10^a
9	23.9	268, 350	Rutin[5]	27.89 ± 1.03^b	56.76 ± 2.13^a	13.98 ± 0.54^d	23.85 ± 2.54^c	21.01 ± 0.50^c
10	24.6	270, 300(sh), 361	Flavonol derivative[5]	27.33 ± 0.87^b	56.45 ± 0.84^a	16.97 ± 0.83^e	18.02 ± 0.52^c	23.03 ± 0.06^c
11	27.5	323	Ferulic acid[6]	2.92 ± 0.12^b	12.57 ± 0.93^a	1.45 ± 0.08^c	0.98 ± 0.17^c	1.18 ± 0.16^c
			Total sum (μg/g)	234.75	492.59	157.39	114.99	153.84

Means on the same row with the same lowercase letters for B-3, B-7, B-8, SJ-1 and SJ-4 are not statistically different at 95% significance by the Scott-Knott test.
[A] Retention time on the C18 Synergi Hydro (4 μm) column.
[B] Solvent: gradient of 0.5% formic acid in water and acetonitrile with 0.5% formic acid.
[C] Peaks were quantified as equivalent of 3,4-dihydroxybenzoic acid[1], chlorogenic acid[2], cyanidin 3-O-rutinoside[3], syringic acid[4], rutin[5] and ferulic acid[6].
[D] Mean ± standard deviation (n = 3, dry basis).

Table 4
Pearson correlation coefficient (r) between the bioactive compounds and the antioxidant capacity of different genotypes of *Oenocarpus distichus*.

Compounds	TEAC	DPPH·
Total phenolic compounds	0.89[a]	−0.76[a]
Total flavonoids	0.88[a]	−0.71[a]
Total flavanols	0.72[a]	−0.78[a]
Total monomeric anthocyanins	0.54[a]	−0.54[a]
3,4 Dihydroxybenzoic acid	0.93[a]	−0.49
Chlorogenic acid	0.96[a]	−0.55[a]
Cyanidin derivative	0.75[a]	−0.49[a]
Cyanidin 3-*O*-rutinoside	0.94[a]	−0.57[a]
Not identified 1	0.70[a]	−0.32[a]
Not identified 2	0.80[a]	−0.49[a]
Not identified 3	0.75[a]	−0.50[a]
Syringic acid	0.59	0.12
Rutin	0.85[a]	−0.45[a]
Flavonol derivative	0.82[a]	−0.40[a]
Ferulic acid	0.90[a]	−0.41[a]

[a] Statistically significant at $p < 0.05$.

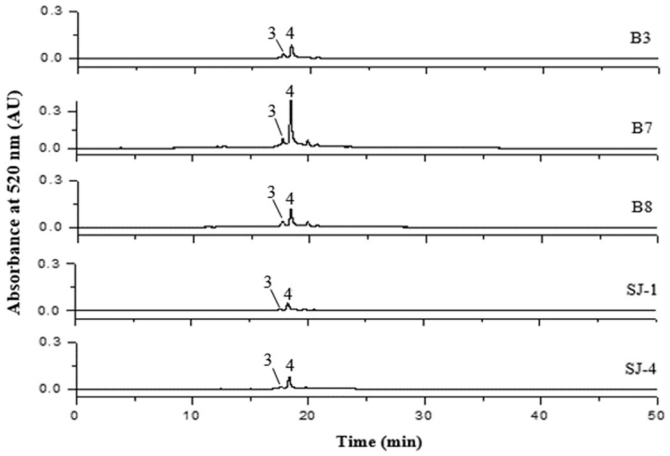

Fig. 2. HPLC-DAD chromatogram at 520 nm of *Oenocarpus distichus* Mart. extracts. Peak: 3) cyanidin derivative, 4) cyanidin 3-*O*-rutinoside.

However, açai fruits were reported to contain 48 μg syringic acid/g fruit (d.b.) (Garzón et al., 2017), while jussara fruits presented 47 μg/g (d.b.) (Bicudo et al., 2014), which were values much higher than those found in this study for *O. distichus* Mart fruits.

For rutin (peak 9), the concentration varied from 13.98 μg/g (B-8) to 56.76 μg/g (B-7) (d.b.), reflecting the great variation in the phenolic composition among the genotypes, particularly B-7, which had the highest concentration. Similar results were reported for *O. distichus* Mart fruits, which also found rutin as the major flavonoid with values varying from 15.24 μg rutin/g fruit to 56.84 μg/g (Carvalho et al., 2016); and for açaí fruits (34 μg/g, d.b.) (Garzón et al., 2017). Peak 10 showed variation among genotypes from 56.45 μg/g to 16.97 μg/g (d.b.) and was assigned as a flavonol derivative compound due to the absorption bands at 270, 300 (shoulder) and 361 nm, which is characteristic of flavonols; whereas, a more precise structure could not be stablished by the applied technique or data available in the literature. Finally, Peak 11 was identified as ferulic acid, with variations among the genotypes from 0.98 μg/g to 12.57 μg/g (d.b.). The highest concentration was found for genotype B-7, which was higher than the value found for another genotype of *O. distichus* fruits (10.8 μg/g) (Carvalho et al., 2016), but lower than the contents exhibited by jussara fruits (33 μg/g, d.b.) (Inada et al., 2015).

The total sum of the phenolic compounds identified in the selected *O. distichus* Mart genotypes highlighted genotype B-7 as the genotype with the highest phenolic content (492.59 μg/g (d.b.).

3.4. Correlation between phenolic compounds and antioxidant capacity

The Pearson correlation coefficients between phenolic compounds and antioxidant capacity are presented in Table 4. The results suggest that the total phenolic compounds contributed most to the *in vitro* antioxidant capacity of the extracts of *O. distichus* Mart fruits since they showed a high significant linear correlation ($r = 0.89$ to TEAC and $r = -0.76$ to DPPH·). The high negative correlation obtained for DPPH · was expected since the lower EC_{50} values represent the higher antioxidant capacity.

The total flavonoid contents were also highly correlated with antioxidant capacity and showed a positive correlation to TEAC ($r = 0.88$) and negative correlation to DPPH· assays ($r = 0.71$). A similar behavior was also observed for total flavanols ($r = 0.72$ to TEAC and $r = -0.78$ to DPPH). The monomeric anthocyanins presented a moderate correlation to the antioxidant capacity ($r = 0.54$ to TEAC and $r = -0.54$ to DPPH·). These results suggests that the antioxidant capacity of *O. distichus* Mart genotypes is not fully associated with the isolated action of the anthocyanins, but possibly with antioxidant effects among different classes of phenolic compounds and other bioactive compounds in the

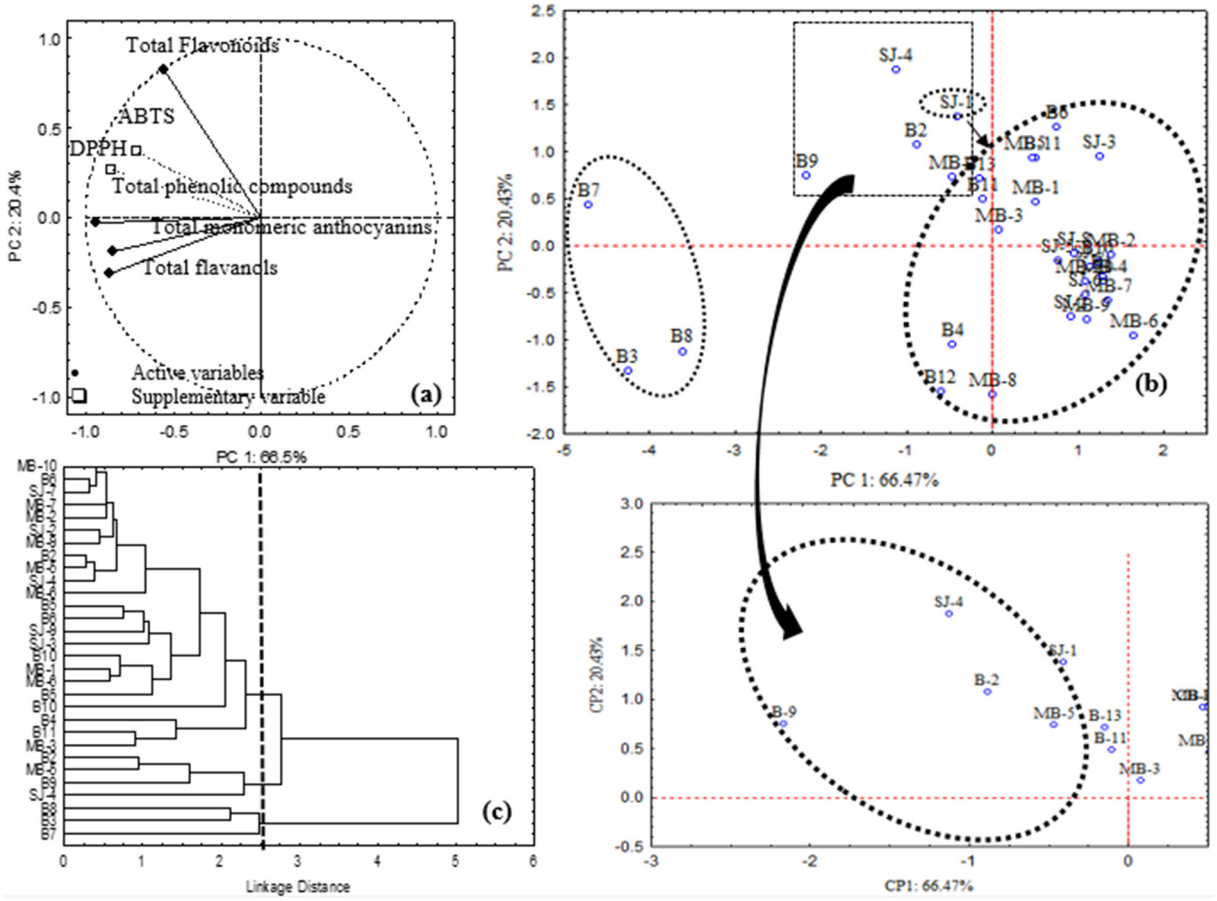

Fig. 3. Classification of *Oenocarpus distichus* Mart. genotypes based on bioactive compound contents (total phenolic compounds, total anthocyanins, total flavonoids, and total flavanols) by multivariate statistical analysis. (a) Variable projection by principal component analysis (PCA); (b) Scatterplot for the *Oenocarpus distichus* Mart. genotypes by PCA with suggested clustering, according to the hierarchical cluster analysis (HCA); (c) dendrogram by HCA.

fruits.

Correlation analysis was used to explore the relationships between the individual phenolic compounds an antioxidant capacity (TEAC and DPPH assays) measured for five genotypes selected to be used in phenolic compounds profile (Table 4). High positive correlation was observed among TEAC and 3,4 dihydroxybenzoic acid ($r = 0.93$), chrologenic acid ($r = 0.96$), cyanidin 3-*O*-rutinoside ($r = 0.94$), rutin ($r = 0.85$), ferulic acid ($r = 0.90$) and others not identified compounds ($r = 0.70$–0.80), except for syringic acid, which showed moderate positive correlation with TEAC assay ($r = 0.59$). Chlorogenic acid ($r = -0.55$), cyanidin 3-*O*-rutinoside ($r = -0.57$), rutin ($r = -0.45$), ferulic acid ($r = -0.41$) and others compounds, they presented a moderate and significant correlation with DPPH· assay, while 3,4 dihydroxybenzoic acid and syringic acid presented low or none correlation with DPPH· ($r = -0.49$ and 0.12, respectively). Chrologenic acid and cyanidin 3-*O*-rutinoside exhibited the highest correlation for both the antioxidant capacity assays (Table 4), which demonstrate their high contribution to the antioxidant capacity of *O. distichus* Mart genotypes. The high correlation of chrologenic acid and cyanidin 3-*O*-rutinoside with antioxidant capacity properties was already demonstrated by other authors (Alvarez, Zielinski, Albert, & Nogueira, 2017; Ozgen et al., 2008).

3.5. Multivariate statistical analyses

The principal component analysis (PCA) performed to assess and classify the phenolic content and antioxidant capacity data in *O. distichus* genotypes enabled condensing most of the information on the original data. The two principal components, PC1 and PC2, had a cumulative percentage of 86.9% of total variance, which is considered high enough to represent all variables. The first principal component (PC1) was able to explain 66.5% of the variation, while the second (PC2) explained 20.4% of the total variation (Fig. 3 (a)).

The scatter plot of the scores of *O. distichus* genotypes for PC1 and PC2 shows the genotypes tended to form clusters, some of which isolated, as evidenced by HCA (Fig. 3c). The data in Fig. 3 (b) shows that the location, the city where the fruits where collected, is a key factor in the composition characteristics of the genotypes studied since B-3, B-7, and B-8, grown in Belém, tended to cluster due to their higher phenolic compound contents. Moreover, of the aforementioned genotypes, B-7 is further away for its higher concentration of phenolic compounds among the genotypes evaluated, which allows differentiating it from the other genotypes in this study. That is relevant for establishing genetic conservation strategies that allow using this genotype as target of study in genetic improvement programs. Furthermore, according to Fig. 3 (b), a cluster of genotypes mostly collected in Marabá tends to be formed. The isolation shown by those relatively highly similar genotypes indicate they might be differentiated from genotypes grown in Belém and São João do Araguaia. On the other hand, the genotypes from São João do Araguaia had greater differences among themselves, which can be seen in the dispersion of results and none clusters were formed. The location of scores related to the largest cluster formed (in the last quadrant), is characterized by featuring the *O. distichus* genotypes with the lowest contents of total phenolic compounds, total flavonoids, total flavanols, and total anthocyanins, which differentiates this cluster from the others.

Therefore, in order to achieve better selection in the genetic improvement program, chemical characterization studies on *O. distichus*

fruits must further investigate the species in order to lay basis for the selection of the best genotypes according to cultivation site in relation to the chemical composition of interest.

4. Conclusions

According to the results, the genetic variability among *O. distichus* fruits and cultivation location significantly influenced their phenolic contents and composition; and the phenolic compounds of *O. distichus* fruits were strongly correlated with the exhibited antioxidant capacity. The genotypes B-3, B-7, and B-8, from Belém, and SJ-4 and SJ-1, from São João do Araguaia, were highlighted for their high phenolic compound contents and antioxidant capacity values, which makes them promising genotypes that may be used in genetic improvement programs. The HPLC-DAD analyses identified cyanidin 3-*O*-rutinoside as the major phenolic compound in *O. distichus* fruits and, for the first time, chlorogenic acid was identified and quantified in the pulp of those fruits.

Conflicts of interest

The authors have no conflict of interest.

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