**In vitro** assessment of the antihyperglycemic and antioxidant properties of araçá, butiá and pitanga

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**A R T I C L E   I N F O**

**Keywords:**
Brazilian native fruits
α-Glucosidase inhibition, Antioxidant activity
Phenolic compounds
Carotenoids
Sugars

**A B S T R A C T**

Native fruits have been known as a great source of bioactive compounds with potential biological effects. The aim of this study was to investigate the α-glucosidase inhibition and antioxidant activities of araçá (*Psidium cattleianum* Sabine), butiá (*Butia odorata* (Barb. Rodr.) Noblick) and pitanga (*Eugenia uniflora* L.) and relate their chemical composition with the observed biological activity. Samples of mature fruits were extracted with ethanol and the observed biological activities were dependent of the species and concentration as well. Araçás were the strongest α-glucosidase inhibitors (IC50 value of 25.4 ± 0.7 and 31.8 ± 0.7 µg/ml, respectively). Pitangas showed antiradical activities against DPPH, hydroxyl and nitric oxide radicals. Orange pitanga and butiá from Santa Vitória do Palmar were the most active concerning anion superoxide radical. All fruits are rich in total phenolic compounds with values in the range of 454.5 ± 17.3-908.3 ± 60.8 mg of equivalents of chlorogenic acid/100 g fresh weight. Purple pitanga stood out due to their levels of anthocyanins and carotenoids. Fruits showed similar amounts of total sugars, araçás and pitangas showed higher levels of reducing sugars while butiás were the richest ones in non-reducing sugars. Fruits with lower IC50 values for α-glucosidase inhibition were correlated with higher concentrations of reducing sugars, phenolic compounds, anthocyanins and carotenoids and grouped by principal component analysis (PCA). The obtained results indicate that these native fruits are promising sources of α-glucosidase inhibitors and antioxidants that can be used to control glycermia in patients with type 2 Diabetes mellitus.

**1. Introduction**

Fruits are an important source of phytochemicals on our diet and its consumption is associated with the lower risk of developing Diabetes mellitus (Muraki et al., 2013). In addition, different studies shows that specific fruits, including exotic fruits, can be used for treatment of various health problems including obesity and diabetes (Balisteiro, Alejandro, & Genovese, 2013; Devalaraja, Jain, & Yadav, 2011). Type 2 Diabetes mellitus (T2DM) is a metabolic disorder, characterized by chronic hyperglycemia that leads to alterations on the metabolism of carbohydrates, fats and proteins resulting from defects in insulin secretion or action, or both. Control of T2DM can be made through rigorous diets, physical activity, and the use of drugs acting for instance on insulin production or inhibition of enzymes associated with degradation of carbohydrates such as α-glucosidase. Different Brazilian native fruits have been exploited as potential source of biological active compounds (Bagetti et al., 2011; Beskow et al., 2014; Denardin & Parisi, 2014; Denardin, Hirsch et al., 2014; Medina et al., 2011) and some of these research are focusing their application as α-glucosidase inhibitors to control diabetes (Gonçalves, Lajolo, & Genovese, 2010). α-Glucosidase inhibitors (e.g. acarbose, miglitol, and voglibose) are agents used by diabetic patients, responsible to reversible inhibit the α-glucosidase enzyme in order to retard the absorption of dietary carbohydrates and suppress postprandial hyperglycemia (Li, Qian, & Li, 2010). Nevertheless, due to huge diversity of native fruits in Brazil there are different species that remains to be studied. From these fruits, we can highlight three native species with increase commercial interest namely *Psidium cattleianum* Sabine (Myrtaceae), *Butia odorata* (Barb. Rodr.) Noblick (Areaceae) and *Eugenia uniflora* L. (Myrtaceae) (Hoffmann, Barbieri, Rombaldi, & Chaves, 2014; Mitra, Irenaues, Gurung, & Pathak, 2012).

*P. cattleianum* fruits, known as strawberry guava and particularly in Brazil as araçá, araçá-amarelo, araçá-vermelho or araçá-do-campo, are ovoid or oblong, weighting less than 20 g, and can have yellow or red peel with white pulp. Fruits are rich in vitamin C and phenolic compounds, being epicatechin and gallic acid their main constituents (Medina et al., 2011). Its fruits are known to possess antioxidant activity (Medina et al., 2011; Nora et al., 2014; Lozano, Vélez & Rojano, 2013), analgesic effect (Alvarenga et al., 2013), antimicrobial (Medina et al., 2011), antidiabetic (Alezandro, & Genovese, 2013; Devalaraja, Jain, & Yadav, 2011) and some of these properties are focused on this study.
et al., 2011), antiproliferative effect (Medina et al., 2011), anti-glycated activity (Yan, Lee, Kong, & Zhang, 2013).

*B. odorata*, known as butiá, produces ovoid to depressed-globose fruits, ranging from yellow to orange to red in colour, with a sweet, acidic taste and whose pulp is source of fibres, pro vitamin A, vitamin C, potassium and caroteneoids (Beskow et al., 2014). *B. odorata* is poorly exploited about their biological potential, however, antioxidant (Beskow et al., 2014) and probiotic activities (Cruxen et al., 2017) have been reported to them.

*E. uniflora*, also known as pitanga, Brazilian cherry or Surinam cherry is a globoid fruit, with 3 cm in diameter, and presents eight to ten longitudinal grooves, looking like a small pumpkin (Celli, Pereira-Netto, & Beta, 2011). The fruits colour depends on the variety, but ranges from orange to purple, and the fruit has an exotic flavour, sweet and sour taste (Lima, Melo, & Lima, 2002). The fruits are a source of anthocyanins, flavonoids and carotenoids and antioxidant activity (Bagetti et al., 2011; Denardin, Hirsch et al., 2014; Pereira et al., 2012; Vissotto, Rodrigues, Chisté, Benassi, & Mercadante, 2013), antiproliferative (Denardin & Parisi, 2014) and anti-inflammatory (Soares et al., 2014) activities have been reported.

Considering the suggestion that native fruits are a good source of biological active compounds and some species shows potential as inhibitors of carbohydrases-hydrolyzing enzymes, the present work aims to determine the chemical composition and evaluated the a-glucosidase inhibitory properties and antioxidant activities of three Brazilian native fruits extracts, namely aracá (*P. cattleyanum*), butiá (*B. odorata*) and pitanga (*E. uniflora*), in order to use this natural resource in the treatment of type 2 Diabetes mellitus.

2. Materials and methods

2.1. Standards and reagents

Reagents were purchased from different suppliers. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), ethylenediaminetetraacetic acid disodium salt (EDTA-Na), iron sulphate heptahydrate, salicylic acid, sodium chloride, sodium bicarbonate, sodium nitroprussiate (SNP), phosphoric acid, phosphothioic acid (PMT), nitroblue tetrazolium chloride (NBT), 5,5′,6-trithioloes(2-nitrobenzoic acid) (DTNB), sulphanilamide, n-(1-Naphthyl) sodium salt (EDTA-Na), iron sulphate heptahydrate, salicylic acid, sodium methosulphate (PMS), sodium bicarbonate, sodium nitroprussiate (SNP) and sodium thiosulphate (SNP) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium carbonate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium carbonate was used for the preparation of the standard solutions.

2.2. Samples

Yellow and red selections of aracá (*P. cattleyanum*) and orange, red and purple-fleshed selections of pitanga fruits (*E. uniflora*) were obtained from the Active Germplasm Bank of native fruits at Embrapa Climado Temperado. Samples of butiá (*B. odorata*) were collected in three different locations, one sample from Herval (32°12′4″S, 53°23′44″W), one from Santa Vitória do Palmar (33°31′8″S, 53°23′5″W) and another one from Pelotas (Active Germplasm Bank of native fruits at Embrapa Climado Temperado, 31°40′47″S, 52°26′24″W). The fruits were sampled searching for a mixture of completely ripe fruits. All fruits were harvested in 2015, between March and April. Fruits were selected considering the absence of visible injury and infections, and also colour and size uniformity, and were frozen (−20 °C) until analysis.

2.3. Preparation of extracts

Fruit extract was prepared from the edible portions of fruits: i) skins and pulps of butiá and pitanga; and ii) skin, pulp and seeds of aracá. At least 10 fruits were combined for each sample that were thawed at room temperature and sliced. Samples (5 g) were extracted using 98% ethanol (1:4, w/v) during 5 min using an Ultra-Turrax homogenizer (Ika, Artur Nogueira, São Paulo, Brazil). The homogenates were filtered through paper filter (Whatman no 4) and further evaporated under pressure at 40 °C. Samples were reconstituted in 20 ml of ethanol/water (3:1, v/v) and stored at −20 °C until analysis. Extractions were performed in triplicate and yields are shown in Table 1.

2.4. Chemical composition

2.4.1. Total phenolic compounds

Total phenolic content was measured according to the Folin–Ciocalteu method adapted from Swain and Hillis (1959). Briefly 50 μl aliquot of the extract and the control (50 μl of ethanol) were each combined with 250 μl of 0.25 N Folin-Ciocalteu reagent. After 3 min reaction, 500 μl of Na2CO3 (1 N) was added, the mixtures were incubated for 2 h at room temperature, and the absorbance was measured at 725 nm. The results were expressed as chlorogenic acid equivalents (CAE g/100 g fresh weight) using a chlorogenic acid (0–0.5 mg/ml) standard curve.

2.4.2. Total anthocyanins

Anthocyanins content was achieved using the method adapted from Fuleki and Francis (1968). Briefly, 425 μl of extract was mixed with 75 μl of HCl 1.5 M and measured at 535 nm. The anthocyanins concentration was expressed as equivalent of cyanidin-3-glucoside (C3G g/100 g fresh weight), using cyanidin-3-glucoside standard curve (0–0.4 mg/ml).

2.4.3. Total carotenoids

Carotenoid content was adapted from Talcott and Howard (1999). An aliquot of acetone (0.6 ml) was added to 0.6 ml of extract, hexane (1.5 ml) was further added and vigorous mixed. Samples were allowed to rest during 30 min and the organic layer was read at 470 nm. Carotenoids concentration was expressed as equivalent of β-carotene (β-CE g/100 g fresh weight), using β-carotene standard curve (0–0.4 mg/ml).

2.4.4. Total, reducing and non-reducing sugar contents

Total, reducing and non-reducing sugar contents were determined with the adapted method described by Nelson (1944). Briefly, sample (100 μl) was diluted in distilled water (1 ml) and neutralized to pH 7 (glacial acetic acid). An aliquot (500 μl) of neutralized extract was mixed with Ba(OH)2 (0.2 ml), ZnSO4 (0.2 μl) and distilled water (4.0 ml), vortexed and allowed to stand (10 min) to precipitate proteins. Samples were filtered with paper filter (Whatman 1) and 1.0 ml was mixed with distilled water (1.0 ml) plus cupric reagent (1.0 ml) and heated (100 °C, 20 min). Samples were cool down in ice water bath and arsenomolibdic reagent (1.0 ml) was added plus 5.0 ml of distilled water. Reducing sugars was estimated spectrophotometrically at 510 nm, using a standard curve constructed from a glucose solution (0–180.0 mg/ml). For total sugar content samples were first heated (100 °C, 15 min) with concentrated HCl (1.0 ml of sample to 25 μl of HCl), neutralized with Na2CO3 and followed the protein removing step and analysis of reducing sugars. Non-reducing sugars were calculated by difference of total sugars minus reducing sugars. Analysis were carried out in triplicate and expressed as mg/100 g of sample.
2.5. Anti-hyperglycaemic and antioxidant potential

2.5.1. General

For the evaluation of the anti-hyperglycaemic and antioxidant potential of fruit extracts in vitro assays were performed applying spectrophotometric methods using a Amersham, Model UV Vis Ultrospec 3100 Pro Amersham Bioscience spectrophotometer. The IC50 values were calculated using at least 5 concentrations for each extract. Percentage of inhibition (I %) for each assay was calculated using the following formulae:

\[ I\% = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \]

where \( A_{\text{control}} \) is the absorbance of the control reaction (containing all reagents except the extract), and \( A_{\text{sample}} \) is the absorbance of the tested extract in the reaction mixture.

2.5.2. α-Glucosidase inhibitory activity

The effect on α-glucosidase was assessed using a procedure previously reported (Vinholes et al., 2011) with a slightly modification. Briefly, 20 µl of fruit extract or ethanol (control) was added to a vial with 100 µl of PNP-G (3.25 mM) in phosphate buffer (pH 7.0). The reaction was initiated by the addition of 100 µl of enzyme (9.37 U/ml in phosphate buffer, pH 7.0) and vials were incubated at 37 °C for 10 min. The reaction was stopped by adding 0.600 ml of Na2CO3 (1 M) and the absorbance of 405 nm was measured.

2.5.3. DPPH scavenging activity

The hydrogen atoms or electrons donation ability of the extracts was measured from the bleaching of purple coloured methanol solution of DPPH by adaptation of the methods reported in the literature (Vinholes et al., 2011; Vinholes, Gonçalves, Martel, Coimbra, & Rocha, 2014). Briefly, 100 µl of each extract or ethanol (control) were added to 1000 µl of a 0.6 mM DPPH methanol solution. The reaction was mixed and incubated in the dark for 30 min at room temperature, samples were read at 515 nm.

2.5.4. Superoxide radical scavenging activity

Superoxide radicals were generated by the NADH/PGS (nicotinamide adenine dinucleotide reduced form/phenazine methosulphate) system (Vinholes et al., 2011). The reaction mixture contained 100 µl of different concentrations of extracts or ethanol (control) and NADH (100 µl of 1.89 mM), nitrotetrazolium blue (NBT, 100 µl of 48.92 µM) and the reaction started by the addition of 100 µl of PMS (47.70 µM). Samples were incubated during 5 min at room temperature and read at 560 nm as described by Zou, Lu, and Wei (2004).

2.5.5. Hydroxyl radical inhibition

Hydroxyl radical scavenging activity was measured according to Vinholes et al. (2014). To initiate the reaction, 0.75 ml (3 mM) salicylic acid was added to the individual reaction mixtures containing 0.25 ml of each extract or ethanol (control), plus 1.1 ml of iron sulphate heptahydrate solution (8 mM, prepared in EDTA-Na 20 µM) and 0.5 ml of H2O2 solution (7 mM). After vortexing, the reaction was incubated at 37 °C for 30 min and then the absorbance (515 nm) of 1 ml supernatant was determined.

2.5.6. Nitric oxide radical scavenging activity

The nitric oxide scavenging activity was determined spectrophotometrically according to a described procedure (Vinholes et al., 2011). Briefly, after incubation of sodium nitroprusside (SNP, 20 mM, 100 µl) with extracts (100 µl) or ethanol (control, 100 µl) for 60 min at room temperature, under light, Griess reagent (100 µl) was added. The mixture was then incubated at room temperature in the dark for 10 min and the absorbance of the chromophore formed was read at 562 nm.

2.6. Statistical analysis

All experiments results were performed in triplicate (n = 9) and expressed as ± standard error of the mean (SEM). Pearson correlation coefficients (r) was applied in order to evaluate the strength of the correlation between the variables under study: Total phenolic compounds, Total anthocyanins, Total carotenoids, Total sugar, Reducing sugar and non-reducing sugar contents and the activity (IC50) of the native fruit ethanolic extracts, expressed in mg of extract/100 g of fruit (Table S1). Principal Component Analysis (PCA) was carried out to transform the original variables into a new set of linearly uncorrelated factors, principal components (PCs) that could be ranked based upon their contribution for explaining the variation of the whole data set. Additionally, PCA can also establish relations between samples and variables (Xu, Hagler, Xu, & Hagler, 2002). The first and second PCs corresponded to the largest possible variance of the original variables. The results were represented in a biplot (score plot and loading plot), which showed the distribution of the samples and the correlation of the five original variables to the two PCs. PCA was calculated using the Analyst-it® application for Microsoft Excel program.

3. Results and discussion

3.1. Phytochemicals

Phenolic compounds have been described with a range of therapeutic effects on different diseases such as cancer, cardiovascular diseases, atherosclerosis and diabetes. It is well established by different studies that the total phenolic composition of a matrix gives an idea of how rich this product is in antioxidants, since these parameters are closely related. Fruit samples were extracted using ethanol 95% at 4:1 proportion (v/w) which was considered the most suitable solvent system in order to achieve high concentration of phenolic compounds and α-glucosidase inhibition (data not shown). Levels of phenolic compounds in native fruits varied from 454.5 ± 17.3 to 908.3 ± 60.8 mg of equivalents of chlorogenic acid/100 g fresh weight (Table 1). The total phenolic content was similar between the two araçá genotypes. Phenolic compounds in butiás varied from 454.5 ± 17.3 to 540.9 ± 63.0 mg of equivalents of chlorogenic acid/100 g fresh weight. Butiá collected in Pelotas was the poorest one, while those from Santa Vitória do Palmar and Herval showed similar values (Table 1). Amounts of phenolic compounds in pitanças varied from 517.2 ± 37.3–908.3 ± 60.8 mg of equivalents of chlorogenic acid/100 g fresh weight among the genotypes. Purple pitanga was the richest one, followed by red and orange genotypes (Table 1). The total phenolic compounds obtained for all samples are consistent with those results reported in the literature (Bagetti et al., 2011; Beskow et al., 2014; Denardin, Hirsch et al., 2014; Fetter, Vizzotto, Corbelini, & Gonzalez, 2010; Medina et al., 2011). All these fruits can be considered great sources of phenolic compounds when compared with other more common and widely consumed fruits such as red apple and pear (125 mg/100 g), black grape (213 mg/100 g) and strawberry (199 mg/100 g) (Giada, 2013). Additionally, purple pitanga showed total phenolic values higher than black raspberry, lemon and grapefruit (670, 843 and 893 mg/100 g, respectively), which are in the top of the list of fruits rich in phenolic compounds (Giada, 2013).

Amounts of anthocyanins in the native fruits varied from 0.6 ± 0.1 to 450.4 ± 36.5 mg of equivalents of cyanidin-3-glucoside/100 g fresh weight (Table 1). The concentration of anthocyanins in red araçá was almost 21 times higher than in yellow araçá genotype. Yellow araçá concentrations were 2–6 times higher than that reported in the literature for three araçá accesses, but 5 times lower than one of them (Medina et al., 2011). In the case of red araçá anthocyanins were 4–6 times higher in the present study (Medina et al., 2011). Butiás showed similar anthocyanin concentrations, ranging from 1.2 ± 0.4 to 1.5 ± 0.1 mg of equivalents of cyanidin-3-glucoside/100 g fresh
Results are expressed as mean ± SD of three experiments done in triplicate (n = 9). Values of equivalents of β-carotene/g fresh weight.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Yield (%)</th>
<th>Total Phenolic compounds a</th>
<th>Anthocyanins b</th>
<th>Carotenoids c</th>
<th>Total Sugar d</th>
<th>Reducing</th>
<th>Non-Reducing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araçá</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow</td>
<td>8.55 ± 0.42</td>
<td>603.1 ± 18.9</td>
<td>1.3 ± 0.4</td>
<td>442.7 ± 46.1</td>
<td>8.2 ± 1.7</td>
<td>7.9 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Red</td>
<td>9.47 ± 0.23</td>
<td>606.1 ± 15.3</td>
<td>29.3 ± 1.4</td>
<td>364.4 ± 24.9</td>
<td>7.7 ± 1.3</td>
<td>5.6 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Butiá</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelotas</td>
<td>12.95 ± 0.59</td>
<td>454.5 ± 17.3</td>
<td>1.5 ± 0.1</td>
<td>2865.2 ± 190.4</td>
<td>10.4 ± 0.9</td>
<td>3.1 ± 0.3</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>SVP</td>
<td>12.67 ± 0.53</td>
<td>535.9 ± 56.2</td>
<td>1.9 ± 0.1</td>
<td>1710.3 ± 243.2</td>
<td>14.7 ± 2.1</td>
<td>2.2 ± 0.1</td>
<td>12.6 ± 2.0</td>
</tr>
<tr>
<td>Herval</td>
<td>8.22 ± 0.46</td>
<td>540.9 ± 63.0</td>
<td>1.2 ± 0.4</td>
<td>2155.5 ± 580.5</td>
<td>8.9 ± 0.8</td>
<td>2.1 ± 0.1</td>
<td>6.7 ± 0.7</td>
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<tr>
<td>Pitanga</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Purple</td>
<td>12.12 ± 0.27</td>
<td>908.3 ± 60.8</td>
<td>450.4 ± 36.5</td>
<td>10,116.7 ± 889.6</td>
<td>9.6 ± 0.2</td>
<td>9.4 ± 0.1</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Red</td>
<td>8.97 ± 0.19</td>
<td>517.2 ± 37.3</td>
<td>60.1 ± 5.3</td>
<td>5697.6 ± 820.6</td>
<td>7.5 ± 0.2</td>
<td>7.1 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Orange</td>
<td>12.30 ± 0.13</td>
<td>526.6 ± 40.2</td>
<td>0.6 ± 0.1</td>
<td>9061.3 ± 514.9</td>
<td>10.8 ± 0.6</td>
<td>8.9 ± 0.7</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD of three experiments done in triplicate (n = 9).

a mg of equivalents of chlorogenic acid/100 g fresh weight.
b mg of equivalents of cyanidin-3-glucoside/100 g fresh weight.
c µg of equivalents of β-carotene/100 g fresh weight.
d g/100 g fresh weight.

3.3. α-Glucosidase inhibition

Diabetes mellitus is a chronic disease and a major public health, with high social and health care costs since it affects over 387 million people worldwide causing 4.9 million deaths in 2014, according to the International Diabetes Federation. In Brazil, there are around 13 million people between 20 and 79 years old diagnosed with T2DM, representing 6.3% of the population (SBD, 2013). Under the assay conditions all native fruits ethanolic extracts, with the exception of butiá from Pelotas, showed a dose dependent inhibitory response over α-glucosidase (Fig. 1). Native fruits extracts showed IC50 values varying from 25.4 ± 0.7 to 3264.9 ± 87.1 µg/ml (Table 2). Araçá genotypes were the most active extracts with IC50 13 and 16 times lower than the acarbose (positive control). Butiá extracts were the less effective ones, only the sample from Herval showed results comparable to the acarbose. Purple pitanga was the most effective among pitanga samples, followed by red and orange genotypes with IC50 6, 5 and 2 times lower than the acarbose (Table 2). From these native fruits, as far as we know, only pitanga has been reported with α-glucosidase inhibition properties (Correia, Borges, Medeiros, & Genovese, 2012). Nevertheless, the reported IC50 (1150 µg/ml) is 5–17 times higher than those found in the present study (Table 2). Comparatively to other native fruits from the Myrtaceae family, the same of araçá and pitanga, lower IC50 was observed in the present study (Table 2). From these native fruits, as far as we know, only pitanga has been reported with α-glucosidase inhibition properties (Correia, Borges, Medeiros, & Genovese, 2012). Nevertheless, the reported IC50 (1150 µg/ml) is 5–17 times higher than those found in the present study (Table 2). Comparatively to other native fruits from the Myrtaceae family, the same of araçá and pitanga, lower IC50 was obtained in the present study. Campomanesia phaea Berg. (cambuci), Psidium guineensis Sw. (araçá), Eugenia stipitata Mc. Vaugh (araçá-boi) and Myrciaria dubia Mc. Vaugh (camu-camu) methanolic extracts were reported with IC50 of 300, 600, 1000 and 1300 µg/ml. This difference on activity obtained by these authors can be due the extraction solvent used, in both studies methanol was used instead of ethanol. Both solvents are good solvent extractors of phenolic compounds but ethanol is reported to extract more phenolic compounds with consequent higher biological potential (Do et al., 2014). Besides, ethanol is safe for human consumption.
As recent studies show that T2DM patients have an increased free-radical production and reduced antioxidant defences can lead to different health complications, supplementation with antioxidants agents can be useful in their treatment. Thus, the antiradical potential of native fruit extracts was evaluated.

The DPPH$^*$ assay was used for a first screening of extracts ability to scavenge free radicals. All native fruit extracts were able to scavenge DPPH$^*$ in a concentration-dependent way (Fig. 2A) with IC$_{50}$ varying from 160.4 to 610 µg/ml (Table 2). Butiá from Herval was the most effective extract against DPPH, however, all extracts showed lower inhibition when compared with the positive control quercetin (Table 2).

Differently from DPPH$,^*$ superoxide radical (O$_{2}^{-}$) is a physiological radical capable of generate other reactive species, like hydroxyl radical and peroxynitrite. The extracts exhibited O$_{2}^{-}$ scavenging activity and the observed effect was dependent on the concentration for all extracts, exceptionally for butiá from Pelotas (Fig. 2B). Variation on IC$_{50}$ values were from 83.5 to 518.9 µg/ml, orange pitanga was the most efficient extract (Table 2). Nevertheless, all extracts showed IC$_{50}$ values higher than quercetin. Apart from araçás that were reported with lower IC$_{50}$ (Ribeiro et al., 2014), this is the first report about the O$_{2}^{-}$ scavenging activity of butiás and pitangas samples, as far as we are concerned.

Hydroxyl radicals are physiologically free radicals that are able to induce strand breakage contributing to biological damage, being therefore closely related with the origin of different human diseases. In the present study, native fruit extracts were able to inhibit hydroxyl radicals in a dose-dependent way (Fig. 2C). The calculated IC$_{50}$ varied from 114.7 to 1350.1 µg/ml (Table 2). Inhibition against hydroxyl radicals can be classified as follow: orange pitanga > red pitanga > purple pitanga > red araçá > yellow araçá > butiá from Herval > butiá from Pelotas > butiá from Santa Vitória do Palmar (Table 2). Quercetin was tested as positive control, in the concentration range of 1.5–95.0 µg/ml, however, under our experimental conditions it did not showed hydroxyl scavenge capacity.

Nitric oxide radical (NO) is involved in different physiological functions, for example it control blood pressure, platelet function, vasodilatation, acts as neurotransmitters and is also implicated in antimicrobial defense. Nevertheless, when its presence exceeds the organism antioxidant capacity, different biological damages can occur, especially when it reacts with superoxide radical forming peroxynitrite a major oxidant produced in vivo. Peroxynitrite in the presence of certain reactive centers may form three of the most reactive and damage species in biological systems: hydroxyl radicals; nitrogen dioxide free radical and nitronium cation (Malinski, 2000). In the present study, native fruit extracts were able to mild scavenging activities over NO, although with was dependent of the concentration (Fig. 2D), with IC$_{25}$ of 780–3800 µg/ml. These values were much higher than the positive control (quercetin).

3.5. Correlation and PCA analysis

The Pearson’s correlation coefficients for the chemical composition and observed activities for the native fruit ethanolic extracts are shown in Fig. 3. α-Glucosidase inhibition was positively correlated with total and non-reducing sugars, indicating that samples with high concentrations of both were less effective against the enzyme. The same behavior was observed for anion superoxide radical that correlates positively with total phenolic compounds and total anthocyanins and hydroxyl and nitric oxide radicals with positive correlation with non-reducing sugars. Negative correlations were observed between hydroxyl and nitric oxide radicals and the amounts of non-reducing sugars, indicating that samples with low concentrations of these compounds were more effective antioxidants. PCA was used to evaluate the main sources of variability of the native fruit extracts in order to understand if there is a relation between the variables (chemical composition and their biological activity) and between samples (araçás, butiás and pitangas). Fig. 3 displays the PCA biplot representing the variables using calibrated axes where the observations (points) are projected on to the axes giving an approximation of the real value. The two first dimensions explain 74.2% of the variability being PC1 responsible for 52.0% of the variance and PC2 for 22.2%. According to Fig. 3 native fruits were distinguished into three groups: araçás (AY and AR) and pitangas (PR and PO); purple pitanga (PP); and butiás (BP, BH and BSVP). Araçás...
pelotas, Santa Vitória do Palmar (SVP) and Herval), and pitangas (purple, red and orange) ethanolic extracts and positive controls. In Fig. 3, the extracts activities can be partially explained by their respective nitric oxide, however in a less extent.

Total phenolic compounds, total anthocyanins and total carotenoids are α-glucosidase and hydroxyl radicals. In addition, the amounts of total phenolic compounds, total anthocyanins and total carotenoids compounds. Butiás pitanga from purple genotype was due the high concentrations of total sugars, phenolic compounds, anthocyanins and carotenoids (Fig. 3). A recent study (Table 3). These results indicate that the type and amounts of inferences have demonstrated significant decrease of plasma antioxidants by carotenoids (α- and γ-tocopherol, α and β-carotene, lycopene, β-cryptoxanthin, lutein and zeaxanthin) in the progression of diabetes and its associated complications such as endothelial dysfunction and atherosclerosis (Polidori et al., 2000; Polidori, Stahl, Eichler, Niestroy, & Sies, 2001; Valabhi et al., 2001). As far as we are concerned carotenoids have never been studied as inhibitors of α-glucosidase, however, as previously stated, they are important phytochemicals that could prevent the development of degenerative diseases. This fact is mainly because carotenoids are important antioxidant compounds, they have been reported as active towards anion superoxide and hydroxyl radicals, two deleterious radicals species that can be in the origin of different degenerative diseases which were inhibited by the native fruits extracts in the present study (Trevithick-Sutton, Foote, Collins, & Trevithick, 2006).

Concerning reducing sugars and their role on α-glucosidase inhibition there is no evidences on the literature correlating the amounts of this group of compounds and the α-glucosidase inhibition. However, it was recently reported that α-glucose, β-glucose and fructose are correlated with the α-glucosidase inhibition of Neptunia oleracea Lour. (Lee et al., 2016) and fructose was correlated with the enzyme inhibitory activity observed for Ipomoea aquatica Forssk. (Sajak, Abas, Ismail, & Khatib, 2016). These results indicate that the enzyme inhibition and antioxidant activity might be probably due to interaction between the different compounds present in the fruit extracts.

4. Conclusion

The present study revealed the α-glucosidase and antioxidant properties of three Brazilian native fruit (aracá, pitanga and butiá). Yellow and red aracá genotypes where the most promising extracts on the inhibition of α-glucosidase with IC50 values much lower than the positive control acarbose. Nevertheless, purple, red and orange pitangas genotypes also showed potent inhibitory activity against the enzyme and all extracts were also potent antioxidants. These results may be partially explained by the presence and combination of phenolic compounds, anthocyanins, carotenoids and reducing sugars. Nonetheless, an extensive investigation is required to further characterize the compounds behind the α-glucosidase inhibition and to understand the relation between the reducing sugars present in the fruits and their biological role. Overall, this study indicates that the native fruits extracts are excellent source of bioactive compounds that can be used to decrease blood glucose and also protect T2DM patients by improving their antioxidant status.

Conflict of interest statement

None declared.

Table 2

<table>
<thead>
<tr>
<th>Samples</th>
<th>α-glucosidase (µg/ml)</th>
<th>DPPH</th>
<th>Anion superoxide radical</th>
<th>Hydroxyl radical</th>
<th>Nitric oxide radical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Araçá</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow</td>
<td>25.4 ± 0.7</td>
<td>334.3 ± 16.5</td>
<td>173.2 ± 34.6</td>
<td>358.2 ± 50.7</td>
<td>1360.0 ± 190.0</td>
</tr>
<tr>
<td>Red</td>
<td>31.8 ± 0.7</td>
<td>490.3 ± 35.1</td>
<td>218.9 ± 7.4</td>
<td>245.9 ± 34.5</td>
<td>1650.0 ± 220.0</td>
</tr>
<tr>
<td><strong>Butiá</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelotas</td>
<td>–</td>
<td>610.9 ± 4.5</td>
<td>–</td>
<td>1280.9 ± 135.8</td>
<td>3800.0 ± 40.0</td>
</tr>
<tr>
<td>SVP</td>
<td>3264.9 ± 87.1</td>
<td>257.2 ± 16.2</td>
<td>96.0 ± 0.6</td>
<td>1350.1 ± 53.0</td>
<td>2770.0 ± 160.0</td>
</tr>
<tr>
<td>Herval</td>
<td>451.5 ± 14.1</td>
<td>160.4 ± 1.1</td>
<td>228.9 ± 12.6</td>
<td>1142.7 ± 142.9</td>
<td>1790.0 ± 190.0</td>
</tr>
<tr>
<td><strong>Pitanga</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Purple</td>
<td>66.3 ± 2.4</td>
<td>229.9 ± 22.1</td>
<td>518.9 ± 33.6</td>
<td>213.7 ± 31.8</td>
<td>780.0 ± 5.0</td>
</tr>
<tr>
<td>Red</td>
<td>83.2 ± 2.3</td>
<td>212.1 ± 22.4</td>
<td>195.4 ± 3.6</td>
<td>184.7 ± 7.4</td>
<td>1060.0 ± 150.0</td>
</tr>
<tr>
<td>Orange</td>
<td>212.2 ± 7.1</td>
<td>317.6 ± 9.6</td>
<td>83.5 ± 7.8</td>
<td>114.7 ± 8.8</td>
<td>1250.0 ± 150.0</td>
</tr>
<tr>
<td><strong>Positive controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acarbose</td>
<td>413.6 ± 20.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>44.6 ± 3.0</td>
</tr>
<tr>
<td>Quercetin</td>
<td>10.5 ± 1.3</td>
<td>2.3 ± 0.1</td>
<td>8.9 ± 0.6</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

*IC50 values (µg/ml) determined for α-glucosidase, DPPH, anion superoxide radical, hydroxyl radical and IC25 (µg/ml) for nitric oxide radical of aracás (yellow and red), butiás (from Pelotas, Santa Vitória do Palmar (SVP) and Herval), and pitangas (purple, red and orange) ethanolic extracts and positive controls.
Acknowledgments

This work was carried out with CNPq support, National Council for Scientific and Technological Development Brazil (process number 400201/2014-3). Authors are thankful to for the financial support of CNPq/ Science Without Borders Program project “Frutas Nativas do Brasil: potencial anti-hiperglicimiante e antioxidante”. J. Vinholes (process number 313712/2014-0) and G. Lemos thanks the Science Without Borders Program (CNPq) for the Young Talent Attraction and Scientific Initiation fellowships.

Fig. 2. Inhibition of DPPH (A), Superoxide anion radical (B), Hydroxyl radical (C) and Nitric oxide radical (D) by araçás (yellow and red genotypes), butiás (from Pelotas, Santa Vitória do Palmar (SVP) and Herval), and pitangas (purple, red and orange genotypes) ethanolic extracts. Values show means ± standard error of the mean (SEM) from three experiments performed in triplicate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).
Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.fbio.2017.06.005.

References


